SOME CONSIDERATIONS REGARDING THE *IN VITRO* BEHAVIOUR OF BASIL (*OCIMUM BASILICUM* L)

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Abstract: *In vitro* cultures of basil were initiated using shoot tips from plants grown in the laboratory. It was noticed that shoot tips and nodes' inoculation on MS medium supplemented with cytokinins, auxins or with cytokinins combined with auxins induces caulogenesis and root generation, providing neoplantlets. Adding only 2,4-D or 2,4-D combined with a cytokinin to the nourishing medium, the main reaction of the explants (nodes, internodes, roots, leaves) was formation of compact, non-proliferative, green-whitish callus. An *in vitro* micropropagation technology was perfected for our investigated species, useful to multiply valuable genotypes.

INTRODUCTION

Basil (*Ocimum basilicum* L.) is a herbaceous, annual plant belonging to the Lamiaceae family. It requires great amounts of light and high temperatures, being resistant to drought (2, 3, 4, 7). Its essential oil comprises estragol, eugenol, linalool, citral, camphor, cineol etc. (5, 6, 7, 9). The main actions of basil volatile oil are: digestive, antispastic, antinauseous, carminative, choleretic, antifungic, stomachic, galactagogue, diuretic (1, 5, 6, 8).
Taking into account that basil is a very important plant from the pharmaceutical and economical point of view, we tested its behaviour in *in vitro* culture, some explants' reaction on several hormonic formuli in order to evince possible valuable genotypes and to elaborate an efficient technology for their micropropagation.

MATERIAL AND METHODS

We initiated the *in vitro* culture of basil using (as explants) shoot tips from a plant brought from Greece and grown in the laboratory. The explants were sterilized with two solutions: mercury chloride (for 6 minutes), then chloramine – T (12 minutes), afterwards they were rinsed twice with distilled sterile water and inoculated on basal Murashige-Skoog (1962) mediumdeprived of growth regulators or supplemented with 0.2-0.5 mg/l BAP. Cultures were grown in Erlenmayer fials of 100 ml (B type). Saccharose (25 g/l) was the charbon source and agar (8 g/l) was used to solidify the nutritive medium. The inoculated fials were incubated in a half-climatised culture room from the University of Bacau (temperature was 23-25^0 C, light – about 2000 lux, continuous illumination). The shoot tips continued their development on the initial mediums and provided neoplantlets, that were the source of sterile biological material to diversify the tests. We used as explants nodes with leaves, shoot tips, internode, leaf and root fragments that were cultivate don varied hormonic formuli of basal MS. The morphogenetic reaction of these explants is presented in table no.1 and the most illustrative aspects from the *in vitro* cultures at this species are displayed in figures 1-8.

RESULTS AND DISCUSSIONS

Initiating the *in vitro* culture of basil did not cause any particular problems, the treatment with $HgCl_2$ and chloramine-T (5%) being very efficient to sterilize the explants taken from plants grown in the laboratory (where the germs are much fewer than in field conditions). The MS medium without hormones or supplemented with small amounts of BAP favoured the shoot growth processes and also their root formation. The neoplantlets obtained represented the sterile explant source to run the tests to observe their morphogenetic reaction in *in vitro* cultures on several hormonic formuli.

We also noticed that nodes and shoot tips inoculation on nourishing media that comprised 0.2-0.5 mg/l BAP led to small callus formation at the contact surface with the medium, the shoots continued their growth and produced a strong net of roots, some of them with secondary roots. BAP also favoured the multiple shooting (fig. 5). As we augmented the BAP

amount within the culture medium (until 2 mg/litre of medium), shoot development was inhibited as well as root formation (compared to 0.2 mg/l).

When we combined BAP and IAA the callus provided at the contact between explants and medium was better developed and the callus was compact and greenish. Multiple shooting phenomenon is very well expressed, but the shoots are small-sized, only sporadically generated roots. When the roots are present, they are very long (up to 10 cm), white, without ramifications (fig. 3). Associating BAP and IBA the reaction was similar to the previously described one, only root genesis was more intense, some explants producing neoplantlets with small thick roots. At the basal part of stems we may often observe short adventitious roots covered by absorbant hairs.

The combination of BAP and NAA facilitated an explant reaction similar to the one with BAPonly than with BAP associated with the other auxins previously described (small callus at explant basal part, multiple shooting, the shoots being smaller than on B_{02}, strong, numerous roots). Combining BAP and 2,4-D we noticed a basic reaction of callus formation (better expressed than on a medium comprising just 2,4-D); caulogenesis was not completely inhibited, but the shoot size is reduced and the callus – compact, green, light green or whitish and with a medium proliferation speed (fig. 7).

