*Bulgarian Journal of Agricultural Science, 13 (2007), 213-219*
*National Centre for Agrarian Sciences*

# Microclonal Propagation of Hyssop (*Hyssopus officinalis* L.)

Z. NANOVA[1], Y. SLAVOVA[2], D. NENKOVA[3] and I. IVANOVA[3]
[1]*Konstantin Preslavsky University of Shumen, BG - 9700 Shumen, Bulgaria*
[2]*Agricultural Institute, BG - 9700 Shumen, Bulgaria*
[3]*Institute of Cryobiology and Food Technologies, BG - 1504 Sofia, Bulgaria*

## Abstract

NANOVA, Zh., Y. SLAVOVA, D. NENKOVA and I. IVANOVA, 2007. Microclonal propagation of hyssop (*Hyssopus officinalis* L.). *Bulg. J. Agric. Sci*., 13: 213-219

A successful trial for tissue culture introduction and micropropagation of the medicinal plant hyssop (*Hyssopus officinalis* L.) has been conducted. Stem tips from plants vigorously growing under controlled conditions were used as initial explants. The introduction and micropropagation were performed in nutritive media with participation of biologically active substances (BAS) as BI (Benzimidazol) - 0.2 or 0.5 mg/l and PP - 40 (derivative of the phenoxy acetic acid and the folic acid) - 0.5 mg/l. On a nutritive medium containing 0.1 mg/l of IAA (indolyl acetic acid) and 0.1 mg/l of BI, rooting percentage of 90.0% ± 3.0% and normal growth of plants with medium sized root and vegetative parts have been achieved.

*Key words:* hyssop, vegetative propagation, nutritive medium, rooting

## Introduction

Hyssop (*Hyssopus officinalis* L.) is a perennial grassy plant of the *Lamiaceae* family. Many stems, 30 - 60 cm high, come out of a single root and become woody at the base. Leaves are opposite each other, lanceolate or linear-lanceolate. Flowers are hermaphrodite, clustered from 3 to 9 in nodes located in the axils of the leaves. The corolla is two-lipped, coloured in blue-violet. It blooms from July through September and bears fruit from August through October (Ganchev, 1995).

The hyssop populations are naturally spread in Central, South and Southeast Europe, the Mediterranean, Caucasia, Central and Southwest Asia, East and West Siberia. In our country this species can be relatively rarely met in certain regions of West Bulgaria (Znepolski, Vitosha regions), inhabiting sunny rocky places with an elevation ranging from 500 to 1000 m above sea level (Anchev, 1989).

It is mainly used as medical plant. By the folk medicine it is recommended against cold, cough, throat pains, etc. Conventional medicine confirms its medical properties, which to a great extent are basåd on the essential oil synthesized by the plant. Hys-

sop has a spasmolitic effect which makes it effective to treat coughs, bronchitis, bronchial asthma, chronic catarrhs and other respiratory diseases (Gruenwald et al., 2000). The pharmacological studies prove its antitubercular effect (Hilal et al., 1978). The tonic effect of the hyssop essential oil on the nervous system makes it suitable for treatment of nervous disorders like state of anxiety, hysteria, depression, etc. There are studies that show that some fractions isolated from the hyssop(coffee acid and a polysaccharide called MAR-10) can inhibit the Human Immunodeficiency Virus (HIV) (Gollapudi et al., 1995; Kreis et al, 1990). Some of the hyssop constituents exhibit high antioxidant activity (Djarmati et al., 1991). Its essential oil possesses also antimycosis activity which is especially effective in the inhibition of the growth of fungi species from the Candida genus - Candida albicans, C. Krusei and C. tropicalis (Mazzanti et al., 1998). The strong aroma essential oil is a component of many liqueurs. It is also used as an ingredient in the production of many brands of colognes and perfumes. In rare occasions hyssop leaves are used in the culinary as flavouring in soups, salads, meat dishes. Hyssop is a valuable honey-bearing plant. Some 70 to 80 kg of honey can be produced from 1 ha under crop.

