Lavandula dentata AND *Lavandula angustifolia* *IN VITRO* ORGANOGENESIS

ORGANOGÊNESE *IN VITRO* DE *Lavandula dentata* E *Lavandula angustifolia*

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ABSTRACT

Lateral buds (0.5 mm), nodal segments (1 cm), shoot tips (1 mm) and shoot apex (0.25 mm) of english lavender (*Lavandula angustifolia* Miller) and French lavender (*Lavandula dentate* L.) were placed on MS culture medium. After *in vitro* establishment, nodal segments of *L. angustifolia* containing two leaves were transferred to culture medium with different combinations of 6-benzylaminopurine (BAP) and gibberellic acid (GA_3) concentrations to induce *in vitro* organogenesis. After two months, the number and length of formed shoots, the percentage of shoots rooted, shoots with callus and the survival of the shoots were recorded. Shoot apex and lateral buds were considered better explants for *in vitro* establishment. Better multiplication rate, callus formation and survival percentage were obtained with the 10 mg L^{-1} BAP combined with 0.5 mg L^{-1} GA_3, while the lower survival percentage and callus formation occurred on media without regulators (control) for *L. angustifolia*. Growth of shoots from *L. dentata* in the applied conditions was not satisfactory.

Index terms: lavender, micropropagation, tissue culture, cytokinin, gibberellin.

RESUMO

Gemas laterais (0,5 mm), segmentos nodais (1 cm), ápices caulinares (1 mm) e ápices meristemáticos (0,25 mm) de alfazema inglesa (*Lavandula angustifolia*) e alfazema francesa (*Lavandula dentata*) foram inoculados em meio de cultura MS. Após o estabelecimento *in vitro*, segmentos nodais de *L. angustifolia*, com duas folhas, foram transferidos para meio de cultura com diferentes concentrações e combinações de 6-benzilaminopurina (BAP) e ácido giberélico (GA_3) para indução de organogênese *in vitro*. Após dois meses, foram avaliados o número e comprimento de brotações, a porcentagem de brotações enraizadas e com calo e a sobrevivência. Ápices caulinares e gemas laterais foram considerados os melhores explantes para introdução do material *in vitro*. A maior taxa de brotação, calogênese e porcentagem de sobrevivência para a *L. angustifolia* foram obtidas com a combinação 10 mg L^{-1} BAP e 0,5 mg L^{-1} GA_3, enquanto menores percentagens de sobrevivência e calogênese ocorreram em meio sem reguladores (controle). O crescimento de brotações de *L. dentata* não foi satisfatório nas condições estudadas.

Termos para indexação: lavanda, micropropagação, cultura de tecidos, citocinina, giberelina.

INTRODUCTION

Lavender is a European native aromatic herbaceous shrub which is currently cultivated worldwide in temperate climate regions. The most cultivated species are *Lavandula dentata*, *L. angustifolia* (formely *L. officinalis* or *L. vera)*, *L. latifolia* (or *L. spica*), *L. stoechas*, and the Lavandin hybrid (*L. angustifolia* x *L. latifolia*) (MARTINS et al., 2000). *L. angustifolia*, the English lavender, is considered the most important species due to its high quality essential oil, which is rich in linalil-acetate and linalool, differently of the highly productive *L. dentata* (French lavender) which is rich in 1,8-cineole, fenchone and canphor (BISSET, 1994; ECHEVERRIGARAY et al., 2005).

Field grown aromatic crops usually are not so productive and present lower secondary metabolites syntheses, not to mention genotypic and phenotypic variations. *In vitro* produced plants would present less variation than seed propagated plants and would better standardizing the produced essential oil. That is the reason why tissue culture techniques have been applied to propagate and expedite multiplication of many aromatic species. Badawy et al. (2003) observed that lavandin crops originated from *in vitro* propagation presented good field productivity and oil quality, being such procedure considered an excellent alternative to deal with problems such as declines, which are observed in conventional lavandin and lavender plantations

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(CHAMBON et al., 1992). Not only that, but with micropropagation, among other tissue culture techniques, it is possible to produce a great number of plants from few plant stock selected individuals, in a short period of time and physically smaller places, while warrants multiplication and conservation of live germplasms (SÁNCHEZ-GRAS; CALVO, 1996; ANDRADE et al., 1999; ECHEVERRIGARAY et al., 2005). Growth regulators are used to manipulate *in vitro* plantlets behavior. 6-benzylaminopurine (BAP), for example, promotes the growth of plantlets shoots (MURASHIGE; SKOOG, 1962); combined to gibberellins (GA_3), it may enhance the procedure since gibberellins are supposed to promote stem etiolation (YOUSSEF; TALAAT, 1998) forming longer internodes shoots making the *in vitro* processes of multiplication and rooting induction easier.

