# THE CAPACITY OF SOME VARIETIES OF OCIMUM TO FORM THE CALLUS IN THE CONDITIONS OF IN VITRO CULTIVATION

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***Abstract***

*The nodal explants of Ocimum basilicum and Ocimum minimum were cultivated on the medium of Ms basal supplemented with auxins 2,4D(6mg/l) and vitamins MS, incubated in conditions controlled by temperature, light and humidity and presented good rates of regeneration over 70% in case of Ocimum basilicum in the conditions of sample of explants from the vegetal material ex vitro and of 100% in case of Ocimum minimum in the conditions in which it was sampled from the sterile vegetal material. During the monitoring (6 weeks) the explants have developed callus of green-yellow-white color translucent pearled, of different sizes that after 3 weeks presented on the surface embryogen cones and at the end of the period in proportion of 96% has generated new plants.*

**Key words:** nodal explant, *Ocimum basilicum, Ocimum minimum,* micropropagation, *in vitro*, *ex vitro*

## INTRODUCTION

The culture of vegetal tissues and cells represent a basic branch of the bio modern technologies, that of vegetal bio technologies, a direction of research that has as purpose the examination of the cells of tissues, of members or of plantlets in the culture *„in vitro",* of the vitro plantlets reaction to the natural conditions of life, in the moment of their transferring *„ex vitro"* (Petruş, 2016) and also a direct commercial importance, by its applying in the fundamental research, as would be the cellular biology, genetics, biochemistry and biotechnology is an evidence of its utility (Gambor, 2002). The culture of tissue not only offers a method of mass propagation, but also makes possible also the production of plants undamaged by diseases and plants genetically modified. Also, it represents an important source of obtaining the secondary products of metabolism (Khosroushahi, 2006; Murashige, 1974).

Despite all the advantages of this technique, there are also some methodological obstacles, mainly the contamination that impedes its exploitation as a technique with maximum efficiency in bio technological research (Ekmekci, 2014). The internal and external contamination of the tissues of plants represents a predominant problem, because, the micro bodies especially the fungi and bacteria can develop faster than the cells of

the plants and can take all the nutrients from the environment impeding thus the growth of the plants (Cassells, 1991).

The basil is a plant from the *Ocimum* type, Lamiaceae family, Lamiales order with origin in India (Viera, 2000) was found in tropical and temperate regions from Southern Asia. The type *Ocimum* is found in Asia, Africa and Central America (Darrah, 1998) but is cultivated also in many European countries being considered that there are between 50 and 150 species and hybrids of basil (Simon et al., 1990).

In Romania the basil prefers the West and South of Romania: here are cultivated improved local varieties, and also cultures adapted to foreign cultures (Roman et al., 2012). Are cultivated for the chemical compounds that are used in the medicine based on plants (in gastro-intestinal and digestive disorders, as anti-inflammatory and antiseptic etc.), in the industry of perfumes and food (Păun, 1988; Robu., 2004; Bei, 2013). It presents a wide range of variations due its complex morphology (Săvulescu, 2010; Simon, 1999; Labra, 2004; Nurzyńska-Wierdak, 2007a), the chemical content and of the composition of the essential oil (Tiţă I., 2008; Marotti, 1996; Vieira, 2000; Sifola, 2006; Nurzyńska-Wierdak, 2007b; Dzida, 2010) and also other biological aspects (Golcz, 2006; Nguyen, 2010).

In the last years, the essential oils and extracts from plants have attracted a great scientific interest, due to their potential as source of natural and antioxidants and active biological compounds. The antimicrobial and antioxidant activity of the essential oils stood at the basis of many applications, including the preservation (Bozin, 2006; Tepe, 2007; Wannissorn, 2005) of fresh and processed food, to the obtaining of some pharmaceutical products used in the alternative medicine and in the naturist therapies (Bozin, 2006; Celiktas, 2007). In medicine the efforts were also made to exploit the potential of some essential oils for the treating of infectious diseases, in order to discover some standard pharmaceutical remedies of substitution (Celiktas, 2007). Traditionally, the basil is widely used for the preparing of the food as flavoring agent (Makri, 2008), in the perfumeries and medical industries (Telci, 2006).

