

AMBERBOA SOSNOVSKYI ILJIN IN VITRO CULTURE OBTAINING

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ABSTRACT

The *in vitro* cultivation conditions for obtaining the isolated culture of *A. sosnovskyi* were firstly developed. Callus culture was obtained on MS (Murashige and Skoog) basal medium, supplemented with 2.0 mg/L indole 3 acetic acid (IAA), 0.2 mg/L kinetin, but further growth was supported on both MS medium and its modified variant with approximately same concentrations of auxins and cytokinins (0.5 mg/L IAA, 0.2 mg/L kinetin and 0.2 mg/L 6-benzylaminopurine (BAP)). Obtained tissues were possess direct and undirect regeneration ability. The influence of nutrient media composition on growth parameters as well as regeneration ability of callus cultures was studied. On the modified medium the content of dry substances of callus culture was 7% in comparison with MS medium where this parameter was estimated as 5%.

Key words: *Amberboa sosnovskyi*, callus culture, plantlet, growth parameter

INTRODUCTION

The genus *Amberboa* contains approximately 8 to 11 species and belongs to the *Asteraceae* family. Traditionally plants of *Amberboa* genus have been used as tonic, febrifuge, anti-diarrheal, antiperiodic, antipyretic, cytotoxic, anti-cough and in skin disorders. Since Zolotnitskaya et al. (1954), the chemical composition of genus *Amberboa* is in the spotlight of scientists when the presence of alkaloid was reported [10]. Further investigations showed the presence of sesquiterpens, which possess cholinesterase inhibitory activity and can be used as neuroprotective agents against Alzheimer's disease [2, 3]. Some species investigated as a source of different terpenes, which have antimicrobial, antioxidant, calcium channel blocking and cholinesterase inhibitory activities [4]. Several substances from *A. ramose* possess tyrosinase-inhibitory effect and can be clinically useful for the treatment of some dermatologic disorders associated with melanin hyperpigmentation [5]. Based on genus peculiarities *A. sosnovskyi* Iljin was found perspective as source of biologically active substances.

A. sosnovskyi has been included in the Red Data Book of Armenia as an endangered species and widely used in folk medicine: it occurs in one floristic region. The extent of occurrence and the area of occupancy are less than 500 km² [1]. In the available literature there is no information on the *in vitro* cultivation of this plant. So, the aim of the present study was to establish experimental conditions for obtaining tissue cultures and plantlets of *A. sosnovskyi*.

MATERIAL AND METHODS

Plant material. The investigated plants *A. sosnovskyi* Iljin were collected from Ararat region (Surenavan) in Armenia (800-820 m above sea level) during the flowering period.

Obtaining of isolated cultures. The leaves, flowers and stems were used as explants. To isolate tissue cultures the explants (approx. diameters of explants - 0.8 to 1.2 cm) were sterilized with solution of 330 mg/L mercuric chloride and 660 mg/L cetylpyridinium chloride for 8-10 min followed by four rinses with sterile distilled water. The sterilized explants were individually aseptically placed into petri dishes, containing 20 mL Murashige and Skoog (MS) nutrient medium [6], supplemented with 2.0 mg/L indole 3 acetic acid (IAA), 0.2 mg/L kinetin, 0.1 g/L myo-inositol, 0.1 mg/L thiamine HCl, 0.5 mg/L pyridoxine HCl, 0.5 mg/L nicotinic acid, 2.0 mg/L amino acetic acid, 20 g/L sucrose and 6 g/L agar. The explants were placed in thermostat at 25°C (for the initiation of proliferation processes). Afterwards the formed primary callus tissues were placed in the flasks (50 mL) then replaced thermostat (in the same conditions).

To optimize medium composition for shoot multiplication, *in vitro* cultivation was performed in MS basal medium (including vitamins, sucrose and agar), supplemented with 0.5 mg/L IAA, 0.2 mg/L kinetin, 0.2 mg/L 6-benzylaminopurine (BAP). This medium was conditionally named MR. Both of media were adjusted to pH 5.8 with 0.1 M NaOH before autoclaving for 20 min at 121°C. The cultures were placed in growth chamber at 25°C and illuminated with a 16-h photoperiod (natural daylight, supplemented with artificial light (approx. 150 μmol quanta m⁻² s⁻¹) provided by a

white fluorescent lamp (Philips Inc., 36 W). Callus tissue further stable growth was supported in both MS medium and its modification. *In vitro* micropropagation of *A. sosnovskyi* was carried out using modified variant of nutrient medium. **Determination of growth and others.** Plant tissues were dried to constant weight at 60°C. Dry weight (DW) of tissues was calculated at the end of every five days up to 30 days by the formula giving below.

$$DW = \frac{\text{Final dry weight of tissue}}{\text{Fresh weight of tissue}} \times 100$$

Growth Index (GI) of callus tissue was calculated at the same terms by the following formula:

$$GI = \frac{\text{Final dry weight of tissue} - \text{Initial dry weight of tissue}}{\text{Initial dry weight of tissue}}$$

Reagents used were of analytical grade.

