

## REACTION OF EXPLANTS FROM LAVANDULA ANGUSTIFOLIA MILLER, CULTIVATED IN VITRO ON THE PURPOSE OF MULTIPLICATION AND CALLUS DIFFERENTIATION

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### Abstract:

*Lavanda angustifolia* Mill. is a honey, medicinal and plant ameliorative specie, which lives in arid and semi-arid climate (of mediteranean origine) and has a great echological plasticity and adjustment capacity.

We studied and followed in vitro culture of apical tissue and of bud tissue cut from mature lavender plant grown on basic MS (Murashige and Skoog, 1962), more complex, with extra aminoacid-glycine, with the variants: Mt = MS + 2 mg/l glycine; V1 = Mt + 1.0 mg/l AIA + 1.5 mg/l BA; V2 = Mt + 1.0 mg/l AIB + 1.5 mg/l BA; V3= Mt +1.0 mg/l ANA + 1.5 mg/l BA.

The culture mediums have been conceived to prove the implication of glycine and of certain auxines in the case of in vitro cultivated lavender plants, in regeneration of plants, callus differentiations, in vitro propagation (given by the number of seedlings/explant and by their structure). After cca. 3 month of in vitro incubation, apex regenerates in 35% on Mt; 55% on V1 and 75% on V2 and forms rootless 2-3 neo-seedlings of 1-2 cm length. On V3 a large mass of callus is differentiated, of 4-5 cmØ at 85% of the explants. The bud has a better regenerating capacity (from 42%, 62% up to 80%) on V3, callus differentiation is 85% as well, the number of differentiated seedlings is double. There are similarities and differences between the evolution of in vitro lavender explants, the bud having superiour values of caulogenesis and even propagation. The results show the effect of glycine aminoacid in the process of in vitro regenerating of lavender plants and also in delaying or even stopping of the auxine's reaction (for inducing rooting). We recomend testing glycine in medium for other species (having economic, ornamental values or spontaneous species, etc), in order to establish if it has or hasn't the capacity to stop or reduce rootedness induced by auxine. On all versions the rooting is blocked by a small collar of callus generated at the bottom of the seedling (similar differentiation). As far as in vitro lavender cultivation is concerned, AIB medium (V2) has the best effect in propagation and, organogenesis and caulogenesis.

**Key words:** *Lavandula angustifolia* Miller.(fam. Lavandulaceae, syn. Lamiaceae), medical, flavoured, phito-ameliorative, propagation, in vitro, MS medium, glycine (aminoacid), inhibition, bud, apex, seedling regeneration rithm, callus differentiation

### INTRODUCTION

*Lavandula angustifolia* Miller, synonym for *Lavandula officinalis* L, (fam. Lavandulaceae, syn. Lamiaceae) is a species well known since antiquity (due to the parfume given by the lavender baths). It is a honey, medicinal and phytoameliorative (contributes to capitalization of eroded land and prevents erosion on lime, sunny downhill) is called „levantica” (Roman et al., 2012). This plant originates from Mediteranean area and is wide-spread in Southern Europe, in the south of France, Spain, Italy, Greece and can be found in spontaneous flora of the Alp Mountains (Paun et al. 1986). In

climate conditions of Romania, this is a cultivated semi-shrub, with a long (up until 30 years) life, remarkable for its greyish-green colour of the leaves and for the lilac coloured flowers (Craciun et al, 1977). The favourable areas for this plant in our country are: Timisului Olteniei, Baraganului Plains, Southern Carpathian Mountains, Tara Barsei (Flora RSR, 1952-1974). In the ecology of this species it is well-known the need for high temperature, beginning with germination (10-12° C) and until maturation and flowering (10-12° C). The plant's demands for humidity are reduced but they are high for light and temperature. High lightness helps growing of the shrub and of the flowers and helps increasing the production of essential oil even three times (Munteanu et al, 2008). Towards the soil, the lavender has few expectations, it prefers the lime ground, faces well the drought but has high demands towards the presence of water, mainly immediately after planting and before flowering (Parvu, 2003). In the origin area, the multiplication of lavender is made by seeds but is also well known the vegetative multiplication through vegetative cuttings taken during the rest period. The hybrids resulting from *Lavandula angustifolia* have unfruitful seeds and can be multiplied only by using vegetative cuttings, in protected areas and on a fertile soil (Encyclopedie universelle de 15.000 de plantes, 1999).

