UDC: 712.41:582.685.4:632.111.8

BIOTECHNOLOGICAL METHODS OF BREEDING STAGHORN SUMAC (*RHUS TYPHINA* L.) *IN VITRO*

BORSCHEVSKYI M.O., postgraduate*

CHORNOBROV O.U., PhD

KLUVADENKO A.A., PhD

National University of Life and Environmental Sciences of Ukraine

Developed technology of reproduction of staghorn sumac (Rh. typhina L.) in culture in vitro. Choose the optimal mode of sterilization explants to study the effect of nutrient medium components on the morphogenetic activity of tissues and organs in vitro.

***Rhus typhina* L., micropropagation, explants, medium, sterilization, aseptic explants, morphogenesis, growth regulators.**

Members of the genus Sumy (Rhus L.) is particularly valuable in terms of decoration, as recently widely used in landscaping system of Kyiv. Staghorn sumac (Rh. typhina L.) and its decorative forms are cultivated in many botanical gardens, various decorative dendro-areas [8]. These decorative quality as life forms, sizes of plants, architectonic crown, shape, structure of leaves and buds, causing cultivation amounts fluffy (Rh. typhina L.) in many cities of Ukraine.

Among introduced in our country in the form of decorative botanical gardens can be met staghorn sumac `*Dissecta*` (*Rhus typhina* L. var. *Dissecta* Rehd.), staghorn sumac `*Laciniata*` (*Rhus typhina* L. var. *Laciniata* Alph. Wood.) i staghorn sumac `*Bailtiger*` (*Rhus typhina* var. *Bailtiger* Tiger eyes). They leaves are cut, which is a special ornament for the environment in which they grow. Unfortunately they are rarely used in landscaping of streets, despite the highly decorative and simplicity to the growth conditions.

---

* Scientific supervisor - Doctor of Agricultural Sciences, Professor N.O. Oleksiychenko

Using in vitro plant propagation allows to solve important problems crop, namely, hundreds of thousands of times to increase the rate of propagation of plants, get genetically homogeneous, healed propagating material. This method can be used in selection work for the reproduction of new hybrid varieties and obtaining transformed plants, and with his help preserve the gene pool of rare and endangered species of natural flora [9].

In the literature there is information about the development and improvement of technology even microclonal multiplication staghorn sumac (*Rh. typhina* L.), but to achieve sustainable results is difficult. Most such studies conducted abroad on species that are not common in conditions of the Kiev region. The success of the introduction of tissue culture of staghorn sumac (*Rh. typhina* L.) depends on many factors: the state of the parent plant and its age, the time of selection and size of explants etc [1, 2, 4].

The aim of our research was the development of staghorn sumac reproduction technology (*Rh. typhina* L.) *in vitro*, with a view to its use as a base for breeding other ornamental cultivars.

Materials and methods. As donor plants used phenotypically normal (without existing anomalies and diseases) annual shoots root shoots (growing place Botanical Garden NULES Ukraine). Initial explants were 3-5 cm long shoots with a few buds that are located in the leaf axils (usually two buds). As sterilizing agents used: 70 % C_2H_5OH (30 s), and 0,2 % $HgCl_2$. We tested several options sterilization of plant material, depending on the exposure (5, 7, 10 min). Aseptically sterilized shoots cut into pieces of length 1.5-2.0 cm explants were released from the remnants of leaves. These aseptic explants were transferred to the culture medium. The bases of the culture medium during cultivation were inorganic salts (macro and micro), vitamins by prescription Murashige and Skoog – MC [3]. Tested different concentrations and ratios of growth regulators: BAP (benzylaminopurine) - 1,0-1,5 mg/l, IBA (β-indolebutyric acid) - 0,2 mg/l and Fe-chelate. To modified nutrient mediums added: mezoinozyt - 100 mg / l, glycine - 2.0 mg / l, 0.7% agar, 3% sucrose. Index of acidity (pH) level was adjusted to 5,7-5,8. For the adsorption of phenolic compounds in the culture medium was added activated carbon at a concentration of 2.0 mg/l.

The plant material was cultivated in the light at room temperature t = 25 ± 1°C, 2.0-3.0 KLK lighting, 16-hour photoperiod and a relative humidity of 70-75%.