RESULTS AND DISCUSSION

The leaves, flowers and stems were used as explants in this investigation. Callus formation was occurred only on the explants of leaf origin. The proliferation begins on 7-8th days on MS medium and the primary callus was formed on 18-20th days of cultivation. Callus tissue of *A. sosnovskyi* had granular consistency and light yellow color growing in dark conditions (Fig. 1). After several passaging light color of callus culture has changed into a light brown with patches of white meristematic zones (Fig. 2a). Such tissue undergoes spontaneous organogenesis under the light (Fig. 2b).

On the MR medium *A. sosnovskyi* callus culture was morphologically different: it has darker color and growth was generally directed over the surface of the agar medium, whereas the growth on MS medium callus tissue was directed upward (Fig. 3 a, b). In addition, after 6-7 passages callus culture gradually lost the ability to regenerate on MS medium, whereas on the MR medium this capacity was preserved.

The *A. sosnovskyi* isolated culture possessed high-frequency regeneration ability on the MR medium, where concentrations of auxins and cytokinins were approximately same (see Material and methods): numerous shoots were formed on the calli cultures which were separated and transplanted to fresh medium (Fig. 4 a, b). On the same medium root formation occurs, sometimes plants reach blooming phase during the June-July period which corresponds to the natural cycle of development (Fig. 4 c).

The leaves and roots of *A. sosnovskyi* regenerated plants were placed on the MS and MR fresh nutrient media. On the MS medium callus culture with high regenerative ability was formed from both leaves and roots (Fig. 5). Numerous regenerated plants were formed on the leaves of plantlets on the MR medium (Fig. 6).

Generally, growth cycle of calli was ended on 27-28th day, but in case with *in vitro* plantlets – on 35-40th day of cultivation.

The study of growth parameters of callus culture revealed that on MS medium the highest accumulation of dry substances was detected at 10-15th and 30th days of cultivation (5%). Reduction of auxin and increase of cytokinin content leads to increase of dry substances accumulation. On the MR medium the content of dry substances was 7%.

Studying the regularity of change of GI during one cycle of cultivation showed that the accumulation of cell mass of callus is not depended on hormonal composition of nutrient medium; the GI of cultures grown in both media was almost identical (Fig. 7).



Fig. 1. Primary callus culture of *A. sosnovskyi* on MS medium

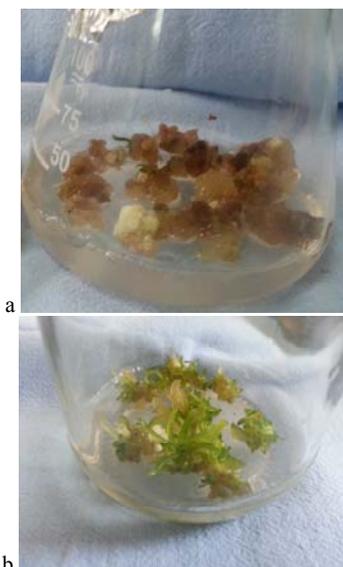


Fig. 2. Spontaneous organogenesis of callus culture *A. sosnovskyi* on MS medium (a; b)

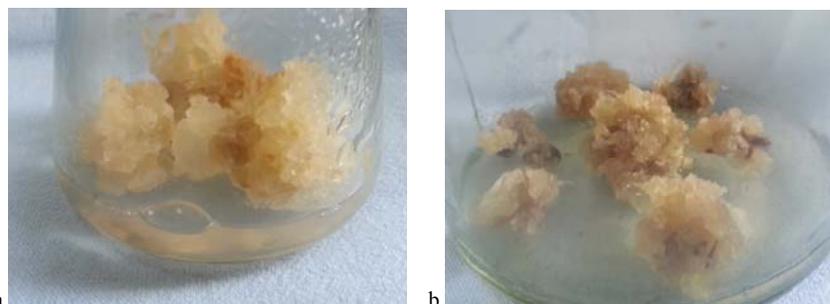


Fig. 3. Callus culture of *A. sosnovskyi*, growing on MS medium (a), growing on MR medium (b).



Fig. 4. Shoot formation on the callus cultures of *A. sosnovskiyi* on the MR medium (a, b), blooming of *A. sosnovskiyi* plantlets on the MR medium (c).