Cytokinins are N^6-substituted adenine derivatives implicated in many aspects of plant growth and development such as cell division, shoot initiation and development, vascular development, leaf senescence, chloroplast differentiation and among them, deetiolation (HUTCHISON et al., 2006).

Optimization of the *L. angustifolia* and *L. dentata* *in vitro* cultivation would not only rapidly increase multiplication rate but also could help to maintain different species germplasms available and improve future crops' homogeneity and productivity, which, specially to aromatic plants, translates in greater probability of obtaining the expected high quality essential oil. Not only that, but biotechnology allows that *in vitro* cells, tissues and organs be genetically manipulated, not only to acquire compounds of interest but also to serve as co-adjuvant to the aromatic and medicinal plants conventional plant breeding (RAO; RAVISHANKAR, 2002).

The objective of this work was to study the establishment and organogenesis of *in vitro L. angustifolia* and *L. dentata*, and to evaluate the effect of the combination of different BAP and GA_3 concentration on the multiplication of both species.

MATERIAL AND METHODS

Lateral buds (0.5 mm), nodal segments with leaf (1 cm), shoot tips (1 mm) and shoot apex with two to three leaf primordia (0.25 mm) were excised from cutting of *Lavandula dentata* and *Lavandula angustifolia* greenhouse stock plants, cultivated located at the Plant Science Department of the Federal University of Parana. Based on Dronne et al. (1999), explants were washed three times of 5 min each in sterilized de-ionized water, soaked in alcohol 70% for 1 min, placed in 20% commercial sodium hypochlorite solution (2% available clorine) with two drops of commercial detergent for 20 min, followed by three 5 min baths in sterile de-ionized water inside a laminar flow hood and aseptically cultivated on MS medium (MURASHIGE; SKOOG, 1962) containing 30 g L^{-1} sucrose, 6 g L^{-1} agar and pH 5.8. Flasks were placed inside growth chambers under the temperature of 25 ± 2° C, 16-h photoperiod e irradiance of 10 - 20 ìmol m^{-2} s^{-1}.

Contaminated or oxidized explants were dismissed while well succeeded ones were further used in the organogenesis experiment. *In vitro* produced nodal segments of *L. angustifolia* containing two leaves each were transferred to MS medium containing 30 g L^{-1} sucrose, 6 g L^{-1} agar and pH 5.8 plus BAP at 1, 3, 5 and 10 mg L^{-1} combined or not with GA_3 at 0.5 mg L^{-1}, respectively, as well as growth regulator free MS medium as control, totalizing nine treatments with 10 flasks containing 10 explants each and four replications per treatment. A completely randomized experimental design was applied. After two months, the number of 0.5 cm longer shoots, the length of longest shoot, the percentage of rooted shoots, percentage of shoots with callus and the survival of shoots were evaluated. Analysis of variance (ANOVA) followed by Tukey Test at 5% of significance were used to statistically analyze the obtained data.

RESULTS AND DISCUSSION

All shoot apex oxidized, probably due to the lesser amount of tissue, as these tiny explants usually suffer a great deal of injury caused by high temperature allied to

excessive cuttings by sharp scalpel in order to remove the external leaves. On the other side, nodal segments, due to their larger size, suffered much contamination, more than 90%, because sanitation tends not to be very effective when these larger explants are used and either external as internal microorganisms present in the tissue may cause held in the tissue. Dias et al. (2002), however, was able to *in vitro* propagate *L. viridis* using nodal segments as explants.

L. dentata did not develop well *in vitro*, few explants formed shoots and these shoots showed slow growing and seems chlorotic. Further tests using different media may be necessary. Similar results were obtained by Jordan et al. (1998) with *L. dentata* in basic MS media indicating that the species probably needs to be introduced in media already containing growth regulators in order to benefit its development. Machado et al. (2011) observed higher percentage of regenerated explants (90%) from shoot tips (5 mm) of *L. dentata* on medium containing 10 μM BAP + 2.5 μM IBA + 0.3 μM GA_3 and concluded that the presence of GA_3 allows shoot more elongated.

Basal MS medium (control) presented the highest mortality percentage (75%). The presence of cytokinin was important for *in vitro* survival of *Lavandula*, which was at least 2-fold better for all the cytokinin containing treatments, reaching 100% survival with combination of 10 mg L^{-1} with 0.5 mg L^{-1} GA_3 (Table 1). Similarly, axillary shoot development and elongation of *in vitro Lavandula* were not observed on growth-regulator free medium in Dias et al. (2002). The synergistic effect of cytokinin on *Lavandula* organogenesis was also confirmed by Chishti et al. (2006) and Dias et al. (2002).