The micropropagation în vitro of *Ocimum basilicum* was concentrated on using different types of explants on mediums of culture with different additions, cultivating the nodal segments and apices, leaves, inflorescences or for the purpose of obtaining different bioactive-rosemary acid compounds, volatile oils, antioxidant compounds and compounds with antiviral activity (Laslo, 2013).

In the context of those mentioned is placed also this paper that has proposed the finding of an optimum protocol of micro multiplying for *Ocimum basilicum* considering the variety, the medium of culture, the experimental factors and the interaction between them.

## MATERIAL AND METHOD

In order to analyze the capacity of some varieties of the *Ocimum* species was initiated in the spring of 2017 an experiment in the laboratory of the Vegetal biotechnology of the Faculty of Environment Protection from the University Oradea. In order to obtain the vegetal material, was placed for germination a greater quantity of seeds of *Ocimum basilicum* because usually they germinate hardly because of the high content of mucilages. After approximately 5 weeks was obtained a sufficient numbers of young plants to initiate the experiment that will be V1. For the second version (V2) were used sterile plants of lime. *Ocimum minimum* being in the collection of the laboratory.

In order to test the capacity of the two varieties of callus formation were selected the apical part of the young plants internodal being obtained thus the explants that were inoculated on the MS medium to which were added vitamins MS (1ml/l) and 2,4D (6mg/l), sterilized medium at 121°C for 15 minutes. After a previous disinfection of the explants with alcohol 70% for 1 minute and hypochlorite 3% for 10 minutes followed by 4 energetic washing with sterile water they were inoculated on the cooled culture medium. For each version (V1, V2) were inoculated 15 tubes (n=15).

After the inoculation the explants were kept in controlled conditions in the chamber of growth with the following parameters: the intensity of the light 50µmol.m-$^{2}$.s$^{-1}$; photo period: 16 hours light 8 dark; the temperature 26°C and humidity of 80%.

## RESULTS AND DISCUSSION

The capacity of callus formation on medium with 2,4D was followed on a period of 6 weeks, interval in which the test of callus was considered and studies from the point of view of the diameter, the consistency of the callus, of the color and its capacity of development on the enriched medium. The results obtained are presented in Table 1.

The diameter of callus after 3 weeks is between 0,8-2,3 cm for V1 and 0,7-1,8 cm for V2 and after 6 weeks between 1,6-3cm for V1 and 1,2-2,6 for V2. The color of the callus varies from green-yellow translucent to yellow translucent and pearled white in case of both versions. In the first period of incubation was found that 27% of the explants from V1 were necrosed fact that is placed on the account that the plants came from and exvitro medium compared to those from V2 that were sampled from sterile medium and to which were not registered any infection or necrosis.

*Table 1*

The differences regarding the capacity of callus formation from the explants of *Ocimum* considered at different intervals of time

| Period of incubation | Version | Diameter of medium (cm) | Color/experimental versions (%) | Consistency | Necrosed % | Capacity of regeneration |
|---|---|---|---|---|---|---|
| **3 weeks** | V1 | 1,9 | 28% - green yellow translucent; 36%- yellow translucent; 36% - pearled white | dense | 27 | 73% Presents embryos on the surface |
| | V2 | 1,6 | 73% - green yellow translucent 27 % - pearled white | dense | 0 | 100 % Presents embryos on the surface |
| **6 weeks** | V1 | 2,2 | 28% - green yellow translucent; 36% - yellow translucent; 36% - pearled white | dense | 27 | 73 % Presents organogenesis |
| | V2 | 1,8 | 73% - green yellow translucent 27 % - pearled white | dense | 1 | 93 % Presents organogenesis |

Regarding the capacity of regeneration of the callus after 3 weeks were observed embryonal buttons and embryos on the surface of the callus in all the tubes from both versions that afterwards have generated new plants with the exception of one from V2. Was also found that at the end of the experimental period it began to appear in the area of contact with the culture medium a mild necrosis.

