# PECULIARITIES OF GETTING ASEPTIC CULTURE OF CENTURIES-OLD TREE TILIA CORDATA MILL. «LINDEN T.G. SHEVCHENKA»

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The actuality of microclonal propagation of historically centuries-old trees are justify. The peculiarities introduction to the culture and capacity for morphogenesis *in vitro* Linden T. G. Shevchenko depending on genotype, explants type and composition of the nutrient medium. Worked out the stage of getting aseptic culture of centuries Linden T. G. Shevchenko which resulted in the efficiency of sterilization reached 70 % of explants that formed regenerants.

***Keywords:*** microclonal propagation, explants, nutrient medium, plant-regenerants, *in vitro.*

Today in Ukraine and the world is not only relevant advanced technology to obtain alternative sources of energy, cloning, genetic engineering and so on. But also a very acute issue preservation, protection and treatment -old trees , historical and cultural heritage, on the verge of death. Existing technologies of reproduction of species of woody plants are not effective in this case because they are not able to provide 100% transfer of genetic material. As an alternative to traditional methods of reproduction is the introduction of high technology, healed, plant material, including microclonal propagation (MCP) [1, 4, 5, 8].

Linden T.G. Shevchenko – centuries-old tree under 1000. *Tilia cordata* Mill. – linden cordata representative of the genus Tilia, a valuable species tree for forest and landscape gardening [3]. From the data of some authors, in their physiognomic type of linden belongs to decorative hardwood (shady) woody plants, decorative features which are as identity leaves and crown [6, 7]. The centuries-old Linden tree T. G. Shevchenko included in the history of "Manor Lyzohub family", which is located at Chernigov city, Chernihiv region, s.m.t. Sedniv str. Shevchenko 11b,

according to the Cabinet of Ministers of Ukraine of 03.09.2009 № 928 "On the Insert cultural heritage sites of national importance in the State Register of Immovable Monuments of Ukraine" (Fig. 1).

Fig. 1 Centuries-old tree *Tilia cordata Mill*. «Linden T. G. Shevchenko» у природі

The method of culture of isolated tissues and organs found its application in obtaining planting material of rare, historically-valuable species of trees to restore and maintain their cultural heritage.

Objective – selection of optimal sterilization conditions for obtaining pure sterile culture centuries Linden T. G. Shevchenko *in vitro*. This is one of the important factors that affect the success of MCP. It should be noted that the purpose of sterilization is not only getting free from pathogens, diseases, viruses, and harmful microorganisms explants, but also to preserve their ability to morphogenesis and callus formation.

Centuries-old tree "Linden T. G. Shevchenka" as an object of MCP had a number of features that reflect the development of methods of sterilization, such as:

- reducing the activity of physiological processes in tissues due to the age of the tree, which reduces the regenerative capacity;
- possibility of viral infection of the original research material at the cellular level;
- infestation of pests and diseases;
- phenol intoxication by the products of secondary metabolism, which inhibit the growth of plants.

In research as explants served stiff pieces of bud shoots and artificially awakened buds. Stems are cut into pieces of 3-5 cm and washed in soapy water for 20 minutes (stirring intensively). After, the samples were transferred to a vessel with sterile distilled water. All subsequent manipulations were carried out in a laminar box.

Sterilization started with dipping the starting material in 70 % ethanol solution (30-40 s). Then, used various sterilizing agents: 0,5-5,0 % solution of sodium hypochlorite (NaClO 10-30 min), 16,5-50,0 % solution of hydrogen peroxide, ($H_2O_2$ 10-15 min), 0,8-1,0 % solution of silver nitrate ($AgNO_3$ 5-10 min). All work on the culture of isolated tissues and organs was performed by standard methods [1, 2].

Before planting on agar nutrient medium explants were cut into segments 1,0-2,0 cm with one or more internodes.

In the process, according to each stage in the study of morphogenetic responses and tissue explants of *T. cordata* used nutrient media (NM) and by prescription of Murasige and Skoog (MS) [9]. To improve the morphogenetic potential of explants and regulation of morphogenesis of the culture media made with different ratios and concentrations of phytohormones cytokinins 6 BAP (0,5-1,5 $mg \cdot L^{-1}$), thidiazuron (TDZ) (0,5-1,0 $mg \cdot L^{-1}$), kinetin (0,25-0,50 $mg \cdot L^{-1}$) type of action. Also, the culture media was added 7 $g \cdot L^{-1}$agar, 0,1 $mg \cdot L^{-1}$ mesoinosytol, served as a source of hydrocarbon feed sucrose 30 $g \cdot L^{-1}$, pH value of the medium - 5,6-5,7. Additionally as component of NM contributed activated carbon at a concentration of 1 $g \cdot L^{-1}$. Its availability adsorbs growth inhibitors which secreted by plant tissues, or contains impurities such monofenilalamins to accelerate growth.

Explants were cultured at a temperature of 24 ± 2 ºC, 16-hour photoperiod at a constant light intensity of 2000-3000 lux.

