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SOME CONSIDERATIONS REGARDING THE *IN VITRO* BEHAVIOUR OF *LAVANDULA ANGUSTIFOLIA* L. SPECIES

Gogu Ghiorghiță, Diana-Elena Maftei, Daniela Nicuță, Irina Toma

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INTRODUCTION

Lavander (*Lavandula angustifolia* L.) is a perennial semi-bushy herb from the Lamiaceae family, of mediterranian origin, grown in Romania for its active principles within its inflorescences, especially for its volatile oil (that comprises mostly linalool and linalil acetate). Lavander active principles have an antiseptic, carminative, sedative, antispastic, diuretic, colagogue etc (1, 2, 4-9). Though lavander cultures can be accomplished with plants provided generatively or vegetatively (by means of cuttings), *in vitro* micropropagation of this species is important in view of eventual valuable genotypes multiplication. Callus production by means of *in vitro* cultures and the selection of cell lines that produce certain substances (e.g. biotine) appear to be up-to-date topics regarding this species, (3, 10, 11). All this being considered, our aim was to investigate the morphogenetic reaction of some lavander explants within *in vitro* cultures, the possibility of inducing indirect organogenesis via callus and somatic embryogenesis, to evaluate the capacity of biomass accumulation in callus cultures, to evince the cytogenetic outcome of the *in vitro* regenerants on varied hormonic formuli, as well as their behaviour in field conditions. This paper presents the reaction of this species *in vitro* and also the estimate of biomass accumulation in lavander callus cultures.

MATERIAL AND METHODS

The source of explants to initiate the *in vitro* cultures of *Lavandula angustifolia* was represented by plants from the Research Centre for Medicinal and Aromatic Plants from Fundulea subsequently cultivated in soil pots in laboratory conditions at the 'Stejarul' Research Centre of Piatra Neamt. The explants (shoot tips) were sterilized for 20-25 minutes in chloramine-T (solution, 5%), then rinsed twice with sterile distilled water and inoculated on

Murashige-Skoog medium, hormone-free or supplemented with 0.2 mg/l BAP. The MS medium was solidified with agar (8.5 g/l) and the carbon source of the nourishing medium was saccharose (25 g/l). The neoplantlets obtained on this culture media represented the source of explants to diversify the experiences of *in vitro* testing on varied hormonic variants of MS. The cultures were initiated in Erlenmeyer vials of 100 ml (B type) and then incubated in a culture room with half-climatised conditions (temperature of 23 to 25º C, light intensity of about 2000 lux, permanent illumination). We also evaluated the capacity of biomass accumulation in callus cultures by means of a callus line provided by stems inoculated on a culture medium supplemented with 2 mg/l IAA. The callus was cultivated in enlightened rooms and also in the absence of light and the data from table 1 represent the average estimate of 3 vials for each hormonic variant. Our tests' results are displayed in tables 1, 2 and figure 1 (a-f).

RESULTS AND DISCUSSIONS

In vitro culture initiation at lavander in the previously mentioned conditions did not raise any particular difficulties. We may state from the beginning that the most significant morphogenetic reaction of the tested explants (shoot tips, nodes, leaves, stem fragments) on the most varied hormonic variants of the MS medium (A, B, BA_1, BB_2, BD_1, BG_1, BN_1, D_2, KN_1, N_2) was callus generation (mostly friable, cream or green), (table 2, fig. 1-a-f).

Sometimes even the shoots transfered on MS medium without growth regulators for enrooting provided roots at base and also a layer of friable callus, more or less developed. The most appropriate medium formuli for callus induction from stems were BG_1, BN_1, BD_1 and B_{02}.

Leaf fragments provided callus intensively on D_2. Stem callus as well as leaf callus proliferated very intensively by transfering it on fresh media supplemented with BAP (0.5-2 mg/l), with BAP (1 mg/l)+IAA (0.5 mg/l), with BAP (1 mg/l)+GA (0.5 mg/l), BAP (1 mg/l)+IBA (0.5 mg/l), kinetine (1 mg/l)+NAA (0.5 mg/l). The callus provided by leaves had no organogenetic capacity. Friable cream or green stem callus grown in light conditions (especially on nourishing media supplemented with BAP) turned green gradually, became more consistent (breakable) and formed caulogenetic isles that generated multiple shoots frequently. The friable cream callus produced at the base of nodes cultivated on media with 2 mg/l IAA

(that was considered a callus line with proliferation peculiarities). Shoots provided by indirect organogenesis (by means of callus) from stem (nodes) callus were resistant to enrooting, disregarding the root-inducing medium formula).