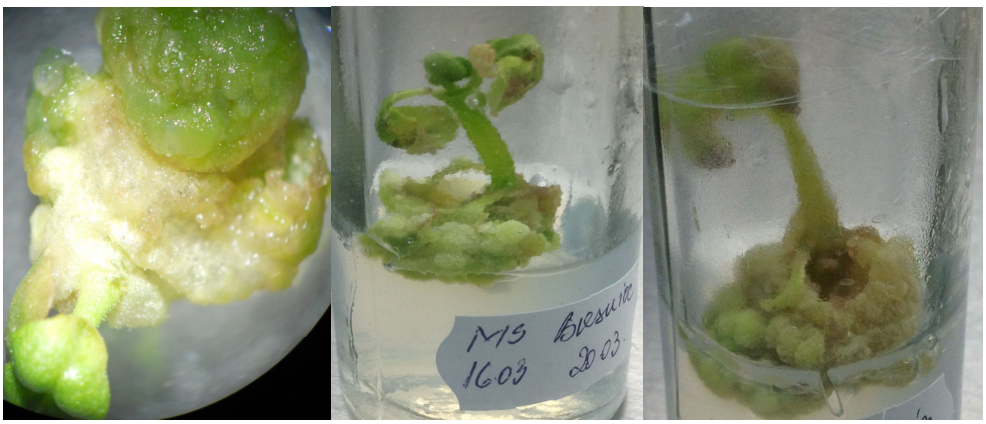

Fig. 1. Images regarding the regenerative aspect of the basil

A special importance of this experiment is that of the regenerative aspect of the callus during the 6 weeks, callus that will be transferred on fresh mediums without 2,4D but with an increased quantity of phytohormones for the differentiation of the plants.

## CONCLUSIONS

From the study accomplished we can reach the following conclusions:

- in 3 weeks after the inoculation 27% of the explants were necrosed in case of *Ocimum basilicum*, a case that we consider that is due to the disinfection and the fact that the vegetal material has *ex vitro* provenience compared to the explants of *Ocimum minimum* coming from sterile plants. It is differentiated also an average size in diameter of the callus of 1,9 cm for V1 and 1,6 cm for V2. Also the callus present a dense aspect and colors in shades of green-yellow-translucent white.
- after 6 weeks was not registered major aspects regarding the consistency and color, in exchange the weight of the callus continued to grow reaching in case of V1 to an average weight in diameter of 2,2 cm and in case of V2 to 1,8 cm.

We recommend the transfer of the cones of growth differentiated on fresh mediums with additions of phytohormones after 30-40 days from the inoculation to have the certainty of obtaining a greater number of plants of higher quality.

## REFERENCES


1. Bei M.F., Popovici R., 2013, The analysis of poor nutrition and of some nutrition dietary imbalance in the human body's iron deficiency. Analele Univ. din Oradea, Fasc. Ecotox, Zoot. și Tehn. de Ind. Alimentară, Vol XII/B , An 12, pp.139-145
2. Bozin B., Mimica-Dukic N., Simin N., Anackov G., 2006, Characterization of the volatile composition of essential oil of some lamiaceae species and the antimicrobial and antioxidant activities of the entire oils. Journal of Agriculture and Food Chemistry, 54, 1822-1828
3. Cassells A.C., 1991, Problems in tissue culture: Culture contamination. In: Micropropagation: technology and application (Eds. Debergh PC and Zimmerman RH), Springer
4. Celiktas O.Y., Kocabas E.E.H., Bedir E., Sukan F.V., Ozek T., Baser K.H.C., 2007, Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus oficinalis*, depending on location and seasonal variations. Food Chemistry, 100(2), 553-559
5. Darrah H.H., 1998, The cultivated Basil. Buckeye Printing Co., Independence, Mo
6. Dzida K., 2010, Biological value and essential oil content in sweetbasil (*Ocimum basilicum* L.) depending on calcium fertilization and cultivar. Acta. Sci. Pol. Hortorum. Cultus. 9(4): 153-161
7. Ekmekci H., Aasim M., 2014, *In vitro* plant regeneration of turkish sweet basil (*Ocimum basilicum* L). The Journal of Animal & Plant Sc., 24(6), pp:1758-1765
8. Gamborg O.L., 2002, Plant tissue culture: Biotechnology Milestones. *In Vitro* Cell Dev Biol Plant 38: 84-92
9. Khosroushahi A.Y., Valizadeh M., Ghasempour A., Khosrowshahli M., Naghdibadi H. et al., 2006, Improved Taxol production by combination of inducing factors in suspension cell culture of *Taxusbaccata*. Cell BiolInt 30: 262-269

