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STUDIES ON THE MORPHOGENETIC REACTION OF *ORIGANUM VULGARE* L. EXPLANTS IN VITRO CULTIVATION

Daniela Nicuță, Irina Lazar

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INTRODUCTION

Origanum genus belongs to the family *Lamiaceae* (Labiate) contein of 42 species and 18 hybrids, widely distributed in Mediterranean areas, Eurasia and Northern Africa. (CHISHTI S., et. al., 2013). *Origanum* species have many activities related to medical, culinary and agricultural importance. Numerous studies on the chemical composition of oregano plants are reported in the literature.

The principal bioactive compounds identified in oregano essential oil are represented by thymol, carvacrol, γ-terpinene, *p*-cymenephenols, (CIULEI I., 1993; BEJAOUI A. et al., 2013; FIGIEL A., 2010, GONCEARIUC M, 2014). Chemical composition and concentrations of the components from essential oil depends on many factors such as genotype, geographical origin, climate, type and soil composition, orientation the development of the plant, harvest time and culture conditions (GARCÍA-BELTRÁN J.M., 2016). Due to bioactive compounds *Origanum* species have many medicinal properties such as antibacterial, antifungal, antioxidant, anti-inflammatory, antitumor, antiparasitic, antiviral, antihyperglycemic and anticholinesterase activities (GARCÍA-BELTRÁN J.M. and ESTEBAN M.A., 2016**).**

Origanum vulgare (L.) is a perennial plant, an important medicinal plant, which is name popular known as Jungali Tulsi or Oregano or Himalayan marjoram.

At this species carvacrol and thymol was reported to be dominant components of its essential oil (CHISHTI S., et. al., 2013). The aroma, flavor and pharmaceutical properties of *Origanum. vulgare* are products its essential oil which consists mostly of monoterpenes and sesquiterpenes (GONG HY, 2014).

Since the resources in spontaneous flora are not always safe, the *in vitro* regeneration method is a safe and rapid way of multiplying valuable oregano genotypes, as well as obtaining new genotypes, richer in bioactive compounds.

Our research has been conducted to identify a safe and high-yield oregano multiplication technology using different concentrations and combinations of phytohormones.

MATERIAL AND METHODS

As biological material for this study we used *Origanum vulgare* seeds purchased from authorized seed producers for the marketing of selected seeds.

The seeds were washed with 70% ethyl alcohol for 2 minutes. They were then subjected to the sterilization step performed on the laminar flow hood. Sterilization consisted in treating the seeds with 5% chloramine T solution for 15 minutes, followed by rinsing them 3 times with sterile distilled water.

Previously, the nutritional variants of the Murashige-Skoog (MS) base medium were prepared according to the preparation recipe, to which various concentrations and combinations of phytohormones were added.

The sterilized seeds were inoculated on the Murashige and Skoog (MS) non-hormone base and the seed pots were placed in the *LEEC* growth room in the dark at 22.5 degrees Celsius for germination. After 4-5 days, the germinated seed culture vessels were transferred under light conditions (2500 lucsi) at 22.5 degrees Celsius to the SANYO growth chamber.

After about 4 weeks of *in vitro* cultivation, the plants obtained on the Murashige-Skoog (MS) without hormones were used as a source of explants (phytoinoculi), represented by apexes and nodes (photo 1).

Both knots and apex sampling were performed in a sterile medium at the laminar flow air niche, placed on phytohormone-supplemented media (Table 1).

Six explants were inoculated into each culture vessel, and for each variant of the tested medium, 4 vessels were inoculated. Pricking out was performed three times for each tested nutrient medium.

To induce the morphogenetic reaction, after inoculation, the vessels were transferred to the growth chamber under controlled light conditions (2500 lx), temperature (22.5° C) and photoperiod (18 hours light / 8 hours dark).

Table 1. Nutritional variants tested

Nutritional variant	Growth regulators (mg/l)		
	BAP	IAA	IBA
MS	-	-	-
BA_1	1 ml/l	1 ml/l	-
BB_1	1 ml/l	-	1 ml/l
BA_2	1 ml/l	0,5 ml/l	-
BB_2	1 ml/l	-	0,5 ml/l

RESULTS AND DISCUSSIONS

The initiation of the *in vitro* culture at *Origanum vulgare* did not pose problems in obtaining sterile plants. Thus, 90% of the inoculated seeds on the MS without hormones germinated, generating vitroplants. The seedlings were maintained on the MS base for 4 weeks, for good development, the length of the stems being about 1cm. The leaves were small, intensely coloured in green. At the base of the stems, 3-4 roots of about 0.4-0.7 cm grew in the nutrient medium.

