USE OF NODAL EXPLANTS IN "IN VITRO" MICRO-PROPAGATION OF MENTHA PIPERITA L.

Laslo Vasile *, Zăpârţan Maria *, Vicaş Simona *, Agud Eliza *

**University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048 Oradea, Romania, e-mail:,vasilelaslo@yahoo.com; mariazapartan@yahoo.com*

Abstract

Nodal explants of Mentha piperita L. were cultivated on the MS basal culture medium, enriched with various combination of auxines and cytochinines. The best rate of scion regeneration (23.9 ± 0.576) was obtained on a culture medium with 0.5 mg/l of Zeatine and 0.5 mg/l IAA. Of the tested culture medium varieties, the best composition for stimulating the number of roots proved to be the one with 1 mg/l BAP and IAA (10.2 roots/explant) and for stimulating growth in lenght the culture environment with 0.5 mg/l Z and IAA.

Key words: Mentha piperita, in vitro micropropagation, culture medium

INTRODUCTION

Peppermint *(Mentha piperita)* is an aromatic perennial plant also known as *Mentha balsamea* Willd. It belongs to the Lamiaceae family, Mentha genus, being a hybrid between *Mentha aquatica* and *Mentha spicata* (Hefendehl and Murray, 1972). It thrives and develops very well in humid areas of the temperate climate, being frequently cultivated in countries in Europa, Asia, USA (Kiran et. al., 2003). The Mentha genus contains a number of approximately 25 species, different in regard of their ploidie levels (Bhat et al, 2002).

Numerous species are cultivated, the plants posessing a high content of flavonoids (12%), polyphenols (19%), carotens, tocopherols, betaine, colines (Gardiner, 2000) and a volatile oil composed of menthol, menotone, mentofuran, carvacrol, thymol, widely used in the cosmetic and pharaceutical industry.

Mentha piperita is characterized by a sterility which is considered practically total. It manifests by the fact that the masculine organs of the flower never develop. Thus, the staminas develop abnormally, remaining short, sometimes proving to be degenerate and withering even from the bud, the polen being abnormally developed. *Mentha piperita* is a completely sterile hybrid. Studies undertaken in recent years have led to the elucidation of some of the causes for peppermint's sterility and have allowed for the production of varieties with a limited degree of fertility.

Because of reduced fertility it is impossible to obtain new varieties with a high production of mint oil by using conventional reproduction techniques.

In this context, the use of *in vitro* culture techniques can ensure both conditions for stimulating somaclonal variability and for speedy multiplication of valuable varieties. The fulfillment of these techniques' potential can only be obtained if repeatable and efficient work protocols can be established. Medicinal plant propagation using nodal segments with axilar buds has proved to be a simple and fiable method for mass production of desired clones (Sunandakumari et al., 2004).

MATERIALS AND METHOD

Young *Mentha Piperita* scions taken from plants authenticated in the Laboratory of Botany and Systematics were washed for 30 minutes under tap water. Afterwards, nodal segments of 3-4 cm in lenght were cut. Under a sterile hood, they were immersed in 70% etilic alcohol for 1 minute, 10% hypochlorite solution with 2 drops of Twenn 80 for 15 minutes and 0.05% mercury chloride solution for 3 minutes, followed by 5 washings with sterile distilled water.

After refreshing the cuts with a scalpel, the explants were inoculated, according to polarity, in dishes with 10 ml of MS (Murashige-Skoog, 1962) culture medium, sterilized at 121^{o}C, at a pressure of 1 atm for 15 minutes, supplemented with different combination of phitohormones (Tab. 1). The cultures were maintained at 25 ± 2 ° C, at a light intensity of 3000 lucs. 10 tubes for each medium variety were inoculated.

In order to interpret the obtained data regarding number and lenght of scions, the ANOVA (variation analisys) test was used.

RESULTS

Obtained results are presented synthetically in Table 1.

After 14 days of cultivation we registered the appearance of the first tips of shoots from the axilar buds of nodal explants, on the culture medium varieties with BAP and Zeatine.