Stem tips of hyssop picked in full bloom are used as herbal drug. Picking them from the natural habitats at one time should not exceed 45-50% of the hyssop populations and the next picking should be after 3 or 4 years. The natural habitats are not sufficient to satisfy the needs of this herb and we therefore recommend its commercial growing (Ganchev, 1995).

Hyssop can be propagated either by seeds or vegetative. Sowing is done at the beginning of March. At normal humidity and temperature levels seed germination starts within 10-12 days. This way of propagation is easy and allows production of a large number of new plants from minimal number of initial plants. However, if we aim to produce plants with particular qualities (e.g. composition of the essential oil) or new variety maintenance, then we prefer the vegetative propagation. It is being done by cuttings or by division of roots in spring or autumn.

The purpose of this research is to explore the possibility of hyssop propagation under in vitro conditions. This will allow the preservation of the natural populations of this species and its propagation in unlimited quantities with well-defined valuable qualities.

## Material and Methods

Experimental work has been conducted at the Tissue Culture Laboratory of the Agricultural Institute in Shumen in the period of 2004-2006. Stem tips 2-3 cm long, picked at the beginning of April from plants vigorously growing under controlled conditions were used as explants for the introduction of the medicinal plant hyssop to *in vitro* culture conditions. Immediately after they had been detached from the initial plant they were sterilized by plunging into a solution of 0.04% $HgCl_2$ and 0.1% Tween for 90 minutes and, subsequently by washing them 3 times with sterile distillated water (each of the tips for 15 minutes). To provoke their growth in tissue culture, over 10 nutritive media were investigated. Appropriate results were received in those of them whose main nutritional composition was of the kind applied for sugar beet rooting (Slavova, 1988). In the latter instead of 1 mg/l ANAA (alpha-naphtyl acetic acid) were added some bio-

logically active substances (BAS) as 0.2 or 0.5 mg/l of BI (Benzymidazol) and 0.5 mg/l of PP- 40 (derivative of the phenoxy acetic acid and the folic acid).

Later on, the same basic nutritive medium, which is a modification of the LePoivre (LP) nutrient medium (Quorin et al., 1977), served for the estimation of rooting influence of the nutritive media with participation of other BASs as À-250 (derivative of the butyric acid, microelements, vitamins), PF-32 (derivative of the phenoxy acetic acid, vitamins), Rutmil in concentrations of 0.5, 1.0, 2.0 mg/l, as well as the auxine-type of growth regulator IAA (indolyl acetic acid). The concentrations used from the latter and the results received are shown in Table 2. Before autoclaving of the media their pH-values were adjusted using NaOH until they reach 5.7 - 5.8.

Cultivation conditions: photoperiod of 16 hours and temperature at 25 ± 1îÑ maintained by LG room air conditioner of the type LS - L 1260/1262 Hz.

Trials were conducted twice with 4-week duration of the subcultivations. The in vivo adaptation of the rooted plants was done under controlled conditions in a soil substrate containing one part of peat and two parts of sand and perlit in 1:1 proportions.

Mathematical treatment of the results was made by Oivin's method (1960).

## Results and Discussion

On the specified nutrient media most of the stem tips initially put on trial with the aim to apply tissue culture techniques to hyssop propagation remained alive. During the 2nd or 3rd sub-cultivations very slight signs of growth were observed. At the end of this stage the *in vitro* adaptation was finished and the explants renewed its normal growth and development without signs of undesired presence of chlorosis, vitrification, necrosis, etc. In the course of one sub-cultivation they took the shape of a plant with 4 to 6 knees which after division provided the hyssop cloning.