For research we used 4 types of modified MS nutrient medium. As a control culture medium used hormoneless MS, subculturing plant material was performed every 7-14 days. For each variant were planted on 15 explants.

During cultivation parts mikropahoniv performed visual analysis of morphogenesis and regeneration ability. For regenerants used type of plant propagation *in vitro*, this is based on the existing activation in intact plant meristem (apex, axillary buds and dormant stems) [10].

During the experimental work using the following methods: culture of isolated cells, tissues and organs of plants [6].

Results. The effectiveness of regeneration of woody plants under in vitro depends on many factors, including proper selection determined by methods of sterilization of explants and culture medium constituents [5, 9].

Sterile material of tree species is very challenging because the surface of infected plant epiphytic bacteria, fungi and their spores. Proper selection of sterilizing agents is to neutralize the epiphytic microflora and not damage plant tissue. In addition, the agent has to penetrate into the tissue and is easily washed off [9].

Since the surface of plants contaminated with spores of various microorganisms and fungi, for the strengthening of sterilizing agents spent handling tissue explants C2H5OH 70% (30 seconds). To achieve this goal using 0.2% HgCl2 and with different exposures (table 1).

1. The effectiveness of sterilization explants staghorn sumac (Rh. typhina L.)

Exposure time, min	Aseptic explants, %	Viable explants,%	Pigmentation of explants	The effectiveness of sterilization, %
5	50	70	green	35
7	80	80	light green	65
10	95	40	light brown	40

The first signs of infection explants staghorn sumac (*Rh. typhina* L.), which was sterilized with a 5-minute exposure, recorded on the third day of cultivation. After 42 days, the infection reached 50% (mostly fungus infection). When sterilization

explants (10 min exposure) on the sixth day of cultivation simultaneously with contamination of plant material (fungus and bacterial) fixed darkening fabric. On 13-15th day of cultivation explants staghorn sumac (*Rh. typhina* L.) in the absence of bacterial and fungal infection was considered aseptic. Thus, the reduction (5 min) or increase (10 min) time sterilization explants resulted in low efficiency of 35% and 40%, respectively. This was due to inhibition of growth processes of microorganisms and spores of fungi (5 min exposure) or a sterilizing agent (10 min exposure). Most effective sterilization time is 7 minutes experimentally determined. In this exhibition viability shoots stored at 80% efficiency sterilization reaches 65%.

Explants of staghorn sumac (*Rh. typhina* L.) were introduced into cultivation *in vitro* in May. According to literary sources in this period of growth the most actively implemented morphogenetic potential [7, 9, 5]. For culturing explants preferred universal basic MS nutrient medium that contains a balanced concentration of nutrients that promotes the growth of many plants isolated tissues *in vitro* [9]. During culturing explants observed the influence of the components of the culture medium at different stages of morphogenesis (Table 2).

2. Stages of morphogenesis staghorn sumac (*Rh. typhina* L.) culture *in vitro*

Variant	The composition of the culture medium	Stages of morphogenesis			
		Start of morphogenesis, day	Start of regeneration of the main shoot, day	Start of multiple shooting, day	Root induction, day
1	½ MS +BAP 0,5 mg/l + 2,0 g/l activated carbon	7-8	11-13	27-32	100-104
2	½ MS + BAP 0,5 mg/l + IMK 2,0 mg/l + 2,0 g/l activated carbon	7-8	11-13	25-30	99-104
3	MS + BAP 0,5 mg/l + IMK 0,1 mg/l +2,0 g/l activated carbon	10-11	15-16	35-40	-
4	Control (MS hormoneless)	12-14	24-25	-	-

On 7-14th day of cultivation explants on all variants of the culture medium noticed swelling buds. Increasing the kidneys led (11 25th day of cultivation) before the main shoot regeneration. Propagation (27-40th day) cultivation were 1,3-1,9 cm long, visually normal (green pigmentation, visual strain is not noticeable). We assume that in the initial stages of morphogenesis in culture explants in vitro do not require exogenous growth regulators, at this stage of the development of all necessary compounds present in tissue explants (Fig. 1).

Fig. 1. Propagation of staghorn sumac (*Rh. typhina* L.), 7-21 day *in vitro*

On the second or third day of culturing explants staghorn sumac (*Rh. typhina* L.) was observed excretion of phenolic compounds from fixed tissue and inhibiting their growth. As you know, due to the high surface activity and high adsorption capacity, activated carbon is used for the decontamination of phenolic compounds [5], so it was added to the culture medium.

