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ASPECTS CONCERNING THE MORPHOGENETIC REACTION OF SOME EXPLANTS OF LAVANDULA STOECHAS 'ANOUK' CULTIVATED IN VITRO

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***Key words**: Lavandula stoechas, in vitro culture, caulogenesis, callusogenesis*

INTRODUCTION

The *Lavandula* genus belonging to the family *Lamiaceae* comprises 39 species of aromatic plants originating in the Mediterranean area. The genus also includes numerous hybrids and about 400 varieties of lavender (Gonçalves S., Romano A., 2013). Many species of this genus are grown in numerous countries, but especially in France, Spain and Italy, both as decorative plants and for the essential oil extracted from flowers and leaves. It is used in the cosmetic industry but also for therapeutic purposes. Lavender essential oil has a chemical composition that varies depending on the genotype, as well as the environmental conditions in which the plant grows (Demissie et al., 2012). Not all lavender species are used in cosmetics and medicine, the most commonly used being *L. angustifolia*, *L. latifolia*, *L. stoechas* and *L. x intermedia* (Cavanagh and Wilkinson, 2002). The properties of lavender essential oil have been known since antiquity, and research on this subject has been reviewed by Woronuk et al. (2011). Due to its therapeutic qualities, the lavender essence can be used both internally and externally, having a calming effect on the central nervous system, as well as a diuretic, colagog, carminative, antispasmodic one, being used in the treatment of convulsive cough, laryngitis, contusions, wounds, burns and insect bites (Pârvu, 1991). Recent research has highlighted the fact that lavender essential oil has beneficial effects in the treatment of dementia (Samallwood et al., 2001, Fu et. al., 2013), and the composition of oil from some subspecies triggers antimicrobial and antioxidant activities.

The multiplication of lavender plants occurs with very low yield. It is carried out vegetatively by woody stem cutting, but their rooting and risks of modification induced by repeated vegetative propagation can be overcome by micropropagation (Soni D.R. et al., 2014). To highlight a good micropropagation protocol, a lot of research has been done over the last 20 years on the *in vitro* multiplication of some lavender species (Gonçalves and Romano, 2013), especially those with valuable essential oils. Most research of this kind has been carried out on the species *L. angustifolia*, *L. latifolia*, *L. officinalis* and *L. vera*

Lavandula stoechas L., known as French lavender or Spanish lavender, is a species mostly used for ornamental purposes. The chemical composition of its ethereal oil was analyzed by Giray et al. in 2008, and research has shown that it can also be used for therapeutic purposes. For example, Cavanagh and Wilkinson (2002) argue that L. stoechas is traditionally used for headache.

In terms of *in vitro* multiplication of lavender species, the literature suggests that the most effective method is propagation from axillary or terminal buds (Gonçalves and Romano, 2013). The purpose of our research was to highlight the morphogenetic reaction of some explants of *Lavandula stoechas (anouk)* to identify the nutrient environments that allow micropropagation of the species.

MATERIAL AND METHODS

To initiate in vitro cultures the Murashige Skoog (1962) medium and its variants were used, including various combinations and concentrations of growth regulators. As carbon source, we used sucrose (25 g/l), and 8.5 g/l agar was added to solidify the medium. After preparation of the nutrient media, the pH was adjusted (corrected) (5.8) and then autoclaved at 121° C for 20 minutes. After autoclaving, the media were dispensed into sterile Type B 100 Erlenmeyer flasks at PBI Space laminar air niche. To foster growth and development of the explants, the inoculated vials were placed in the SANYO growth chamber which provided controlled conditions: 25° C, 2500 lux brightness, 16-hour photoperiod.

The biological material used was represented by young shoots that were harvested in June from the "Anastasie Fătu" botanical garden in Iaşi. The explants (shoot tops and nodes) were sterilized with Chloramine T (5%) solution for 20-25 minutes, after which they were vigorously rinsed three times with sterile distilled water. The sterilized explants were then inoculated on initiation media: hormone-free MS basal medium (Murashige-Skoog, 1962) or MS supplemented with BAP + IAA (1/0.5-1 mg/l), BAP + IBA 1/0, 5 - 1 mg/l). The sterile neoplantules obtained on the initiation media were then used to test the morphogenetic reaction of the shoot tops and

nodule explants (containing leaves) on a series of hormonal formulas of the MS medium (Table 1).