10. Labra M., M. Miele, B. Ledda, F. Grassi, M. Mazzei, M. Sala, 2004, Morphological characterization, essential oil composition and DNA genotyping of *Ocimum basilicum* L. Cultivars. Plant Sci. 167(4): 725-731
11. Laslo V., 2013, Biotehnologiile vegetale şi aplicaţiile lor. Ed. Universităţii din Oradea, pp.316
12. Makri O., S. Kintzios, 2008, Ocimum sp. (Basil): Botany, Cultivation, Pharmaceutical Properties and Biotechnology. J. Herbs Spices Med. Plants. 13(3): 123-150
13. Marotti M., R. Piccaglia, E. Giovanelli, 1996, Differences in essential oil composition of basil (*Ocimum basilicum* L.) Italian cultivars related to morphological characteristic. J Agri. Food Chem. 44(12): 3926-3929
14. Murashige T., 1974, Plant propagation through tissue cultures. Ann Rev Plant Physiol 25: 135-166
15. Nurzyńska-Wierdak R., 2007a, Comparing the growth and flowering of selected basil (*Ocimum basilicum* L.) varieties. Acta Agrobot. 60(2): 127-131
16. Nurzyńska-Wierdak R., 2007b, Evaluation of morphological and developmental variability and essential oil composition of selected basil cultivars. Herba. Pol. 53(3): 255-261
17. Păun E., M., Dunitrescu, A., Verzea M., Coşocariu, O., 1988, Tratat de plante medicinale și aromate cultivate, Vol I și II. Ed. Ceres, București
18. Petruş A., 2016, Biotehnologii vegetale-curs pentru uzul studenţilor
19. Robu T., Constantin M., 2004, Plante medicinale autohtone. Institutul european, Iaşi
20. Roman Gh.V. (coord.), Morar G., Robu T., Tabără V., Axinte M., Borcean I., 2012, Cernea S., Fitotehnie, Vol II/Plante tehnice, medicinale şi aromatice. pp. 372-388
21. Săvulescu E., 2010, Botanică sistematică. 148 pp.
22. Sifola M.I., Barbieri G., 2006, Growth, yield, and essential oil contentof three cultivars of basil grown under different levels of nitrogen in the field. Sci. Hort. 108: 408-413
23. Simon J.E., J. Quinn, R.G. Murray, 1990, Basil: A source of essential oils. In J. Janick and J.E. Simon (eds.). Advances in new crops. Timber Press, Portland, OR. pp.484-489
24. Simon J., E. Morales, M.R. Phippen, W.B. Vieira, R.F.Z. Hao, 1999, Basil: A Source of Aroma Compounds and a Popular Culinary and Ornamental Herb. In: Janick, J. (ed), Perspecctives on new crops
25. Telci I., Bayram E., Yilmaz G., Avci B., 2006, Variability in essential oil composition of Turkish basils *(Ocimum basilicum L.).* Biochemical Systematic Ecology, 34, pp.489-497
26. Tepe B., Daferera D., Tepe A., Polissiou M., Sokmen A., 2007, Antioxidant activity of the essential oil and various extracts of Nepta flavida Hud.-Mor. From Turkey. Food Chemistry, 103, pp.1358-1364
27. Tiţă I., 2008, Botanică farmaceutică, Ed a III-a. Ed. Sintech, Craiova
28. Viera R.F., J.E. Simon, 2000, Chemical characterization of basil (*Ocimum* spp.) found in the markets and used in the traditional
29. Wannissorn B., Jarikasem S., Siriwangchai T., Thubthimthed S., 2005, Antibacterial properties of essential oils from Thai medicinal plants. Fitoterapia, 76, pp.233-236