Table 1 presents the influence on the *in vitro* rooting of different nutrient media (2nd, 3rd and 4th ones) applied for the introduction of tissue culture method. In contrast to their successful performance in cloning of *Stevia Rebaudiana Bertony* plants (95% of rooting when 0.2 mg/l of BI was applied, Slavova et al., 2003) and in cloning of the common hedgenettle ((*Stachys officinalis Trev.* (L.) with 82.9% of rooting achieved on a nutrient medium of 0.5 mg/l of BI and 64.7% of rooting on a nutrient medium of 0.5 mg/l of PP - 40 (Slavova et al., 2005), in our case these media proved to be ineffective. At this stage of study the highest rooting (32.9 ± 3.7%) was observed on a nutrient medium containing 0.5 mg/l of PP - 40 (the 11th one) which is, of course, extremely insufficient. Similar results have also been received under the influence of the nutrient media containing certain concentrations (0.5, 1.0, 1.5 and 2.0 mg/l) of the substances A-250, PF-32 and Rutmil. The concentrations specified turned out to be completely unsuitable at the substances PF-32 and Rutmil. On culture media with participation of the first substance just random and solitary roots were detected, and on the media with Rutmil participation - at the base of plants forming of abundant and loose callus was observed being extremely undesired during the process of in vitro rooting. A- 250 with concentrations of 1 and 1.5 mg/l was the only substance which caused rooting of 29.7 % and 29.6%, respectively. Subsequent de-

**Table 1**

**Influence of BI and PP-40 preparations at various concentrations upon the *in vitro* rooting of *Hyssopus officinalis***

| No | Nutrient media with | | Plants used | Plants rooted | |
|---|---|---|---|---|---|
| | BI | PP-40 | | | |
| | mg/l | | number | % | ±m |
| Control | - | - | 120 | 6.7 | 2.3 |
| 2 | 0.2 | - | 126 | 5.5 | 2 |
| 3 | 0.5 | - | 120 | 9.2 | 2.6 |
| 4 | - | 0.5 | 125 | 0.8 | 0.8 |
| 5 | 1 | - | 124 | 14.5 | 3.2 |
| 6 | 0.1 | - | 185 | 9.2 | 2.1 |
| 7 | 0.05 | - | 176 | 19.9 | 3 |
| 8 | - | 0.2 | 123 | 4 | 1.8 |
| 9 | - | 0.1 | 180 | 5.5 | 1.7 |
| 10 | - | 0.01 | 122 | 4.9 | 2 |
| 11 | - | 0.05 | 158 | 32.9 | 3.7 |

clines in concentrations of these substances showed effect only under the influence of Rutmil substance in concentrations at levels of 0.01, 0.05 and 0.005 mg/l which suppressed the callusogenesis and resulted in rooting percentages of 7.3, 9.1 and 36.1%, respectively. Optimization of the nutrient media for in vitro rooting of hyssop was attained through the next series of trials with inclusion of the auxine IAA and the biologically active substance BI. The results are shown in Table 2.

On the control nutrient media (the 1st one with no IAA and BI) there were a small number of rooted plants (10.7 ± 2.9%). Roots were single and had slightly grown up, the vegetative part which had grown at an average extent of 21.1 ± 0.8 mm was pale and susceptible to leaf roll infection. Adding 1.0 mg/l of IAA to the 2nd nutrient medium increased the rate of rooting to 63.7 ± 4.5%, but taking into consideration the effect on the whole-plant development we cannot support its use. Plants have grown twice larger than the control plants (42.3 ± 2.1 mm) due to the elongation of internodes. They were pale green, thickened at the base with callus of 0.8 to 10.0 mm in diameter formed on some of the plants. Roots could easily be separated from the stem. Similar performance was also recorded for plants cultured on the next two nutrient media (3rd and 4th), where IAA applications were 0.5 and 0.1 mg/l, respectively.

Regardless the higher (71.3 and 77.0%) rooting percentages reached on these media, because of the undiminishing intensity of callus growth at the base of plants and roots which were easy to break, the results on these media were worse than results received with the application of 0.1 mg/l of IAA and 0.1 mg/l of BI in the 5th nutrient medium. Such concentration of BI