Sterilization is an integral part of the early stages of microclonal propagation. Its quality is largely dependent on sterilization substance, its concentration and exposure. Optimal selection of sterilizing substance is that it neutralize pathogens and as little harm plant tissue. There were difficulties in sterilizing objects with cracks, depressions, injuries. In this case, not only the surface sterilization, but penetration into the sterilizing solution negatively affected the growth of tissue *T. cordata in vitro*. It is

therefore necessary to select sterilizing solution that is easy launder of tissue with distilled water or decomposed so as not to contaminate the fabric. In the main performance indicator sterilizing agents was made of explants that developed normally in culture *in vitro.*

As a result, tested several options depending on the type of sterilization sterilizing substances and exposure time for centuries *T. cordata*.

Growth and development of primary explants varied depending on the sterilizing substances used at the beginning of administration and type of explants . Also important was repeated often transplant plants through phenol intoxication, every 2 days during the first week and then every week for a month. These measures yielded a higher percentage of primary regenerants from the initial explants.

Artificially awakened buds best subjected to sterilization for use as solution for sterilisation of $H_2O_2$ (50 %) of 5 min exposure and triple washing in sterile distilled water (5 min ) (Fig. 2).

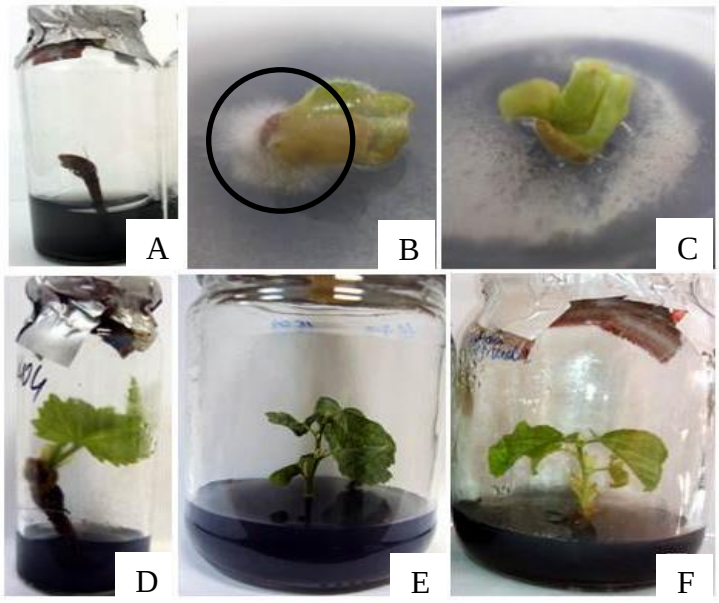


Fig. 2 Aseptic culture of centuries *T. cordata*: A, C - aseptic explants; B - infected explants; D - aseptic primary microshoots *in vitro*; E, F - plants-regenerants obtained by direct morphogenesis

Under these conditions the 55 % aseptic and capable of regenerating explants Linden T. G. Shevchenka. In contrast, stiff stems were more prone to injury. As it turned out infection, manifested on day 3, the effectiveness of sterilization thus

amounted to 20 %. As noted in the cultivation of such culture over the next transplantation accompanied by endogenous fungal and bacterial infection.

Most effective for the fragments of lignified shoots appeared to be comprehensive sterilisation using 70 % solution of ethanol (30 sec.), 1 % solution of $AgNO_3$ (7 min), with a single washing in sterile water (1 min), followed by 25 % solution of $H_2O_2$ (10 min) and one-time washing (5 min). The effectiveness of sterilization thus reached 70 %. All aseptic explants obtained in this way, through 25-27 days observed activation of axillary buds, and after 35-40 days – the formation of primary aseptic microshoots (Fig. 2).

To maintain in culture in vitro produced sterile microshoots transplanted to MS medium that contained vitamins and growth regulators of cytokinin BAP (0,5 $mg \cdot L^{-1}$), KIN (0,25-0,50 $mg \cdot L^{-1}$) and TDZ (0,25-1,0 $mg \cdot L^{-1}$). In the early stages of micropropagation effective was the NM with the addition of TDZ at a concentration of 0,25 $mg \cdot L^{-1}$ and NM with 0,5 $mg \cdot L^{-1}$ BAP with the addition of activated carbon in all cases. Transplanting primary microshoots successfully showed the ability to morphogenesis.

So, as a result of research, the best explants for the introduction of *in vitro* culture is as stiff and artificial revival of centuries-old shoots Linden T. G. Shevchenka personal use, provided the method of sterilization. According to lignified – complex of sterilisation with using 70 % solution of $C_2H_5OH$ (30 sec), 1 % solution of $AgNO_3$ (7 min), with a single washing in sterile water (1 min), then 25 % solution $H_2O_2$ (10 min) and one-time washing (5 min) to artificially awakened – 25 % solution of $H_2O_2$ – term (10 min).

Thus, a successful getting of aseptic explants and primary transplanting plant – regeneration set the stage for the study of the characteristics and selection of components NM for further mass regeneration root formation in vitro and selection substrate for adaptation to *ex vitro*.

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