INVESTIGATION OVER THE IN VITRO MORPHOGENETIC REACTION OF LAVANDER (*LAVANDULA ANGUSTIFOLIA* L.) EXPLANTS

INVESTIGAȚII PRIVIND REACȚIA MORFOGENETICĂ "IN VITRO" A EXPLANTELOR DE LAVANDĂ (*LAVANDULA ANGUSTIFOLIA* L.)

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Abstract. *Lavander (Lavandula angustifolia L.) is a perennial semi-bushy herb that belongs to Lamiaceae family with a mediterranian origin. The plant is grown in Romania for its active principles contained in its inflorescences, more specific for its volatile oil. The importance of this plant is due to its antiseptic, carminative, sedative, antispastic, diuretic, colagogue effect. Due to the fact that lavender cultures can be accomplished with generative or vegetative multiplicated plants (though cuttings) the "in vitro" multiplication is quite an important and challenging task. In our study, different combinations of growth hormons were tested in order to achieve regeneration structures from young tissues of lavander. Kinetin (KIN) and α-naphthaleneacetic, Kinetin (KIN) and indolilacetic acid (IAA), or benzilaminopurine (BA) and NAA or IAA, zeatine were added to basic medium composed of full strength MS salts, MS vitamins, 3% sucrose and 0,8% agar-agar. Young explants (shoot tips, binodal explants and leaves), derived from mother plants grown in controlled conditions, were cultivated on these media. The morphogenetic response is highly dependent on the initial explants, hormons used in the media, genotype, and environmental conditions.*

Key words: micropropagation, uniformity, stability, homogeny, BAP.

Rezumat. *Lavanda (Lavandula angustifolia L.) este o plantă ierboasă semi-arbustă, perenă care aparține familiei Lamiaceae, având o origine mediteraneeană. Planta este cultivată în românia datorită principiilor sale active conținute în inflorescențe, mai specific datorită uleiurilor sale volatile. Importanța plantei este datorată proprietăților sale antiseptice, carminative, sedative, antispastice, diuretice, cu efect colagog. datorită faptului lavanda poate fi cultivată pe cale generativă şi vegetativă (prin butaşi) multiplicarea „in vitro" este un obiectiv important fiind de-a lungul timpului scopul a numeroase cercetări. În studiul nostru au fost testate diferite combinații de hormoni de creştere în scopul regenerării de plante noi porinind de la explante tisulare de lavandă. Astfel diferite concentații şi combinații de kinetină (KIN) şi -naphthaleneacetic, kinetină (KIN) şi acid indolilacetic (IAA), sau benzilaminopurină (BA) şi NAA sau IAA, zeatină au fost adăugate mediului de cutură de bază MS, vitamine MS, zaharoză 3% şi agar 0,8%. Explantele de tip apex, explante uninodale şi frunze au fost recoltate de la plante mamă crescute în condiții controlate şi apoi au fost cultivate pe aceste medii. Răspunsul morfogenetic a depins atât de explantul inițial cât şi de hormonii utilizați, genotip sau condițiile de mediu.*

Cuvinte cheie: micropropagare, uniforme, stabile, omogene, BAP.

INTRODUCTION

Lavender species of commercial importance are native to the mountainous regions of the countries bordering the western half of the Mediterranean region of Europe. The name "lavender" comes from the Latin verb lavare "to wash" or "to bathe." There are approximately 20 species of lavender with hundreds of various genotypes differentiated by variations ranging from growth form to chemical composition of essential oil. The traditional uses of lavender range from use as a perfume to a antimicrobial agent. This powerful and potent herb has been utilized throughout antiquity and is still retained as a common household ingredient today. Recent studies have found that essential oils from this extraordinary species can replace chemical methods currently in use to suppress sprouting in potato tubers for storage (Vokou, 1993). In bioactivity studies in India, lavandula species have been proven to show potent activity against insect pests (Sharma et al., 1992). Another study in Austria provided evidence of the sedative effects of the essential oil of lavender after inhalation (Buchbauer et al., 1992). Currently, the majority of lavender products are utilized for essential oil production and for their aromatic properties.

The growing demand for natural products has intensified studies on the selection of native *Lavandula* plants and their economic exploitation. *In vitro* cultures have been employed for the production of secondary metabolites, medicinal and aromatic compounds of a number of *Lavandula* species (Segura and Calvo, 1991).

Micropropagation is used routinely to generate a large number of high-quality clonal agricultural plants, including ornamental, medicinal and vegetable species. Micropropagation has significant advantages over traditional clonal propagation techniques. These include the potential of combining rapid large-scale propagation of new genotypes, the use of small amounts of original germplasm (particularly at the early breeding and/or transformation stage, when only a few plants are available), and the generation of pathogen-free propagules.