**Table 2**

**Data on the rooting of *Hyssopus officinalis* in nutrient media with the participation of the IAA-auxine and the biologically active substance BI**

| No | Nutrient media with | | Plants used | Plants rooted | | Root length | | Length of the vegetative part | |
|---|---|---|---|---|---|---|---|---|---|
| | IAA | BI | | | | | | | |
| | mg/l | | number | % | ±m | mm | ±m | mm | ±m |
| Control | 0 | 0 | 112 | 10.7 | 2.9 | 3.1 | 1.1 | 21.1 | 0.8 |
| 2 | 1 | 0 | 113 | 63.7 | 4.5 | 2.9 | 0.3 | 42.3 | 2.1 |
| 3 | 0.5 | 0 | 108 | 71.3 | 4.4 | 4.4 | 0.5 | 45.1 | 1.9 |
| 4 | 0.1 | 0 | 148 | 77 | 3.5 | 12.7 | 0.9 | 69.4 | 3 |
| 5 | 0.1 | 0.1 | 100 | 90 | 3 | 7.7 | 0.5 | 30.5 | 1.2 |
| 6 | 0.1 | 0.05 | 144 | 52.8 | 4.2 | 3.7 | 0.5 | 17.4 | 1.1 |
| 7 | 0.05 | 0.05 | 154 | 63.4 | 3.9 | 12.1 | 1.2 | 64.3 | 3.1 |

in this medium suppressed the calluso-genesis and provided normal plant development (30.5 ± 1.2 mm). Plants were dense green, leaves enlarged with no signs of vitrification, with a greater number of roots tightly joined to stem (Figure 1).

These traits as well as the higher rate of rooting - 90.0 ± 3.0% compared to those received on the other media (the rooting rate was mathematically proved with high reliability ($P<0.001$), make this nutrient medium fit for use. IAA and BI concentrations applied proved to be chosen optimally and by the results obtained on the next two nutrient media ($6^{th}$ and $7^{th}$) which along with 0.1 and 0.05 mg/l of IAA also contain 0.05 mg/l of BI. The lower content of BI has proven insufficient to suppress the negative effect detected at single participation of 1.0, 0.5 and 0.1 mg/l of IAA in the initially applied nutrient media ($2^{nd}$, $3^{rd}$ and $4^{th}$). Plants grew pale-coloured and crooked, with leaves curled and callus formed at the base, which makes these nutrient media certainly unfit for use.

Table 2 gives us an idea about the influence of specified IAA and BI concentrations and ratios on the lengths of root and vegetative part. They had the lowest size (2.9 and 42.3 mm, respectively) on the 2nd nutrient medium, where the IAA concentration was at highest level - 1.0

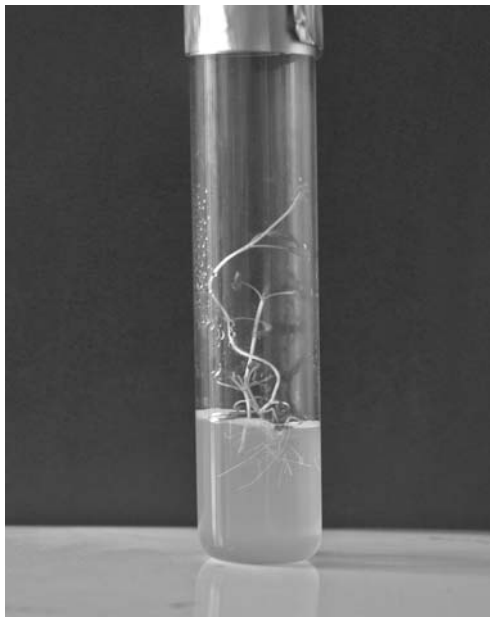

**Fig. 1. Rooting of Hyssop under *in vitro* conditions**

**Fig. 2. *In vitro* cloned hyssop in field conditions - 2nd year**

mg/l. As long as concentrations decreased the values of the lengths increased to 12.7 and 69.4 mm, respectively, on the medium containing 0.1 mg/l of IAA (the 4-th one). Participation of both 0.1 mg/l of IAA and 0.1 mg/l of BI in the 5th nutrient medium (proven to be most favorable among all of the investigated media) has induced plant formation with medium- sized roots (7.7 ± 0.5 mm) and vegetative part (30.5 ± 1.2 mm). They facilitated the in vivo adaptation under controlled conditions directly into soil substrate. Within 45 to 60 days of the adaptation process plants were ready for field transfer (Figure 2).