The lavender is well known as a decorative plant, used alone or in association with other floral species, widely used and very decorative in parks and gardens (BOTANICA – Encyclopedie de botanique et d'horticulture, 1997). The lavender is a honey species, very valuable and important for human and animal cure, cosmetics and for industry of essential oil (Parvu, 1988). The inflorescence of *Lavandula angustifolia* contains essential oil up to 1,4%. The maximum of quantity is found at the beginning of flowering and during 09-02 a.m. hours of the day (Munteanu et al, 2007). The phytotherapeutical importance of the essential oil can be summarized for the internal use (normalizes the heart functions through adjustment of excitability state of organism) and for external use as well, through dropping the pain and eliminating toxins from the organism). The essential oil is recommended in migraine, headache, heart diseases, fever, flu, etc (Bojor and Alexan, 1983). We can mention here a few of the effects of lavender essential oil: light sedative, treats the insomnia, antispasmodic, digestive stimulant, calming bathes by using a concentrated emulsion (Nazad – coord, 2000).

For classical multiplication – vegetative cuttings – we can use mature plants (cca 3-4 years old), which is a long-drawn method. Due to this fact, we use micro-multiplication in vitro, in the same manner (Cachita, 1987) as for other economic, ornamental or phytotherapeutical important and valuable plants, such as potato species, in which we can obtain seedlings – the tubercles (Agud et al, 2010; Agud, 2011). The advantages of vegetative

bio-technologies are well known in different plants, just to mention obtaining of a great number of plants from one explant, in a short amount of time, identical to the donor mother plants (Laslo, 2013). At the end of the experiment, this method ensures the obtaining of a superior seedling material, both as quantity and quality (Cachita et al, 2004), and this might be many times the only quick method to introduce new plant species in culture, in certain areas (Cachita et al, 2009). The advantage of micro-propagation and *in vitro* multiplication of plants species have made possible conservation of genetic resources (Bajaj, 1986) and even establishing of special methods for conservation of rare, endemic species which were in danger of extinction from our country's flora (Laslo et al, 2011). It was also possible to extend this kind of unconventional propagation method as a conservation technique as a contribution to preservation of botanical elements from existing protected areas (Agud, 2014).

#### MATERIAL AND METHOD

The lavender culture has been initiated at the end of April, optimal period for classical multiplication of *Lavandula angustifolia* Miller, as well as for *in vitro* micro-propagation. The vegetative material cut out from mature plants consisted of cusp apex (apical meristem) of cca. 0,2-0,3 mm, a tissue that has proven a good regenerative reaction *in vitro*. This kind of tissue has proven a very good regenerative reaction in other species that we have experimented, aboriginal species of economic interest (Agud, 2001). We also have used young, unformed bud tissue, uncoloured, cut out from young inflorescence, from a mature plant, grown in solar or in hotbed or field. Starting from previous experiments with other medicinal and flavoured plants, we established that other *in vitro* cultivated types of tissues have good regeneration capacity, based on the type of species and on type of medium structure (Zapartan et al, 1994). In case of *Menta piperita*, the reaction of knob explant was due to hormone balance in the environment and due to supplementary intake of substances with the role of induction of organogenesis and *in vitro* propagation (Laslo et al, 2011).

Culture medium used for *Lavandula angustifolia* Miller, had the composition of basic MS (Murashige and Skoog, 1962), just more complex, with extra amino acid- glycine – well known as having the effect of growing the capacity of regeneration and stimulation of organs forming in potato (Agud et al, 2010). This complex medium has been considered as control version (Mt): to this we added phyto-hormones as follows:

**MT**=MS+ 2 mg/l glycine, **V<sub>1</sub>**=Mt+1,0 mg/l AIA + 1,5 mg/l BA,  
**V<sub>2</sub>**=Mt+1,0mg/l AIB+1,5mg/Lba, **V<sub>3</sub>**=Mt+1,0 mg/l ANA+1,5 mg/l BA

The medium formulae in variants were conceived to establish which was the implication of the aminoacid in the medium and how balanced concentrations (0,1 mg/l auxines, 1,5 mg/l IBA) and auxines (AIA, AIB, ANA) and benzylaminopurine (BA) influence the results of our experiments.