The beginning of proliferation was recorded after 20 days. None of the cytochinine varieties showed the formation of callus. The experimental varieties with 1 and 1.5 mg/l BAP respectively proved to be more prolific, with 12.1 and 11.1 shoots/explant respectively, confirming in fact numerous reports especially recommending the use of nodal explants when a high rate of proliferation is desired in herbaceous species (Roy et. al., 1995; Travers et. al., 1996; Chakraborty et. al., 2004; Chishti et. al. , 2006; Laslo & Zapartan et.al., 2010). This effect of cytochinine presence is known and it can be explained because of the role the have in organogenesis, stimulating the formation of axilar shoots by inhibiting apical dominance.

Tabel 1

Experimental varietis and obtained results in nodal explant cultures of Mentha Piperita

Nr. var.	Regulators mg/l			No. of shoots buds/explan	Shoot length/expl. cm
	BAP	Z	IAA		
1	0.5	-	-	8.0 ± 0.765	7.4± 0.752
2	1.0	-	-	12.1 ± 0.190	6.2± 0.165
3	1.5	-	-	11.1 ± 0.345	6.3± 0.365
4	2.0	-	-	6.5 ± 0.230	5.5± 0.235
5	-	0.5	-	9.1 ± 0.345	7.6± 0.355
6	-	1.0	-	8.1 ± 0.411	7.8± 0.365
7	-	1.5	-	7.5 ± 0.617	8.0± 0.665
8	-	2.0	-	6.7 ± 0.595	8.2± 0.564
9	0.5	-	0.5	8.1 ± 0.491	7.7± 0.625
10	1.0	-	1.0	10.3 ± 0.230	6.1± 0.435
11	1.5	-	1.5	14.3 ± 0.472	5.8± 0.231
12	2.0	-	2.0	11.9 ± 0.293	6.2± 0.245
13	-	0.5	0.5	23.9 ± 0.576	4.0± 0.487
14	-	1.0	1.0	17.7 ± 0.195	4.5± 0.589
15	-	1.5	1.5	15.5 ± 0.231	5.7± 0.376
16	-	2.0	2.0	13.7 ± 0.345	6.0± 0.654

Regarding lenght of the shoots grown on the Zeatine culture medium varieties, it has been observed that they show better developement, they are bigger and more robust, cu shorter inter-nodal spaces and with mean lenghts ranging from 7.6 to 8.2 cm.

From the culture medium varieties with different combinations of cytochinines and auxines, the best results regarding the number of proliferated shoots were recorded on the medium with 0.5 mg/l Z and 0.5 mg/l IAA. As a mean, on this particular variety, we obtained 23.9 shoots with a lenght of 4.0 cm. Very good results were obtained on the varieties with 1 and 1.5 mg/l Z and IAA/l respectively.

In varieties with 2 mg/l IAA, after 32 days of cultivation we recorded the formation of adventive roots on the scion nodes.

Concerning the number and lenght of the roots, the results are shown in Fig. 1.

Of the tested culture medium, the best composition for stimulating number of roots proved to be the one with BAP and IAA 1 mg/l (10.2 roots/explant).

The lenght increase of roots was vigurously stimulated (14.2 cm) on the Z and IAA 0.5 mg/l each culture medium. None of the experimental varieties in which we used auxines yielded any callus.