## Conclusion

Stem tips from plants vigorously growing under controlled conditions were successfully used for in vitro culture of hyssop. Nutrient media specified for introduction and micropropagation contain 0.1, 0.5 mg/l of BI or 0.5 mg/l of PP- 40.

It was found out that nutrient medium containing 0.1 mg/l of IAA and 0.1mg/l of BI has significant influence on the rooting success - up to 90.0 ± 3.0% and cause normal growth of plants with medium sized root and vegetative parts.

The results obtained represent a definite method for introduction of hyssop micropropagation with possible further optimization of some stages.

## References

**Anchev, M.**,1989. Genus Hyssopus L. In : Kuzmanov, B. (Ed.). Flora of the Peoples Republic of Bulgaria. Vol. IX, S., *Bulg. Acad. Sci*., pp. 283 - 284 (Bg).

**Djarmati, Z., R. M. Jankov, E. Schwirtlich, B. Djulinak and A. Djordjevic**, 1991. High antioxidant activity of extracts obtained from sage by supercritical CO<sub>2<|sub> extraction. JAOCS, *Journal of the American Oil Chemists Societe*, **68 (10)**: 731 - 734.

**Ganchev, G.**, 1995.Hyssopus officinalis L. In: Bondev, I. (Ed.). Chorological Atlas of the medicinal plants in Bulgaria. *Acad. Press*, Sofia, pp. 111 - 112 (Bg).

**Gollapudi, S., H. A. Sharma, S. Aggarwal, L. D. Byers, H. E. Ensly and S. Gupta**, 1995.

Isolation of a previously unidentified polysaccharide (MAR-10) from Hyssop officinalis that exhibits strong activity against human immunodeficiency virus type 1. Biochem. *Biophys. Res. Commun.*, **210**:145-151.

**Gruenwald, J., T. Brendler, C. Jaenicke (eds)**, 2000. PDR for Herbal Medicines. Montvale, NJ: Medical Economics, pp. 414-415.

**Hila, J., H. Sayed, El-Alfy, S. Taha, El-Sherei and M. Moshera**, 1978. Investigation of the volatile oil of Hyssopus officinalis. Egypt Pharm. Sci., **19**: 177-184.

**Kreis, W., M. H. Kaplan, J. Freeman, D. K. Sun, and P. S. Sarin**, 1990. Inhibition of HIV replication by Hyssop officinalis extracts. *Antiviral Res.*, **14**: 323-337.

**Mazzanti, G., L. Battinelli and G. Salvatore**, 1998. Antimicrobial properties of the linalool - rich essential oil of Hyssopus officinalis L. var. decumbens (Lamiaceae). *Flavour and Fragrance Journal*, **13**: 289 - 294.

**Oivin, I. A.**, 1960. Statistical treatment of experimental results. *Pathological Physiology and Experimental Therapy,* **6** (4) : 76 - 85 (Ru).

**Quorin, M., Ph. Lepîivre and Ph. Boxus**, 1977. Un premier bilan de 10 annees de resherches sur les "cultures" de meristems et la multiplication *in vitro* de fruitiers ligneux. C., R., Resh., 1967 - 1977 et Rapports de Synthese. Stat. Cult. Fruit et Maraich. Gembloux, pp. 93 - 117.

**Slavova, Y.**, 1988. Developing of the vegetative propagation methods and obtaining of the haploid sugar beet plants in vitro. PhD Thesis, Agric. Acad., Sofia (Bg)

**Slavova, Y., D. Nenkova and I. Ivanova**, 2003. Study on the influence of the substance of benzymidazol upon Stevia rebaudiana Bertoni, cultivated *in vitro*. *Bulg. J. Agric. Sci.,* **9** : 225 - 228.

**Slavova, Y., D. Nenkova and I. Ivanova**, 2005. Influence of some new, biologically active substances on in vitro rooting of medicinal Betony (*Stachys officinalis Trev*. (L.). *Bulg. J. Agric. Sci*., **11**: 135 - 139.

*Received November, 12, 2007; accepted February, 12, 2007.*