Plant regeneration in vitro is dependent on the manipulation of the inorganic and organic constituents in the medium, as well as the type of explant and the species. In most plants, successful regeneration from the callus or directly from the explants takes place after a series of subcultures in various media, in a sequence which is often specific to the species, variety, or the newly introduced genotype. The determining factors are the combination of the concentration in relation to medium volume and the composition of growth promoting and retarding regulators in the medium, the physiological status and competence of the cells and their capability for morphogenetic expression.

Although *Lavandula* species can be vegetatively propagated, the poor rooting ability of stem cuttings, as well as the lack of selected clones, limits its industrial exploitation (Segura and Calvo, 1991).

In order to achieve these important goals, a primordial condition is the establishment of a viable and rapid multiplication technology, specific for each species, which should allow the regeneration of a sufficient number of plants in the shortest period of time.

MATERIAL AND METHODS

The source of explants utilised for the initiation of the "in vitro" cultures of *Lavandula angustifolia* is represented by plants supplied by Vegetable Research and Development Station Bacau.

For initiating the "in vitro" culture, the lavander explants were sterilized for 15 minutes in 0.1 % mercury chloride solution; subsequently, they were washed three times with sterile distilled water and inoculated on the ground nutritive medium Murashige-Skoog (1962) - fig. 1 and 2.

Fig. 1. Explants excised from mother plants

Fig. 2. Sterilization of explants

For testing the morphogenetic reaction, mature plants grown under controlled conditions (temperature, humidity, light, etc) were used as mother plants. Different types of explants were excised: shoot tips, binodal explants and leaves.

Explants were cultivated on MS medium solidified with 8.0 g/l of agar, having succrose (30 g/l) as carbon source; the medium was supplemented with growth regulators in various concentrations and combinations (table 1).

The pH was adjusted to 5.8 prior to the addition of the agar and autoclaved at 121°C (1.06 kg/cm^2) for 25 min.

Cultures were then incubated at 26±1^{o}C, a 16-h photoperiod, and 5000 lx light intensity.

Table 1

Variants of nutritive medium with different hormonal factors utilized for "in vitro" cultivation

Components	**L_1**	**L_2**	**L_3**	**L_4**	**L_5**	**L_6**	**L_7**	**L_8**
Macro elements	Murashige and Skoog, 1962							
Microelements								
Vitamins	Murashige and Skoog, 1962							
KIN	2.0	2.0	2.0	-	-	-	-	-
BAP	-	-	-	2.0	2.0	-	-	-
Zeatine	-	-	-	-	-	2.0	2.0	2.0
NAA	-	0.5	-	0.5	-	-	0.5	-
IAA	-	-	0.5	-	0.5	-	-	0.5

The rooted plantlets obtained on different nutritive medium were transferred to hydroponics conditions in bottles for acclimatization. The pots with the hydroponic solution (that contained Previcur 0.15%) were covered with clear bags to provide 100% relative humidity. They were placed in an acclimatization room under a 16/8 h photoperiod at 20 - 23°C. The acclimatized plants were planted in a potting mixture of

sterilized sand + vermiculite (1:1 ratio) in plastic cups, hardened in a mist chamber (80% relative humidity) for 2 weeks before transfer to green house.

RESULTS AND DISCUSSIONS

The morphogenesis potential of shoot tips, binodal explants and leaves of lavander cultivars was estimated. Occurrence of the first regenerated shoots was observed 13 - 18 days after cultivation.

We investigated the morphogenetic reaction of the shoot tips, under different hormonal formulae. Not all the inoculated explants had the same morphogenetic reaction, due to the fact that there are functional differences between the similar morphologic explants. In this stage of ontogenetic development there are different particularities in the organogenesis reaction of the explant, particularities that lies on the specific totipotence of each explant.

On the other hand, a part of the tips were eliminated due to their gradual degeneration (reaching to necrosis), or to the secondary contamination of the recipients. The morphogenetic reaction of *Lavandula angustifolia* explants was favorable, the initiation and development of the regenerative structures were followed by the rapid development of the neopropagules.

On the medium supplemented with KIN, the explants did not show a remarkable response. At the contact of the stem with the nutritive medium that contains NAA a small, compact, cream-coloured callus sporadically appeared. In spite of the increasing size of the apexes, L1 and L2 medium, characterized through the presence of kinetin inhibited the organogenesis.