Table 1. The hormonal variants used to highlight the morphogenetic reaction of *Lavandula stoechas 'Anouk'* explants

No.	Hormonal formula	GROWTH REGULATORS (ml/l)				
		BAP	IAA	IBA	NAA	2,4-D
1	MS	-	-	-	-	-
2	BA_1	1	0,5	-	-	-
3	BA_2	1	1	-	-	-
4	BB_1	1	-	0,5	-	-
5	BB_2	1	-	1	-	-
6	BN_1	1	-	-	0,5	-
7	BN_2	1	-	-	1	-
8	BD_1	1	-	-	-	0,5
9	BD_2	1	-	-	-	1

RESULTS AND DISCUSSIONS

As mentioned earlier, the most numerous attempts at lavender micropropagation have been performed on *L. angustifolia*, *L. latifolia*, *L. officinalis* and *L. vera*. Most researches have shown that the rate of multiplication was very low, and there were very frequent phenomena of callusogenesis (Sánchez-Gras and Calvo, 1996; Nobre, 1996, Ghiorghiță et al., 2009) and formation of shoot hyperhydricity during in vitro vegetative propagation (Ziv, 1991; Debergh et al., 1992, cited by Andrade et al., 1999).

In our investigations, too, we highlighted callus formation on most of the tested nutritional variants. Its consistency and rate of proliferation varied according to the hormonal combination of the tested nutrient media (Table 2).

Table 2. The morphogenetic reaction of explants of

Crt. No.	Hormonal formula	Type of explant inoculated	The morphogenetic reaction
1	MS	Nodes, apexes	Caulogenesis (++) from the apex explants
2	BA_1		Caulogenesis (++); vitrified stoots; friable callus, cream (+), degenerates easily
3	BA_2		White and friable callus (+++); caulogenesis (++), 1-2 shoots/explant.
4	BB_1		Cream callus (+), caulogenesis (+); some shoots vitrified
5	BB_2		White and light green callus (++); caulogenesis (++)
6	BN_1		White and half-compact callus (+) with roots
7	BN_2		White and half-compact callus (++) with roots
8	BD_1		Callusogenesis (+++), white and friable callus
9	BD_2		Callusogenesis (+++), green and friable callus

The best callusogenetic reaction was highlighted on media supplemented with 2.4D, as also reported in specialized papers on other lavender species (Ghiorghiță et al., 2008; Claudine M., Santos D., Biasi, Luiz A, 2012) but, in our case, also on nutritional variants supplemented with IAA and IBA – (1 mg/l) in the presence of BAP.

The obtained callus generally showed a friable consistency, having a cream, whitish-cream or light-green color. On the culture media supplemented with BAP and NAA (0.5 - 1 mg/l) or NAA (2mg/l) roots were formed at callus level. The callus did not show regenerative capacity on any nutritional variant.

From our observations, we found that the callus transfer should have been done within 2-3 weeks from the culture, otherwise the callus loses its ability to multiply and undergoes necrosis rapidly. This observation was also reported by other researchers, who mentioned the fact that the proliferation of the callus stagnated after three weeks (Claudine M., Santos D., Biasi, Luiz A, 2012). Experiments conducted by other researchers highlighted that the absence of light is an important factor in the survival and proliferation of the callus. Thus, the periodically pricked out callus, cultivated under dark conditions, allowed the maintenance of its proliferation capacity (Ghiorghiță et al., 2008).

The subcultivation of the callus obtained by us allowed pricking out only 3-4 times (under light conditions - 2500 lx, 16-hour photoperiod). After this period, the callus degenerated, even if it had been transferred to the same nutritional variant. After a longer period of subcultivation, the callus changed its consistency, it became brittle, semi-compact, granular, and cell multiplication capacity was totally lost.