In case of combining a cytokinin (kinetine) and an auxin (NAA), the response was much alike with the combinatiosn NAA-BAP, a compact, small-sized, brown callus being provided, this hormonic formula enhances both caulogenesis and root formation.the newly-generated neoplantlets were endowed with longer shoots and the multiple shooting was less expressed. The roots (adventitious ones included) were stronger than on BN (table 1, fig. 6)

The hormonic formula with kinetine and NAA induced very vigorous, white roots deprived of secondary branches, grouped in clumps.

We tested the explants reaction on a nutritive medium containing two cytokinins in higher concentrations (BAP 2 mg/l and kinetine 1.5 mg/l). We noticed a moderate caulogenesis and also multiple shooting, neoplantlet growth being slower. Basal leaves were obviously larger than the other ones; the leaves in touch with the medium produce a small amount of callus. Roots were absent on this culture medium.

The shoot tips and nodes were also cultivated on media supplemented only with auxins (2 mg/l). When the auxin was IAA (fig. 1, 2), the explants provided neoplantlets with 2-4 shoots/explant, root genesis being very intense (the most intense), including the adventitious one. The roots appeared as fascicles. On a nutritive medium with IBA the obtained shoots were longer than in the case of IAA, but the number of shoots and roots/explant was more reduced than on A_2 medium (supplemented with IAA).Using NAA, we observed that a small callus was formed, 1-2 shoots per node appeared and root genesis was less expressed (shorter roots, some of them thin, other thick). When we included 2,4-D in the nutritive medium, the main reaction was callus generation (compact, green, light green or whitish callus). Caulogenesis was not completely inhibited, but the shoots provided by axillary buds did not elongate. Transfering this callus on a medium supplemented with BAP (0.5 mg/l) facilitated its obvious proliferation, but it did not cause its differentiation and it gradually degenerated. In only 5 situations out of 30 some of the transfered calluses provided roots (few and short).

We also used internodes as explants. Inoculating internode fragments (of about 1-1.5 cm in length) on a medium supplemented only with 2,4-D, they produced a layer of compact, low or medium proliferative, green and cream callus on their entire surface. When it was passed on other hormonic formuli, no organogenetic processes occurred.

Leaf fragments cultivated on a nourishing medium comprising 1 mg/l BAP and 0.5 mg/l 2,4-D provided a compact, high proliferative, light-green or whitish callus on their entire surface. On a medium with 2 mg/l IBA the leaf explants generated a small, compact, cream callus just in the petiole region. We obtained a similar response on a medium where BAP was combined with NAA, a friable granulated green callus appeared at the basal part and also on the leaf explants. The association of kinetine and NAA led to compact, cream-greenish callus formation that sporadically generated offshoots and roots.

Finally, when we tested the reaction of root explants, we noticed that a compact, cream-greenish, low proliferative callus appeared on the medium supplemented with 2,4-D. The hormonic variant with IBA induced the formation of a thin layer of callus, that provided a net of thin secondary roots of different sizes (table 1).

Thus, using shoot tips and nodes as explants the reaction was neoplantlet generation on numerous hormonic formuli of basal Murashige-Skoog medium, including MS deprived of growth regulators. Our tests evinced the fact that the species is quite sensitive to hyperhidry, which is very difficult to control after its appearance, that is why it requires to be prevented. We need to mention a very interesting fact: the shoots affected by hyperhidry provided numerous roots, but their accommodation to the septic environment is extremely difficult, if not impossible.

The neoplantlets that were not affected by this unwanted phenomenon were accommodated to *ex vitro* conditions in a hydroponic system (fig. 8), without significant losses of biologic material. The accommodation took up to 2 weeks, depending on the temperature from the culture room (the accommodation is faster and more efficient at lower temperatures - 18°C). Regenerants’ transfer to field did not raise any particular problems either, their survival rate being almost 100%. They formed many secondary branches in field conditions.

CONCLUSIONS

The preliminary data concerning the in vitro behaviour of basil showed that:

- Nodes and shoot tips’ inoculation on MS medium supplemented with cytokinins, with auxins or combinations of the two phytohormones in varied concentrations enhance caulogenesis and also root formation, providing neoplantlets. Sometimes the explants produce a small - size callus at the contact surface with the nutritive medium. When the auxin was 2,4-D, the main reaction of the explants (nodes, internodes, leaves and roots) was callus generation.
- The callus obtained on media supplemented with 2,4-D or with BAP and 2,4-D was transfere don media comprising only BAP, which maintained its initial features, but did not lead to its differentiation.
- The neoplantlets provided on several hormonic formuli, including on hormone-free MS medium were easily accommodated at septic conditions in a hydroponic system. There were very few losses of biological material among the regenerants transfered in field.
- MS medium supplemented with small amounts of BAP (0.2 mg/l) is the most indicated medium formula to induce multiple shooting; nodes and shoot tips are the mostr appropriate types of explants for the micropropagation of eventual valuable genotypes of basil.