The medium L7 (zeatine – 2 and NAA – 0.5), determined shoot development and the appearence of the true leaves, but the process of caulogenesis is still weakly represented. The leaves became white-yellowish and they dried in time. On all these variants, the size of the shoots, at the same age, is smaller than on medium with BAP alone or in combination with IAA.

Fig. 3. Explants inoculated on the culture medium with zeatine

The nutritive medium supplemented with 2 mg/l^{-1} zeatine (L6) – fig.3, provided a good growth and development of the shoots, which present a vigorous aspect. On all medium variants that contained benzilaminopurine as the main growth regulator the morphogenetic reaction is focused toward the regeneration of new shoots that evolutes rapidly in fully formed plants.

The shoots regeneration was accomplished through the neoformation of adventive shoots (at the basis of the inoculated tips) as well as through the multiple axillary sprouting (through the development of pre-existent meristematic centers). A part of the regenerated sprouts were cultivated on fresh cultivation mediums in order to continue the regenerative processes. Gradually the shoots that were at the best stage of development were inoculated on rooting medium, which should allow the initiation and development of roots.

The morphogenetic reaction of binodal explants fragments on the medium MS supplemented with several hormonal formulae was mainly oriented toward the obtaining of new shoots. Contrary to expectations based on the results obtained on other plant species the weakest reaction of this type of explants was recorded on the MS medium supplemented with 2.0 mg/l^{-1} kinetine. The best morphogenetic reaction showed the binodal explants grown on nutritive medium supplemented by BAP and zeatine alone or in combination with 0.5 mg/l^{-1} IAA. The green colour of the explants became more intense and none of the explants presented at this time callus.

The leaf explants placed on MS medium supplemented with 2 mg/l^{-1} kinetin formed a quite friable callus, cream-coloured, with reduced proliferation. IAA induced, on the other hand, an intense root generation. The supplementation of the nutritive medium with zeatin influenced callus generation processes and organogenesis processes. On all the tested nutritive variants the morphogenetic reaction of leaf explants was low and is not recommended for the initiation of an "in vitro" culture focused toward the regeneration of true to type plants.

Regarding the influence of the hormonal formuli, the best morphogenetic reaction was obtained on the variants that contained as growth regulators the BAP and IAA. These hormones allowed both in tips and hypocotyls the development of meristematic centres that rapidly evolved in shoots. Direct bud formation was observed in explants cultured on MS medium added with BAP – fig. 4. The combination of BAP with IAA increased the percentage of regeneration and the development of the explants. The substitution of IAA with NAA was not active in a medium added by BAP. While, the substitution of BAP with KIN (associated with NAA or IAA) determined the initiation of callogenesis without bud formation.

Optimum values for shoot induction, both from shoot tips and uninodal explants were obtained on MS medium, supplemented with BAP - 3 mg/l^{-1} and 1 mg/l^{-1} IAA – fig.5. One hundred percent of explants cultured on this medium turned green and showed a good differentiation.

Regenerated plants were transferred on hydroponics medium and kept about four days covered with a plastic foil, in the culture room. Subsequently, they were day by day acclimatized to room atmosphere.

Fig. 4. Morphogenetic reaction on medium with BAP

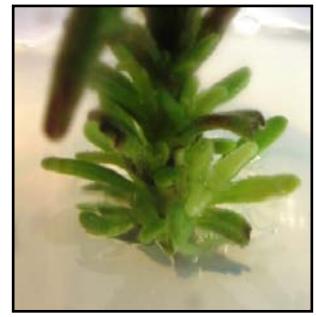

Fig. 5. Direct regeneration of shoots from binodal explants

CONCLUSIONS

Our investigation regarding the "in vitro" morphogenetic reaction of *Lavandula angustifolia* showed that the best explant for the initiation of the cultures are the shoot tips.

The researches finalize through the obtaining of viable plants through direct organogenesis. The capacity of regeneration is strongly depending on the type and quantity of exogenous hormones. The best morphogenetic reaction was obtained on the variants that contained as growth regulators the BAP and IAA. These hormones allowed both in tips and hypocotyls the development of meristematic centres that rapidly evolved in shoots. Optimum values for shoot induction, were obtained on MS medium, supplemented with BAP - 3 mg/l^{-1} and 1 mg/l^{-1} IAA. One hundred percent of shoot tips cultured on this medium turned green and showed a good differentiation. Good results were also obtained on medium variants supplemented with zeatin alone or in combination with IAA.

The obtained experimental results encourage the continuation of the researches for the determination of all the factors that can influence the regeneration process (genotype, explant etc). This should allow the establishment of a rapid and efficient propagation technology that permit the regeneration of a large number of plants, in short term, plants that have the same genetic background as the parental plants.

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