Federal State Funded Institution of Science
"The Labor Red Banner Order Nikita Botanical Gardens – National Scientific Center of the RAS", Yalta, Russian Federation

IN VITRO REGENERATION OF *HYSSOPUS OFFICINALIS* L. AND PLANT GENETIC FIDELITY

I.V. Bulavin, N.N. Ivanova, I.V. Mitrofanova

The research was conducted in the framework of State Task No. 0829-2019-0038 of the FSFIS "NBG-NSC", and done on the basis of the Unique Scientific Installation "Scientific Center of Plant Biotechnology, Genomics and Conservation" (USI "PHYTOBIOGEN").

Introduction

Hyssopus L. is a small Mediterranean genus of perennial undershrubs are utilized as aromatic and medicinal plants. Traditionally, hyssop plants are propagated by seeds, however this method has some limitation for perspective forms and cultivars. Today, biotechnological methods are important for mass multiplication and conservation of the valuable forms obtained by selection breeding. At the same time a growth regulator effect on the plant material *in vitro*, especially on genetic stability is discussed.

The objective of our investigation was: 1) to develop the direct *in vitro* regeneration and 2) to determine the genetic fidelity of plants obtained *in vitro*.

Material and methods

Hyssop plants (*Hyssopus officinalis* L. cv. Nikitskiy Beliy) grown *ex situ* at the collection plot of the Nikita Botanical Gardens were used for the investigation. Shoot single-node segments were used as explants.

Single-node segments

Regeneration in vitro

Modified MS culture medium with half-strength of macro- and microsalts, vitamins, 30 g/L sucrose, 0.5-1.0 mg/L 6-benzylaminopurine (BAP), 0.1 mg/L β-indolylbutyric acid (IBA), and 9.0 g/L agar.

Cultivation in the phytochambers

T 24±1°C, 16-hour photoperiod and 37.5 µM m^{-2} s^{-1} light intensity. Total cultivation period *in vitro* was six months

Further sub-cultivations

Modified MS medium with the same composition and 0.3-0.9 mg/L BAP.

DNAs isolation

Classical method with trimethylammonium bromide (2×CTAB) and 2% polyvinylpyrrolidone (PVP).

PCR

With RAPD (OPA1-4) and ISSR (UBC807, UBC818, UBC836, GR215, HB12, X10) primers.

Horizontal electrophoresis

1.7% agarose gel with 0.5×TBE buffer at 85 V during 1 h

Results

In vitro regeneration

Initiation of hyssop vegetative bud development *in vitro* was observed on modified MS medium with 0.5-1.0 mg/L BAP and 0.1 mg/ IBA by 8-10 days (Fig. 1). After 15 days, first leaves were formed. Microshoot was developed through 21 days, and axillary buds were formed.

