

CONSERVATION OF BIODIVERSITY OF POME ROOTSTOCKS IN THE REPUBLIC OF MOLDOVA

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Summary. The results of long-term research on the preservation and maintenance of a healthy biobank of the main zoned and promising apple rootstocks for the Republic of Moldova using the *in vitro* tissue culture method and *in vivo* conditions of the depository are presented. The described plant cultivation methods eliminate the possibility of reinfection of the original material and guarantee the availability of pure, virus-free biodiversity of pome rootstocks. Special attention is given to the possibilities of accelerated micropropagation of these genotypes to meet the needs of local nurseries.

Keywords: biobank preservation, rootstocks, apple, virus-free planting material, *in vitro* propagation.

Introduction

The modern course of fruit nursery development in the Republic of Moldova, in line with European quality standards, is aimed at introducing a certification system for the production of orchard planting material (2). Certification will guarantee high biological quality of the planting material, compliance with established standards, and expand potential sales markets. This will positively impact the country's economy. While reducing phytosanitary risks associated with the import of planting material. In the process of achieving these goals, the primary task is to maintain the availability of basic original plants of each genotype that are of interest to the country's nurserymen and fruit growers to ensure their varietal and phytosanitary purity.

The Laboratory of Virology and Phytosanitary Control at the Scientific and Practical Institute of Horticulture, Viticulture and Food Technologies (Moldova, Chisinau) maintains a strategic assortment of virus-free clonal apple rootstocks for the Republic of Moldova (5). Apple is an important industrial pome crop, but in recent years, apple plantations in Moldova have been gradually decreasing due to the uprooting of old orchards and the weak establishment of new plantations. The limiting factor is also the insufficient production of high-quality rootstock material. Maintaining a bank of valuable virus-free biotypes of clonal apple rootstocks and using the potential of microclonal propagation technology can open new prospects for the industrial cultivation of this fruit crop.

Materials and Methods

The research was conducted in the Laboratory of Virology and Phytosanitary Control of Fruit Crops at the Scientific and Practical Institute of Horticulture, Viticulture and Food Technologies (Moldova, Chisinau). The objects of our research were clonal apple rootstocks: M 26, MM106, 62-396, Pi-80, M 9, M 27, M 4, 54-118. The rootstocks were preserved *in vivo* and *in vitro*. Under greenhouse conditions, two plants of each genotype were maintained, planted in soil in 17-liter containers. Plant maintenance in the depository was organized with isolation to prevent reinfection by diseases and pests. The initial material for creating the *in vitro* collection was shoot

tips from actively growing plants (April-May) measuring 4-8 mm. The sterilization of plant material was carried out using chlorine-containing agents and hydrogen peroxide. The cultivated explants were kept in a culture room with regulated environmental conditions - at a temperature of +22+24°C, relative humidity of 70%, light intensity of up to 3-5 thousand lux, and a light period duration of 16 hours. Transfer of explants to fresh nutrient medium was performed every 4-8-12-16 weeks. For introducing explants *in vitro*, the nutrient medium by Murashige-Skoog (MS, 1962) with the addition of sucrose 30 g/l, vitamins (inositol 100 mg/l, B6-0.5 mg/l, B1-0.5 mg/l, PP-0.5 mg/l, ascorbic acid - 10 mg/l), cytokinin BAP -1 mg/l, agar-agar - 6 g/l, pH of the nutrient medium 5.8 was used. To maintain regenerative capacity to preserve isolated material, nutrient media WPM (McCown B., Lloyd G., 1981), or MS (Murashige and Skoog, 1962) with reduced nitrogen salt content were used. For proliferation, nutrient media based on MS (4), WPM (3), B5 (Gamborg O., Eveleigh D., 1968) (1) supplemented with sucrose 30 g/l, inositol 100 mg/l, B1, B6, PP -0.5 mg/l, ascorbic acid - 3 mg/l, BAP -0.5-3 mg/l, auxin IBA -0-0.5 mg/l, agar-agar - 6 g/l, pH of the nutrient medium 5.8 were tested. At the rhizogenesis stage, the concentration of macronutrients in the nutrient medium was halved, and IBA (Indole-3-butyric acid) was used as a growth stimulator. The adaptation of rooted plants was carried out in plastic tunnels at 100% air humidity during the first weeks of cultivation.