Also, we did not face the phenomenon of infection of crops, which indicates that all conditions of sterility have been ensured during the handling of crops.

The node and apex explants inoculated on the tested hormone variants showed a good morphogenetic reaction, only two organogenetic phenomena being detected. The best response highlighted was caulogenesis followed by rhizogenesis. The varied intensity of the phenomena observed in the inoculated explants depended on the interaction between endogenous factors (explant genotype) and exogenous factors (type of explant and hormonal formula used).

The nutrient medium supplemented with BAP and 1ml IAA (variant BA1) determined a good caulogenetic reaction for most inoculated explants (photo 2). There regenerated 2-4 shoots / explant. These showed a good development with a faster growth rate than BB1 and BB2.

The length of the shoots varied between 1.2 and 4.5 cm. At the same time, it was noticed that the leaves were of large size and dark green colour, and the vigorous stems with short internodes. At the base of some shoots there was observed the phenomenon of rhizogenesis; the few roots were long and thin, with no side branches or absorbent trichomes (photo 2).

Also, in case of the BA2 nutritional variant, (photo 3) caulogenesis was well-highlighted at the level of inoculated explants, although a small percentage of them degenerated.

The newly formed shoots have shown a good growth and development rate on this nutritional variant. In the case of the BA2, the length of the shoots varied between 0.5 and 8 cm. As with the BA1 environment, the leaves were larger in size, intensely green, and short stalk internodes. The roots formed at the base of some shoots were thin, of small size (0,2 - 1 cm), 2-4 in number on each shoot.

On the BB1 nutritional variant (1ml BAP + 1ml IBA) all inoculated explants responded. The main morphogenetic reaction was caulogenesis, which was expressed with a very good intensity (photo 4).

The regenerated shoots were well developed, they had a vigorous appearance, long internodes, but their growth in length was slower compared to the BA1 and BA2 variants. The size of the shoots ranged between 0.5 and 6 cm. The number of the regenerated shoots per explant varied between 1 and 2.

The leaves were large and green but of a lighter green compared with the shoots regenerated on the IAA-supplemented media; at the base of some shoots there was highlighted the rhizogenesis; the roots were short, numerous, thin and branched. In some culture vessels, some shoots had chlorophyll deficiencies.

On the BB2 medium (photo 5) most of the inoculated explants reacted, the percentage of reaction being 90%.

There was a good to very good caulogenetic reaction, its intensity varying from one nutritional vessel to another. Most of the shoots were well-developed, but some of them looked tender. The length of the regenerated shoots varied between 1.0 and 5 cm and their number / explant was 1-2. Rizogenesis was highlighted in some culture vessels, namely at larger shoots, the roots being short, 0.2 to 0.5 cm, thick and 2-4 in number / explant, covered in absorbent trichomes.

On the MS environment the apexes and nodes explants have generated new shoots, namely 1-2 / explant.

The shoots were close in length to those obtained on phytohormones supplemented media, but their growth was slower. The leaves were smaller but normally coloured. At the base of the apex and node explants, there developed short roots with many trichomes in the form of tussocks.

After 2.5 months from inoculation, on the MS, BA1 and BA2 variants there were observed secondary shoots, found at the basal nodes of the first shoots formed from the initial explants (). In some culture vessels the presence of adventive roots of about 0.5 cm in length was observed.

The development of a powerful root system is advantageous because it is not necessary to transfer the shoots to other nutrient environments to induce rhizogenesis.

Thus, the shoots can be accommodated directly to the *ex vitro* environment (photo 6), to continue growing in greenhouses or fields.