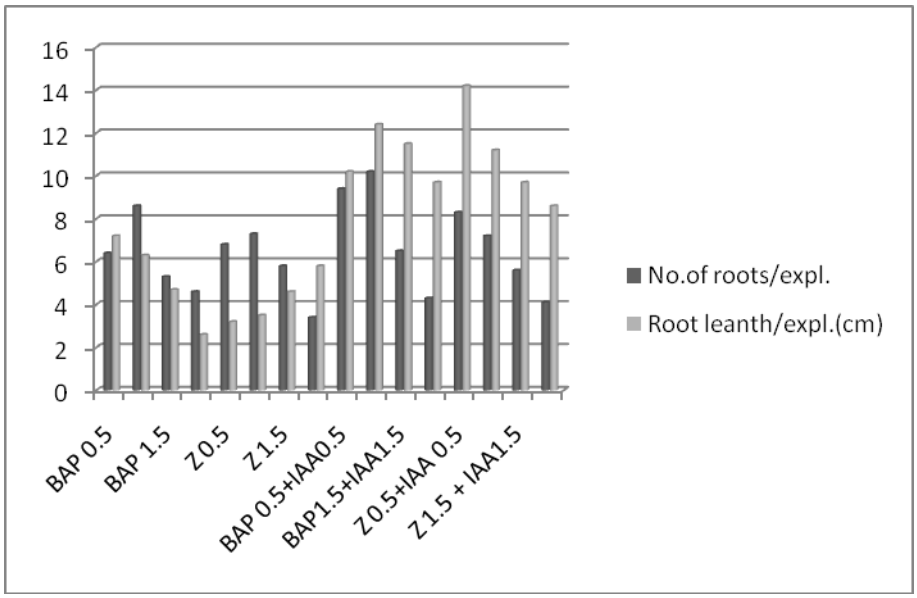

Fig. 1 The number of roots and lenght dynamic in relation to the phitohormonal composition of the culture medium

Root growh on Z and IAA 0.5 mg/l each medium (Foto 1). Stem developement on the Zeatine 1 mg/l medium (Foto 2) (strong vigor can be observed in these stems)

Foto 1

Foto 2

Good stem growth but short roots could be observed on the Zeatine 0.5 mg/l and IAA 0.5 mg/l medium. (Foto 3). Image which shows the formation of roots on the stem nodes (Foto 4).

Foto 3

Foto 4

REFERENCES

1. Chakraborty, A., and Chattopadhyay, S., 2008, Stimulation of menthol production in *Mentha piperita* cell culture, In Vitro Cellular and Developmental Biology - Plant 44(6),518-524.
2. Chishti, N., Shawl, A.S., Kaloo, Z.A., Bhat, M.A., Sultan, P., 2006, Clonal propagation of *Mentha arvensis* L. through nodal explant. Pak. J. Biol. Sci., 9, 1416-1419.
3. Gardiner P., 2000, The Longwood Herbal Task Force (http://www.mcp.edu/herbal/default.htm) and The Center for Holistic Pediatric Education and Research (http://www.childrenshospital.org/holistic/) Peppermint (Mentha piperita)
4. Ghanti Kiran et al. 2004, Rapid regeneration of Mentha Piperita L . from shoot tip and nodal explants, Indian Journal of Bio, 3, 594-598.
5. Hefendehl, F.W., and Murray, M.J., 1973, Monoterpene composition of a chemotype of Mentha piperita having high limolene. Planta Med. 23, 101-109.
6. Laslo, V., Zăpârţan, M., Vicaş S., 2010, *In vitro* behavior of certain varieties of *Vitis vinifera* L. Grown on Hormonally balanced culture medium. Abstracts „International Symposium „Trend In The European Agriculture Development" May, 20-21, 2010, Timisoara, p. 258.
7. Murashige, T., Skoog F., 1962, A revised medium for rapid growth and bioassays with tobacco tissue cultures, Physiol. Plant, 15, 473- 497.
8. Sunandakumari, C. et. al. 2004, Rapid axillary bud proliferation and ex vitro rooting of herbal spice, Mentha Piperita L, Indian Journal of Biotechnology,Vol. 3, 108-112.
9. Roy SK, Roy PK, & Rahaman M, Clonal propagation of Rauwolfia serpentina through *in vitro* culture, *Acta Hortic,* 390 (1995) 141-145
10. Savithri Bhat, Priti Maheshwari, Sushil Kumar, Anil Kumar, 2002, Mentha species: In vitro Regeneration and Genetic Transformation, Molecular Biology Today 3(1): 11-23.