Root induction occurred in the control media and for the combination of 1 mg L^{-1} BAP with 0.5 mg L^{-1} GA_3, but not in the other treatments. Endogenous auxin was probably responsible for root induction in the basic medium treatment while cytokinin (BAP) present in the other treatments probably blocked the effect of the auxin since these two regulators are known to influence each other's action, as postulated by Murashige and Skoog (1962).

Even though not statistically detected among treatments of shoot length (Table 1), it was possible to visualize a probable cytokinin induced formation of more compact and shorter shoots BAP concentration increased (Figure 1). The 1 mg L^{-1} BAP + 0.5 mg L^{-1} GA_3 and 10 mg L^{-1} BAP + 0.5 mg L^{-1} GA_3 combinations were the ones which formed more shoots, being the last one the concentration which also formed the greater amount of calli aggregated

TABLE 1 – Different combinations of BAP and GA_3 concentrations for *Lavandula angustifolia in vitro* organogenesis.

Treatments	Shoot number[1]	Shoot length (cm)[1]	Roots (%)	Callus (%)[2]	Survival (%)[2]
Control	1.00 b[3]	1.61 ns	8.6	2.8 c	25.7 b
1 mg L^{-1} BAP	3.05 ab	1.87	0	11.4 c	51.4 b
3 mg L^{-1} BAP	3.93 ab	1.65	0	25.7 bc	71.4 ab
5 mg L^{-1} BAP	4.09 ab	0.95	0	11.4 c	65.7 ab
10 mg L^{-1} BAP	3.72 ab	0.97	0	28.5 bc	79.9 ab
1 mg L^{-1} BAP + 0.5 mg L^{-1} GA_3	6.49 a	1.15	2.8	31.4 bc	65.7 ab
3 mg L^{-1} BAP + 0.5 mg L^{-1} GA_3	4.43 ab	1.36	0	34.2 bc	54.2 b
5 mg L^{-1} BAP + 0.5 mg L^{-1} GA_3	2.42 b	0.93	0	59.9 ab	77.1 ab
10 mg L^{-1} BAP + 0.5 mg L^{-1} GA_3	8.09 a	1.18	0	88.5 a	100.0 a
C.V. (%)	19.5	11.3	-	49.1	28.9

[1]Original data transformed into the root of (x + 1).
[2]Original data transformed into the arc sen root of (x/100).
[3]Average numbers followed by similar letters, on the same column, do not differ statistically accordingly to Tukey's test at 5%.

to the shoot bases (Table 1) as also observed by Tyub et al. (2007). In Dronne et al. (1999) it seemed that not only the amount of BAP but the combination of BAP with GA_3 *per se* induced calli formation, it is considered interesting because they can be used for germplasm maintenance as well as in cell suspensions to future secondary compounds elicitation, for quantitative or qualitative analysis of growth responses and metabolism of natural products, as well as for studies of cell cycle under standard conditions (XU et al., 2008) and *in vitro* breeding by protoplast fusion as improved cultivars could be developed via genetic transformation or somatic hybridization (BONA et al., 2007). Calli and cells suspensions were obtained from *L. angustifolia*, under light, with the combination of 2.4-D + GA_3. A blue pigment which may be interesting as a dye in the food industry was present in the media (BONA et al., 2012).

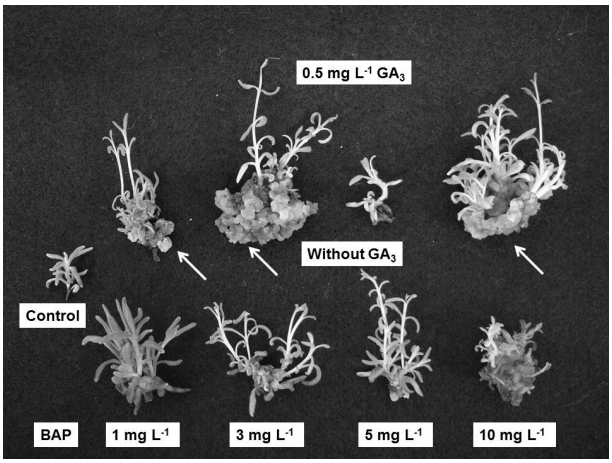

FIGURE 1 – *Lavandula angustifolia* organogenesis induced by different combination of BAP and GA_3 concentrations. Arrows indicate callus.

CONCLUSIONS

Shoot apex and lateral buds were considered the best explants option for organogenesis of *L. angustifolia* e *L. dentata* compared to nodal segments and shoot tips.

The presence of BAP combined with GA_3 in the culture medium was important for the *L. angustifolia* organogenesis, as shoots formed on growth regulator-free media (control) were visibly inferior to all the other treatments.

Organogenesis of *L. dentata* was not successfully achieved in the conditions tested herein.

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