## RESULTS AND CONCLUSION

The observations have been made after 2,5-3 months from in vitro inoculation of explants originated in the apex and bud and the results have been presented by comparison and have been summarized in Table 1, marking the evolution of the specie according to the nature of in vitro inoculated explant. The evolution between V<sub>1</sub> and V<sub>2</sub> is similar in the case of apex originated explant, with greater percentage of the regenerative capacity for V<sub>2</sub>. As for V<sub>3</sub> the evolution is uniform, showing a very large mass of callus as a consequence of differentiation. The callus starts being necrotic if not passed, it doesn't evolve towards regeneration. Passing of callus had to be done on two mediums: for plant regeneration on a medium with cca. 2mg/l cytokinin +0,5 mg/l auxin and for friable callus on a suspension with 8mg/l 2,4D + 5 mg/l BA.

*Table 1*

In vitro reaction of the apex tissue and of bud tissue grown on glycin medium  
(after 2,5-3 months)

Var.	APEX			BUD			Observații privind diferențierea tesutului calusal
	% reg.pl.	No.pl./cm	Nr. răd.	% reg.pl.	No. pl./ cm	Nr. răd.	
Mt.	35	2pl/1,5cm	-	42	2 pl./ 2cm	-	Similar evolution for apex and bud cuttings. Two plants/expl are regenerated, with cca 1-2mm Ø callus at the bottom, without roots.
V <sub>1</sub>	55	3pl/2.5cm	-	62	5pl./2cm	-	Similar evolution, the bud is superior, with 3-5pl/expl, without roots to develop from basic callus 1,5cm Ø
V <sub>2</sub>	75	3pl/1cm	-	80	6pl./2,5cm	-	Better evolution, even, with 3-6pl/explant coming from basic callus of 1cmØ de la bază.
V <sub>3</sub>	85%callus	-	-	85%calus	-	-	A callus mass of de 4-5cmØ

Differences in evolution of regenerative capacity (caulogenesis and callusogenesis) of apex tissue and bud tissue are present as well as differences regarding the number of regenerated plants –table 1, the multiplication percent. Apical tissue on Mt, V<sub>1</sub> and V<sub>2</sub> generates 2-3 explants from a piece of callus of 1-2mm Ø up to 1-1,5cm Ø. On V<sub>3</sub> there is a callus mass of 4 cm. In fig. 1 you can see the media values of the numbers and height of in vitro obtained seedlings.

The bud has better evolution but similar to apex, generates a greater number of neo-seedlings/explant, 2 to 6 neo-seedlings (the double number) of 2-2,5 cm length (on V1 and V 2 as well) and has the same alluses collar that seem to inihabate the rooting process: on V3 we find a callus collar of 5 cm Ø.

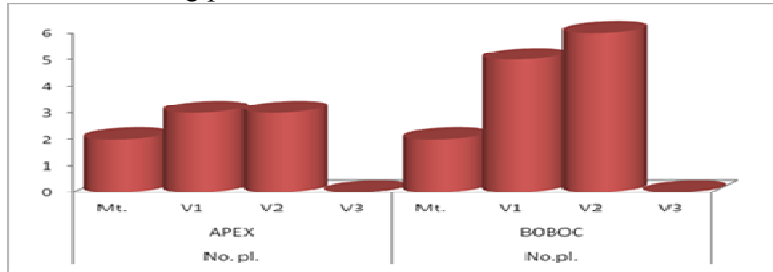


Fig.1. Average plant apex differentiated buds and lavender (after 2.5-3 months)

These results entitle us to affirm that the explants have the same reaction on all versions, presenting favourable differencies for the bud, regarding the number of neo-seedlings. On all versions the rooting process is inhibited by the callus collar forming at the bottom of the seedling. We can find the callus collar in all versions of seedlings (so we have similar differentiation). Due to this lack of rooting induces by the callus we observed this experiment for a longer period of time. As watching this fact in the case of the lavender seedlings from our collection, we ascertained two small roots of cca. 0,4 cm only on V2, in the presence of indolil-butiric acid - AIB (V2).