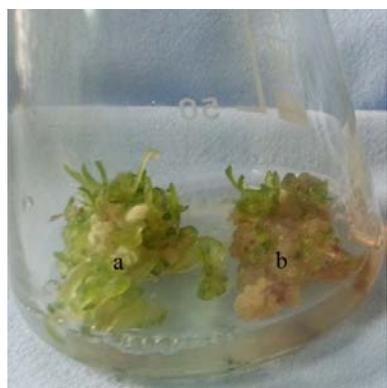


Fig. 5. *A. sosnovskiyi* callus culture of leaf (a) and root (b) origin.



Fig. 6. Direct organogenesis on the leaves of *A. sosnovskiyi* regenerated plants

Summarizing data of our investigation it might be possible to conclude that isolated culture of *A. sosnovskiyi* can be obtained using MS medium and its modified variant which contains the approximately same concentration of auxins and cytokinins. Callus cultures have high growth parameters and regeneration ability on both media, but the latter ability was preserved only in case of using modified MR medium. Previous investigations of our group showed the high metabolic activity of isolated cultures of different plants, growing in Armenia [7-9], so this investigation also can be useful for further studying of metabolic activity of *A. sosnovskiyi* *in vitro* cultures as well as for preservation of the natural plant sources, using method of micropropagation.

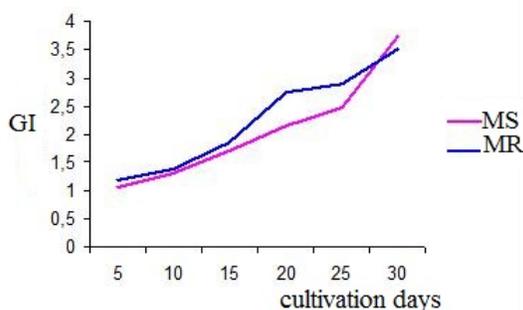


Fig. 7. Growth indices of *A. sosnovskiyi* calli cultures growing on MS and MR nutrient media.

REFERENCES

- [1] Fifth national report of the Republic of Armenia to the convention on biological diversity, 2014. 106 p. <http://www.cbd.int/doc/world/am/am-nr-05-en.pdf>.
- [2] Ibrahim M., Farooq T., Hussain N., Gulzar T., Hussain I., Akash M.S.N., Rehmani F.S., 2013. Acetyl and butyryl cholinesterase inhibitory sesquiterpene lactones from *Amberboa ramose* / Chemistry Central Journal, №7: 116.
- [3] Ibrahim M., Imran M., Ali B., Malik A., 2012. A secondary metabolite amberin from *Amberboa ramose* / Journal of Asian Natural Products Research, №14: 281-285.
- [4] Iqbal S.M., Mushtaq A., Jabeen Q., 2014. Antimicrobial, antioxidant and calcium channel blocking activities of *Amberboa divaricate* / Bangladesh Journal of Pharmacology, №9: 29-36.
- [5] Khan S.B., Haq A., Afga N., Malik A., Khan M.T.H., Shah M.R., Choudhary M.I., 2005. Tyrosinase-inhibitory long-chain esters from *Amberboa ramose* / Chemical and Pharmaceutical Bulletin, v. 53, №1: 86-89.
- [6] Murashige T., Skoog F., 1962. A revised medium for rapid growth and bio assays / Physiologia Plantarum, №15: 473-479.
- [7] Petrosyan M.T., Shcherbakova Y.N., Popov Yu.G., Trchounian A.H. 2013. Isolated culture of St.-John's wort elongated (*Hypericum elongatum* Ledeb.) as the source of biologically active compounds / REPORTS of National Academy of Sciences of Armenia, v. 113: 195-201.

- [8] *Sahakyan N.Zh., Petrosyan M. T., Popov Yu G., Volodin V.V., Matistov N.V., Gruzdev I.V., Shirshova T I., 2010.* Content of neutral lipids and fatty acids in callus cultures and leaves of intact plants of *Ajuga genevensis* and *Ajuga chia* / Second Balkan Conference On Biology, Special edition: 87-90.
- [9] *Sahakyan N.Zh., Petrosyan M. T., Volodin V.V., Volodina S.O., Agajanyan J.A., Popov Yu.G. 2008.* Isolated culture of *Ajuga genevensis* L. as a potential source of biological active substances / The New Armenian Medical Journal Vol.2, №4:65-74.
- [10] *Zolomitskaya S.Y., 1954.* New alkaloid-bearing plants of the Armenian flora / *Izvestiya Akademii Nauk Armyanskoy SSR. Biol I Sel. Khoz. Nauki*, v.7, № 5:27-29. (in Russ.)