Shoot tips and nodes inoculated on varied hormonic formuli also generated roots, forming neoplantlets: sporadically on media with cytokinins and a high frequency of enrooting on media with auxins (IAA, NAA) or cytokinins and auxins (BAP+IAA, BAP+NAA, kinetine+NAA), (table 2). The highest percentage of root formation was registered on MS medium supplemented with 2 mg/l NAA. This medium formula also favoured the obtaining of the most vigorous neoplantlets that had an average of 5 to 7 basal shoots. A quite satisfactory frequency of enrooted shoots was observed on hormone-free MS medium (1d).

We consider that in order to micropropagate this species the best solution is to initiate and maintain the culture only on hormone-free MS medium as lavander is very sensitive to hyperhydria. This phenomenon brings a great difficulty in shoots enrooting which is sometimes impossible. Supplying the culture medium with phytoregulators obviously increases the risk to induce hyperhydria. At the same time the use of hormone-free MS medium only inhibits callus formation at shoot base, as well as root generation. Researches to discover the most appropriate hormonic formula for enrooting the shoots obtained by indirect organogenesis via callus are in progress. Neoplantlets' accommodation to septic environment took a short period of time and the biological material losses did not exceed 10 %, (fig. 1f).

Fig. 1. a – Friable foamy callus provided by nodal explants on B_{02} culture medium; b – Caulogenetic callus generated by nodal explants on medium with IAA and transfered on media comprising Kinetine and IBA; c – Regeneration of shoots from nodal callus passed on media supplemented with BAP; d – Indirect multiple shooting (from friable nodal callus obtained on A_2 formula) on MS basal medium; e – Neoplantlets with callus formation provided on IBA medium; f – *In vitro* regenerants accommodated in a hydroponic system

Table 1. Morphogenetic reaction of some lavander explants on varied hormonic formuli of Murashige – Skoog medium

Var	Type of explant	Hormonic formula	Growth regulators (mg/l)							Morphogenetic reaction and proliferation speed
			BAP	GA	IAA	IBA	NAA	KIN	2,4-D	
1.	Nodes and shoot tips	A			2.0					Vigorous neoplantlets (++) with multiple shoots (+++); friable cream callus (+++) at stem basis
2	"	B	0.2							Neoplantlets (+); compact green-brownish callus (+) at contact surface with nourishing medium; multiple shooting (+++)
3	"	BA	1.0		0.5					Multiple shooting (+); neoplantlets sporadically (+); friable green callus (++) that sporadically differentiated shoots (+)
4	"	BB	1.0			0.5				Friable cream callus (++) that degenerated in time; caulogenesis was inhibited on this medium formula
5	"	BD	1.0						0.5	Friable cream and green callus (++) that degenerated in time
6	"	BG	1.0	0.5						Multiple shooting (+++); friable cream callus (+++)
7	"	BN	1.0				0.5			Neoplantlets (+) with multiple shoots (++); cream and green callus (++)
8	"	IB				2.0				Neoplantlets (++), cream friable callus (+)
8	"	N					2.0			Vigorous neoplantlets (++) with multiple shoots (++)
9	"	KN					0.5	1.0		Neoplantlets (++), multiple shooting (++); friable but also hard consistency (granulated) cream and green callus (++)
10	"	MS								Neoplantlets (++) - multiple offshoots (+), cream greenish friable callus (+)
11	Internodes	BD	1.0						0.5	Friable cream and green callus (++) on the entire explants' surface
12	"	D							2.0	Quite friable callus (++), cream and green callus on the whole explants' surface
13	Leaves	BD	1.0						0.5	Compact cream and green callus (++) on the entire explants' deprived of organogenetic capacity
14	"	D							2.0	Friable cream and green callus (++), no organogenetic capacity
15	Node callus provided on A medium	B	0.2 -0.5							Friable green callus (++) that frequently provides multiple shoots (+++)
16	"	KN			2.0		0.5	1.0		Friable foamy cream callus (++++), high proliferative
17	"	N					2.0		2.0	Friable foamy cream callus (++++), high proliferative
18	"	MS								Friable cream-whitish callus in darkness, green in light (++++) that frequently provided shoots in light conditions (++) and seldom in dark conditions (+)
19	Node callus provided on KN	MS								Callus cultures maintained in darkness displayed a good proliferation speed (++), turned cream-whitish, semi-compact and generated frail shoots sporadically (+)
20	"	B	0.2							Friable callus of hard consistency (++) that frequently produced multiple shoots (++)
21	Leaf callus generated on D2	B BG	0.5 1.0	0.5						Friable green callus (++++) with cream-brownish or white callus isles, no organogenetic capacity

A=IAA; B=BAP; BA=BAP+IAA; BB=BAP+IBA;BD=BAP+2.4-D; BN=BAP+NAA; D=2.4-D; BK=BAP+kinetine; BG= BAP+giberellic acid; IB=IBA; KN=Kin+NAA; N=NAA;
(+) poor reaction; (++) good reaction; (+++) very good reaction

During our researches we offered a special attention to two stem callus lines that were very semblable as consistency and colour (friable, cream-greenish), though their proliferation capacity was very different. They were provided on MS medium supplemented with 2 mg/l IAA (A_2) and respectively on media supplemented with 1 mg/l kinetine and 0.5 mg/l NAA (KN_1). The friable cream callus obtained on A_2 transfered on BA_1 (1 mg/l BAP+0.5 mg/l IAA) enhanced its luxurious multiplication, it gained a foamy aspect and this character was maintained for about 2 years. Our purpose was to use this 2 callus lines to induce somatic embryogenesis. In this view they were cultivated on hormone free MS, in a culture room with no light (fig.1b).