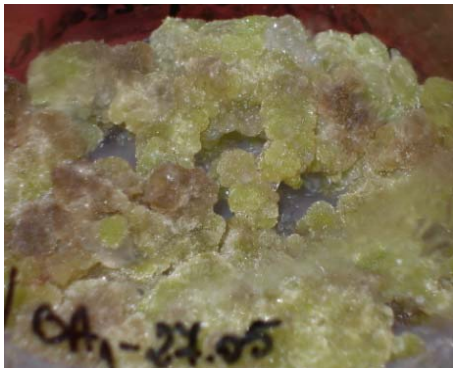

Photo 1. Callusogenesis on BA_1 medium

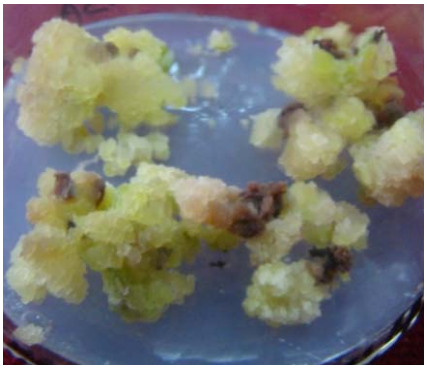

Photo 2. Light green callus on BB_2 medium

Photo 3. White and friable callus on BD1

Photo 4. Callusogenesis on BD_2 medium

Photo 5. Compact callus from the nodes explants, with roots on BN_2

Another important aspect highlighted in the callus cultures was the appearance, in the culture medium, of a blue-violet compound, as also reported in the literature, which is due to the L-cysteine content. This compound confirms the high capacity of callusal cells to produce rosmarinic acid, used in the pharmaceutical and cosmetic industry (Claudine, 2012).

Another marked morphogenetic reaction was caullogenesis. The vegetal explants that allowed the regeneration of new shoots were the apexes. The phenomenon occurred, however, with low intensity, the number of regenerated shoots/explant being 1-2. The nutritional media where caullogenesis was observed were characterized by the presence of a low concentration of auxin (IAA, IBA - 0.5 mg/l) in the presence of BAP cytokinin (1mg/l) and the hormone-free MS base. The shoots obtained, although grown in length, were very fragile and showed very slow growth. Some newly formed shoots have highlighted the hyperhidrosis phenomenon. Various protocols for micropropagation of the species (including the use of a modified base medium – a 1/4 salt strength MS medium) have been presented in the literature, but hyperhidrosis has been reported frequently. This is why some authors consider that this phenomenon may be a limiting factor in the propagation of lavender plants (Nobre, 1996).

Photo 1. New shoots on basal medium (MS)

Photo 2. Shoots from nodal explant on BB2 medium

Photo 3. Caulogenesis on BA2 medium

Photo 4. Vitrified shoots on BA1 medium

Photo 5. Calus and shoots from nodal explants (BB1)

Photo 6. Calus and shoots on BN1

CONCLUSIONS

- Initiation of in vitro culture at the species *lavandula stoechas (anouk)* did not pose problems, this being possible using the MS basal medium and the shoot tops as explants.
- The main morphogenetic reaction was callusogenesis. The consistency, proliferation rate and callus colour varied according to the combination and concentration of phytohormones in nutrient environments. Maintaining the callus in culture could not be achieved more than 3 prick outs on the same nutritional variant, even if the transition occurred at intervals of 2-3 weeks. With no nutritional variation, the callus did not show any regenerative capacity.
- The emergence of shoots is a phenomenon that occurred with low intensity, callusogenesis being observed only on media supplemented with a reduced amount of auxins, in the presence of BAP. The shoots showed very slow growth.
- Rhizogenesis was only highlighted in the calluses developed on NAA supplemented media.

ABSTRACT

The *Lavandula* genus belong to the family *Lamiaceae* and comprises 39 species of aromatic plants. Many species of this genus are grown both as decorative plants and for the essential oil extracted from flowers and leaves.

Vegetative propagation of lavender plants is produced with very low yield.

Numerous researches have attempted to identify a protocol for micropropagation of different species of lavender and these suggests that the most effective method is propagation from axillary or terminal buds.

Our observations led to the following conclusions: the best reaction highlighted was callusogenesis followed by caulogenesis. Lavender callus formation was highlighted in the medium supplemented with 2,4 D in various concentrations. On various hormonal formulas, the callus generally has a good proliferative capacity, but no regeneration capacity of new shoots.

The emergence of shoots is a phenomenon that occurred with low intensity, caulogenesis being observed only on media supplemented with a reduced amount of auxins, in the presence of BAP.

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