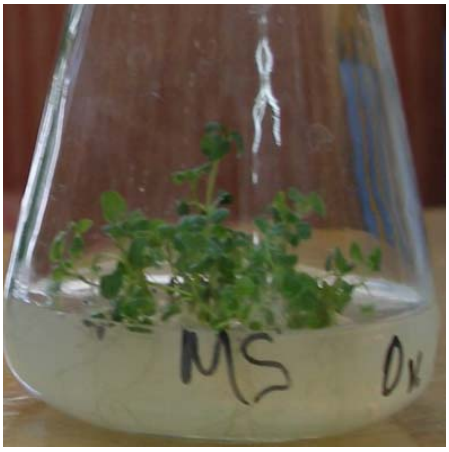

Photo 1. Vitroplants on MS

Photo 2. Morphogenetic reaction on BA1 variant

Photo 3. Morphogenetic reaction on BA2 variant

Photo 4. Morphogenetic reaction on BB1 variant

Photo 5. Morphogenetic reaction on BB2 variant

Photo 6. Vitroplants adapted to the ex vitro environment

CONCLUSIONS

The initiation of the *in vitro* culture at *Origanum vulgare* was easily accomplished using apex and node explants obtained from vitroplants from seeds germinated on MS medium without hormones.

The node and apex explants presented as morphogenetic reaction the generation of shoots (caulogenesis) as well as roots (rhizogenesis).

The shoots obtained on all tested variants generally showed good and very good growth, depending on the phytohormonal combination present in the nutritive medium.

On the hormone-free MS, the morphogenetic response was satisfactory compared to that identified on growth supplemented environments.

The best shoots yield was found on the media supplemented with BAP and IAA.

The development of a good root system at the level of in vitro regenerators allows for rapid adaptation to the ex vitro environment.

ABSTRACT

The species *Origanum vulgare* has been known since Antiquity as a plant with a high content of bioactive compounds, especially in plant leaves and flowers. The biochemical analysis of the vegetative parts of oregano plants revealed the presence of essential oregano oil. It contains mainly two phenols, carvacrol and thymol, of great economic importance. Due to the chemical composition of essential oil, oregano plants are used in the pharmaceutical, cosmetic and food industries.

Our research was aimed at in vitro cultivation of Origanum vulgare explants. Apexes and nodes were grown on several nutritional variants supplemented with BAP, IAA and NAA, at different concentrations.

The main morphogenetic reaction was caulogenetic reaction. At the base of the shells formed from the explants, roots were formed.

The shoots obtained on all tested variants generally showed good and very good growth, depending on the phytohormonal combination present in the nutritive medium. The best shoots yield was found on the media supplemented with BAP – 1ml/l and IAA – 0,5 ml/l.

REFERENCES

1. CIULEI I., GRIGORESCU E., STĂNESCU U., 1993 - Plante medicinale, fitochimie i fitoterapie, vol. 1, Ed. Medicală, Bucureşti, 276p.;
2. GARCÍA-BELTRÁN JM, ESTEBAN MA., 2016 - Properties and Applications of Plants of *Origanum Sp*. Genus. SM J Biol.; 2(1): 1006;
3. GONCEARIUC M., BALMUŞ Z., UNGUR N.,DRAGALIN I., 2014 - Genotipuri perspective de *Origanum vulgare* ssp. *vulgare* L. si *Origanum vulgare* ssp. *hirtum* (Link) Ietswaart. Buletinul AŞM. Ştiinţele vieţii. Nr. 1(322);
4. GONG HY., WH. LIU, GY. LV, XIAOYING ZHOU, 2014 - Antioxbdant properties of oregano (*Origanum vulgare*) leaf extracts. Rev Bras Farmacogn 24(2014): 25-32;
5. ICONOMOU-PETROVICH G.N., NIANIOU-OBEIDAT I. 1998 - Micropropagation of *origanum vulgare* Subsp. *hirtum* (Mt. Taygetos). In: Tsekos I., Moustakas M. (eds) Progress in Botanical Research. Springer, Dordrecht;
6. SULEYMAN KIZIL, KHALID MAHMOOD KHAWAR, 2017 - Efficient mass propagation of *Origanum acutidens* (Hand.-Mazz.) Ietswaart under in vitro conditions. Bangladesh J. Bot. 46 (2): 667-673.

AUTHORS' ADDRESS

NICUŢĂ DANIELA, LAZAR IRINA - "Vasile Alecsandri" University of Bacau, Department Biology, Ecology and Environmental Protection, e-mail: dana_nicuta@yahoo.com.