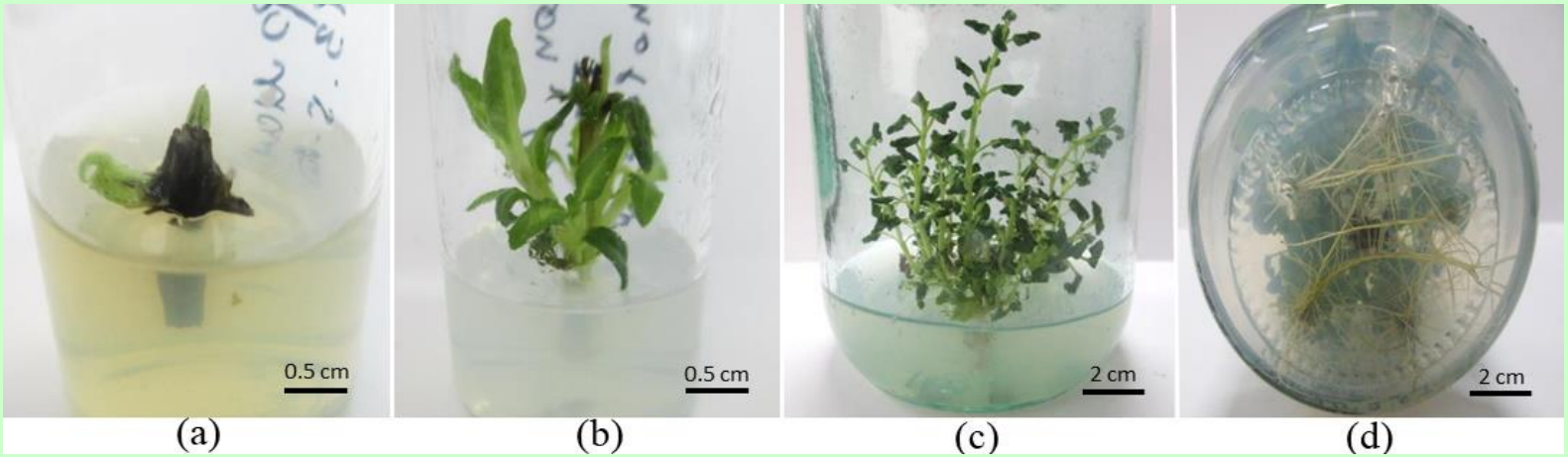

Figure 1. Stages of hyssop regeneration *in vitro* from single-node segments on modified MS medium with BAP: a – initiation of the microshoot formation on the primary explant; b –microshoot formation; c – multiple microshoot formation; d – spontaneous rhizogenesis.

Results

RAPD-, and ISSR-PCR

- All investigated primers gave reproducible bands.
- The band number varied from 6 (OPA3) to 10 (OPA10) (RAPD-PCR) and from 2 (UBC807) to 8 (GR215) (ISSR-PCR).
- RAPD amplicons ranging in size from 260 bp (OPA6) to 3000 bp and slightly more (OPA2).
- ISSR amplification product lengths varied from 250 bp (HB12) to 1750 bp (X10).
- Primers produced amplicons, which were monomorphic for all investigated *ex situ* and *in vitro* plants and no polymorphism was detected.

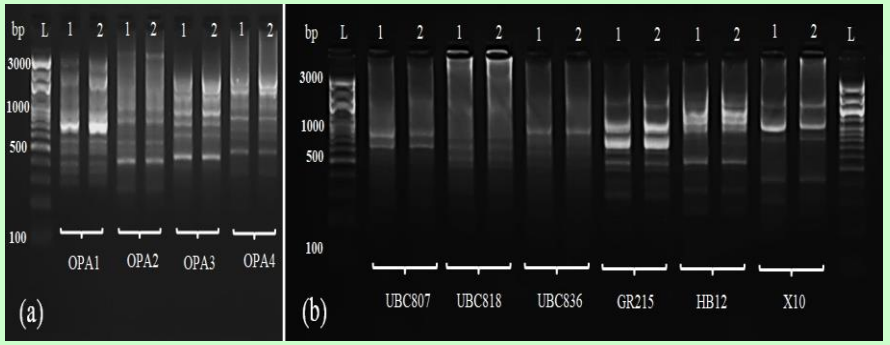

Figure 2. Electrophoregram of the amplified products with RAPD (a), ISSR (b) primers and DNA isolated from leaves of *ex situ* (1) and *in vitro* (2) plants of *Hyssopus officinalis* (cv. Nikitskiy Beliy). L – ladder, bp – base pairs.

Conclusion Our results clear demonstrated that *in vitro* regeneration of *H. officinalis* cv. Nikitskiy Beliy from shoot single-node segments on modified MS medium supplemented with 0.5 mg/L BAP and 0.1 mg/L IBA gave regenerants/clones with normal morphology, genetically identical to mother plants *ex situ* that is very important for the future application of biotechnological method for mass propagation of valuable genotypes, cultivars and plant breeding forms.