Starting from the 25th day of cultivation observed lag in the growth of the explants. To further induce multiple shooting and regeneration of the root system required exogenous growth regulators BAP and IBA in a certain ratio. The study proved the most optimal combination of BAP and IBA (2,5:1). Experimentally determined that the concentration of components is reduced by half of the culture medium positively affects shooting and root induction. The intensity of the regeneration of shoots versions nutrient mediums number 1 and number 2 occurred 3 times more intense compared with the control.

During further cultivation on the environment mikropahoniv number 1 and number 2 passed their development as manifested in increasing the length and

thickness of the shoot and leaf blade area, number of leaves and internodes. Start of regeneration of roots were fixed at 99-104 days of cultivation. With further culturing mikropahoniv observed a gradual increase in the length, thickness and number of roots (Fig. 2).

Fig. 2. Formed root system staghorn sumac (Rh. typhina L.), 130 days in culture in vitro

So, as a result of the research was obtained plant regenerated staghorn sumac (Rh. typhina L.) which will later be used for adaptation to the open ground.

Acknowledgements. The method of sterilization of explants staghorn sumac (*Rh. typhina* L.) by application of 0.2% solution of $HgCl_2$ within seven minutes of getting 65% of viable aseptic cultures.

It is established that for mass propagation staghorn sumac (*Rh. typhina* L.) *in vitro* microshoots advisable to cultivate ½ MS with added BAP and IBA (2,5:1). The technology of reproduction staghorn sumac (Rh. typhina L.) in vitro will be involved to produce plant-regenerants cultivars of ornamental species.

References

1. Behboodi, B. S. H. (2002) Tissue culture results of all wildspecies and some cultivars in Iran. ISHS Acta Horticulturae 591: III International Symposium on Pistachios and Almonds, Spain.

2. Doroudi, H. Akbarinia, M. Jalali, S and Khosroujerdi E. (2008) Effects of cutting diameter and media on rooting and survival of Sumac cuttings (Rhus Coriaria. L.). Iranian Journal of Biology Spring; 21(2):271-277.

3. Murashige T., Scoog F. A revised medium for rapid, growth and bioassays with tobacco tissue cultures // Physiol. plantarum. – 1962. – V.15. - N. 3. – P. 473.

4. Tissue culture in medicinal plant of Sumac (Rhus coriaria) / Abbas Safarnejad, Sayedeh Bi Bi. Layla Alamdari // International jornal of science and nature. Vol. 2 (4). – Society for science and nature, 2011. P. 760-763

5. Біотехнологічні прийоми розмноження малини (rubus hispidus l.) в умовах in vitro / А. А. Клюваденко, О. Ю. Чорнобров, П. Ю. Дрозд, М. Д. Мельничук //Науковий вісник Національного університету біоресурсів і природокористування України. Вип. 134. Ч. 3. Серія "Біологія, біотехнологія, хімія, екологія" : Збірник наукових праць / редкол. Д. О. Мельничук . - К. : НУБПУ, 2009. - С. 369-377

6. Калинин Ф. Л. Методы культуры тканей в физиологии и биохимии растений / Калинин Ф. Л., Сарнацкая В. В., Полищук В. Е. - К.: Наукова думка, 1980. – 488 с.

7. Катаева Н. В. Клональное микроразмножение растений / Н. В. Катаева, Р. Г. Бутенко. - М.: Наука, 1983. - 96 с.

8. Кохно Н.А. Основные ландшафтообразующие интродуценты парков Украины / Н.А. Кохно // Оптимизация структуры парковых насаждений с использованием интродуцентов. – К.: Наук. думка, 1988. – С. 67-75.

9. Кушнір П. Г. Мікроклональне розмноження рослин: теорія і практика / Г.П. Кушнір, В.В. Сарнацька. – К.: Наукова думка: Інститут фізіології рослин і генетики, 2005. – 269 с.

10. Мельничук М.Д. Біотехнологія рослин : підр. (для студ. агробіол. та біол. спец., наук., викл., асп.] / Мельничук М. Д., Новак Т. В., Кунах В. А. - К.: Поліграфконсалтинг, 2003. - 520 с.