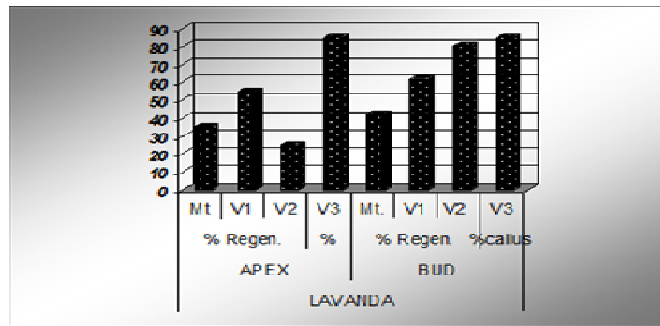


Fig.2. Percentage plant regeneration of *Lavandula angustifolia* apex and freshman Mill (after approx. 2.5-3 months)

The in vitro regenerative capacity of *Lavandula angustifolia* Miller explants, put in percentage average, has been manifesting in a similar way as the bud explant. In table 1 we can see that the apex and the bud regenerates seedlings in 75-85% on V2 and form callus in 85% on V3. The evolution of the explants is similar and even, having differences between the two explants expressed in fig.2, in favour of bud in vitro cultivated explants, which has a superious evolution.

The results that we obtained in the case of *Lavandula angustifolia* Miller *in vitro* cultivated tissues, helped us to establish that, the presence of aminoacid in the culture medium, delays or even inhibits auxin's effect and for our species the differentiation of roots is inhibited. The presence of glycine in the medium is rather a helper for the differentiation of callus at the bottom of the explant in the form of a callus collar of 0,5 cmø, which has the capacity to generate neo-seedlings (on Mt, V2 and V3). In combination with ANA (  $\alpha$  naftil acetic acid), glycine stimulates formation of a large mass of callus, cca. 4-5cmø, which, after cca 20 days has regenerative capacity (Foto V2 and V3).

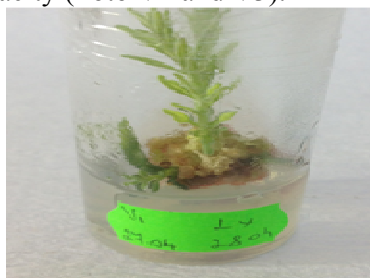


Photo. 1 - Evolution in V1 (Mt + 1 mg / LA + 1.5 mg / BA)

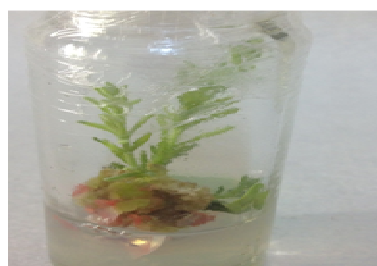


Photo. 2 - Mt (MS + 2 mg / 1 glycine) Photo 3 - V2 (Mt + 1 mg / LA + 1.5 mg / BA)

We can notice that after a long period of time the callus get necrotic and we recomend due to this situation, the quicker passing of the callus to fresh medium. We can state that glycine in the case of lavender substitutes the effect of 2,4D auxine as we know the implication of auxine in stimulating callus formation at the majority of *in vitro* cultivated plants (Deliu, et all. 1993-1994). Neo-seedlings un-rooted can be passed as wholes or cuttings can be used (1-2 nodes) on MS medium, simple, without using amino-acid but with a auxine present which will stimulate rooting in cca. 12-14 days, in combination with a citokinine in the composition, in small dose(0,5mg/l) in order to stimulate propagation.

## CONCLUSION

1. The presence of glycine in the basic culture medium fosters the formation of regenerative callus after cca. 2 months from inoculation: friable or regenerative callus differentiates according to medium composition.
2. Differentiated neo-seedlings have not formed roots. The cause has to be looked for in the presence of amino-acid in the culture medium which might inhibit rooting but fosters regeneration of plants from regenerative embryogen callus. Neo-seedlings can be cut from the callus mass and can be cultivated on a simple MS medium having 1,0mg/l AIB și 1mg/l BA which foster rooting and propagation.
3. The differences in evolution of the three auxines (in the combinations of the experiment versions) are obvious: on the AIB and AIA mediums the evolution is similar (with very small, insignificant differences) with stimulation of callus regeneration that generates plants:ANA stimulates forming of a friable callus mass that needs to be passed on a fresh, improved with cytokinine culture medium in order to stimulate regeneration of plants.
4. It is clear that the presence of the amino-acid in the culture medium delays or inhibits the effect of auxines (stimulator of the differentiation of roots).

We recommend therefor, keeping the aminoacid in the medium but using a greater dose of auxines for stimulating rooting or removal of glycine if we wish for a completely formed, greater number of plants. It is possible to maintain the aminoacid in order to stimulate generation of callus. We also recommend passing at cca. 3 weeks of formed callus on a fresh medium with a high dose of 2,4 D and of benzilaminopurine (BA), in order to increase the regenerative capacity of the callus tissue.

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