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APPENDIX

Table no. 1 – The morphogenetic reaction of some basil explants on varied hormonic formuli of Murashige – Skoog medium

Var.	Explant	Hormonic formula	Growth regulators (mg/l)					The morphogenetic reaction and proliferation speed
			BAP	IAA	IBA	NAA	KIN	2,4-D	
1.	Nodes and shoot tips	A		2.0					Neoplantlets (+++), multiple shooting (++), vigorous, fascicled roots with very few secondary branches (++++); adventitious roots from the upper branches
2.	"	B	0,2-2.0						Compact, cream-greenish callus at the contact surface with the nourishing medium; multiple shooting (+++) best represented on media with higher amounts of BAP (2 mg/l), which inhibit root formation; speed growth of shoots is quite low; net of roots within the medium (+++), some of them with secondary roots
3.	"	BA	1.0	0.5					Multiple shooting (+++), small-size shoots; compact, green callus within the medium, at the basal part of the shoots; root formation sporadically (+); when the roots are present, they are very strong, elongated, white, without secondary ramifications
4.	"	BB	0.5		0.5				Compact, cream-greenish callus (++); multiple shooting (++) less represented than on BA, short offshoots; root genesis (++) more intense than on BA, filamentous roots deprived of ramifications
5.	"	BD	1.0					0.5	Compact, green-whitish callus (+++); poorly represented caulogenesis (+); roots are absent
6.	"	BN	1.0			0.5			Compact, green-brownish callus (+);intense root formation (+++), multiple shooting (+++)
7.		BK	2.0				1.5		Caulogenesis (++), moderate reaction; multiple shooting, the offshoots' growth was very slow; basal leaves are obviously larger than the other ones; the leaves in touch with the culture medium provide a small amount of callus; roots are absent on this hormonic formula
8.	"	KN				0.5	1.0		Long shoots (+++) with 1-3 basal branches; semi-compact, cream-greenish callus (++) surrounding the basal part of shoots; a great number of fascicled roots (+++) with many secondary roots; adventitious roots (++) from the inferior nodes of the stem
9.	"	D						2.0	Compact, green, light-green, whitish callus (++), poorly represented caulogenesis (+)
10.	"	IB			2.0				Neoplantlets (++) with long and strong shoots; friable, green callus at the contact between shoots and medium; strong, fascicled net of roots within nourishing medium (+++); short, adventitious roots with absorbant hairs sporadically
11.	"	N				2.0			Compact, cream callus (+); 1-2 offshoots/node, more frail than on IB; short, fascicled roots (++), some of them filamentous, other quite thick
12.	"	MS							Neoplantlets (+++) with many axillary shoots on the main stem (+++); numerous fascicled, but frail roots (+++); sometimes multiple shooting; no callus provided on this medium
13.	Internodes	D						2.0	Compact, cream and green callus (+) on the entire explant surface
14.	"	N				2.0			Quite friable, cream and green callus (++); roots generated by callus sporadically (+)
15.	Leaves	BD	1.0					0.5	Compact, light green and whitish callus (+++), on the whole limb of leaves
16.		BN	1.0			0.5			Callus generation at the explant base (++); friable, granulated, light green callus
17.	"	IB			2.0				Compact, small-size and cream callus (+) around the petiole, that provides roots (++)
18.	"	KN				0.5	1.0		Compact, cream-greenish callus (++); shoots (+) and roots (+) are rarely generated
19.	Roots	A		2.0					Callus (+) and numerous, thin roots (++)
20.	"	D						2.0	Compact, cream-greenish callus (+)

Fig. 1 – Neoplantlets of basil on A_1 medium

Fig. 2 – Intense root formation on A_1 medium

Fig. 3 – Multiple shooting and adventitious roots on BA_1

Fig. 4 – Very intense root genesis on BA_1

Fig. 5 – Multiple shooting and callus on culture medium with BAP (B1)

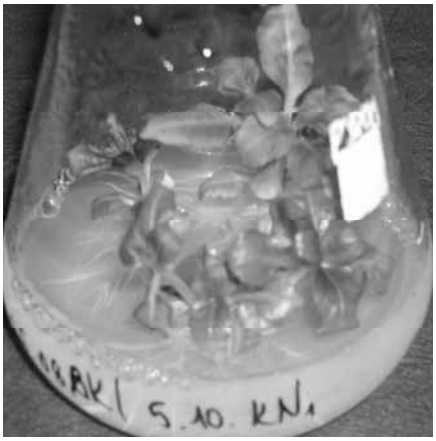

Fig. 6 – Vigorous neoplantlets and roots on KN_1 medium

Fig. 7 – Granulated friable callus on BD medium

Fig. 8 – Neoplantlet accommodation (hydroponic system)