The A_2 line (kept in darkness) maintained its features though it was repeatedly passed for 4 months: high proliferation speed (in a period of 14 days it filled about half of the culture vial and deprived the medium of its nutrients) and light-cream colour. The attempts to induce somatic embryogenesis were unsuccessful. Growing this type of callus in light and its repeated transfers on MS formula with BAP (0.2-0.5 mg/l) or with BAP (0.5 mg/l)+kinetine (0.5 mg/l) improved its consistency. It turned green and caulogenetic callus isles appeared on its surface, leading to shoot formation subsequently, (fig. 1c). Even after 2 years of *in vitro* subcultivation, A_2 callus line maintained its caulogenetic capacity.

Table 2. Biomass evaluation in *in vitro* lavander callus cultures

Hormonic variant	Growth regulators (mg/litre)				Biomass supply (g/vial)
	BAP	Kinetine	GA	NAA	
A. *Callus cultures exposed to permanent illumination*					
MS	-	-	-	-	9.4452
BK	0.5	0.5	-	-	13.5589
BG_1	1.0	-	0.5	-	14.3526
KN_1	-	1.0	-	0.5	11.9016
B. *Callus cultures maintained in darkness conditions*					
MS	-	-	-	-	12.7420
B	0.5	-	-	-	11.6152
BG_1	1.0	-	0.5	-	14.9952
KN_1	-	1.0	-	0.5	12.2952

The high proliferation speed of A_2 callus cells determined us to evaluate its capacity of accumulating fresh biomass within a fortnight, in light and also darkness conditions on MS (control) medium and on certain hormonic formuli in view of its potential utility – production of important active principles. It was very interesting issue is that callus biomass values achieved in light and also in darkness conditions are very similar on the same medium formuli. It was ascertained that on hormone free MS medium a greater callus biomass was obtained compared to the one produced in the absence of light. In the first case some of the nutrients are probably consumed during the cell differentiation processes and not only for their own multiplication. The medium formula that provided the highest biomass both in light conditions (14.35 g/vial) and darkness (14.99 g/vial) was the one comprising 1 mg/l BAP and 0.5 mg/l GA (BG_1), (table 1).

The KN_1 callus line cells were also unable to produce somatic embryos by their repeated cultivation on MS medium (in the absence of light). In this case callus displayed a lower cell proliferation speed; it turned friable and cream, compact and whitish and some callus regions generated shoots (etiolated) even in darkness conditions, shoots that got their natural colour.

In a period of 3 months this callus line lost its cell multiplication capacity gradually.

SUMMARY

The main reaction of explants (shoot tips, nodes, leaves) of Lavandula angustifolia L. on varied harmonic formuli of MS medium was callogenesis. Nodes and shoot tips provided neoplantlets only on hormone-free MS and sporadically on MS with B_{02} (0.2 mg/l), A_2 (2 mg/l IAA), KN_1 (1 mg/l kinetine and 0.5 mg/l NAA) and N_2 (2 mg/ NAA). A friable cream callus, high proliferative in light and also in darkness was obtained on A_2. Its capacity of fresh biomass accumulation was tested on varied hormonic formuli.

CONCLUSIONS

Our investigations regarding the *in vitro* behaviour of *Lavandula angustifolia* L. evinced that:

- Nodes and shoot tips provided neoplantlets on hormonic formuli that comprised cytokinins (BAP and kinetine) and auxins (IAA, IBA and NAA) alone or combined; the top efficiency was accomplished on MS medium supplemented with 2 mg/l NAA;
- Callogenesis process is very frequent at this species and was induced from all the plants parts on every hormonic formula tested, the callus was friable, cream or green and caulogenetic;
- A stem callus line (with a high proliferation speed, biomass efficiency and caulogenesis) was isolated. This line maintained its features even after two years of *in vitro* subcultivation.
- We consider that the the results of our research depended very much on the genotype of the biological material used to initiate the *in vitro* cultures of *Lavandula angustifolia.*

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AUTHORS' ADDRESS

GHIORGHIȚĂ GOGU, NICUȚĂ DANIELA - University of Bacau, Calea Mărăşeşti Street, 157, Bacău, Romania.

MAFTEI DIANA-ELENA, TOMA IRINA - "Al.I.Cuza" University, Carol I Blvd., 20A, Iasi, Romania.