Results and Discussions

The preservation of virus-free clonal apple rootstocks in the depository has been conducted for 20 years. Annual spring pruning is performed. During the vegetation period, daily watering is required. From March to September, protective measures are taken every 7-14 days. Annual retesting of rootstocks for the absence of viral and mycoplasma diseases is conducted. It should be noted that during this period, the death of individual plants was observed. According to regulations, the initial material is renewed every 8-10 years. The presence of only two original plants for further propagation in the certification system chain does not allow for rapid multiplication of the necessary rootstock. The preservation of virus-free pome rootstocks under *in vitro* laboratory conditions has been conducted for several years (6). Currently, the collection includes eight main clonal apple rootstocks. The initiated material is maintained in minimal quantities - 10-30 microplants and is renewed annually to avoid genetic changes due to long-term exposure to physiologically active substances. Cultivation of plants on nutrient media with reduced nitrogen concentration (WPM, MS with ½ N salt) and cytokinins allows for the preservation of quality material without transplanting for about 8 weeks, which halves the number of subcultures and minimizes the cost of maintaining the biobank *in vitro*. Extending the period between passages to 12-16 weeks leads to partial or complete necrosis of shoots.

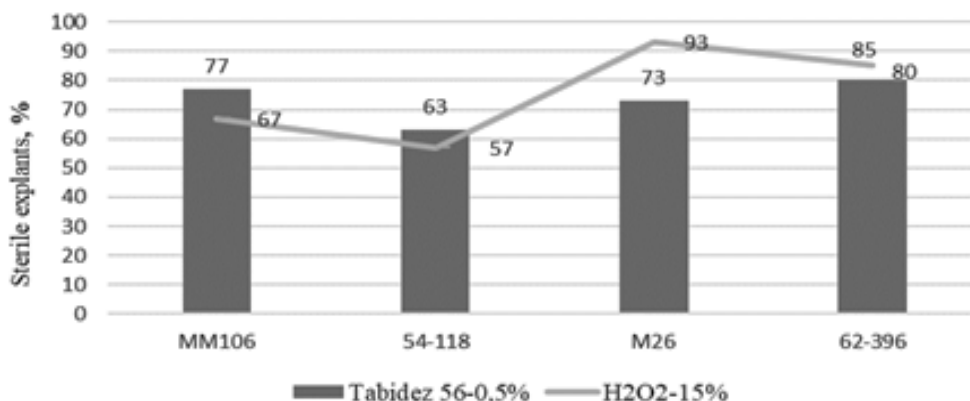


Figure. Results of sterilization of explants of clonal apple tree rootstocks

The availability of such a collection allows for the rapid multiplication of high-quality rootstock material at any time of the year. Microclonal propagation of plants is a highly productive method widely used in modern horticulture. Research conducted in the virology laboratory has developed effective *in vitro* propagation techniques for many clonal apple rootstocks, achieving a multiplication rate of $10^{-3-3.5}$ from a single original explant. As a result of the research, optimal sterilization schemes for isolated explants were selected.

The use of 15% hydrogen peroxide and 0.5% sodium dichloroisocyanurate allowed obtaining more than half of the clean explants capable of further development (Fig.). Clonal apple rootstocks M 26, 62-396 showed increased regenerative ability.

The mineral composition of the nutrient medium, as well as the composition and concentration of physiologically active substances, had a significant impact on the formation of new microshoots and their parameters. For rootstocks MM106, M 26, and 62-396, high quality of newly formed plants along with intense proliferating effect was achieved when cultivated on MS nutrient medium. For 54-118 and 62-396, good results were also obtained on WPM medium. The multiplication rate differed significantly between rootstocks depending on the biotype, combination, and concentration of cytokinin. Cultivation of microplants on nutrient media with the concentrations of growth stimulants indicated in table allowed obtaining an average of 4.8-5 additional shoots per passage (about 4 weeks) in rootstocks 54-118 and 62-396. For MM106, this value reached 4.3, and for M 26 - 3.8 new shoots per original. Rhizogenesis processes in clonal apple rootstocks were successfully conducted in the presence of IBA on a nutrient medium with reduced macronutrient content. Rootstocks 54-118 and 62-396 rooted 80 and 85% of shoots respectively, at an IBA concentration of 0.5 mg/l. Rhizogenesis within 83% was achieved in rootstock M 26 at an IBA concentration of 1 mg/l and within 75% in rootstock MM106 at 3 mg/l IBA. The average number of roots varied between 2.5-6 per shoot.

Table

Average number of additional microshoots obtained during micropropagation of apple rootstocks over 4 weeks of cultivation

Rootstock	BAP: IBA concentration in nutrient medium, mg/l	In average number additional shoots	Of average shoot Height, cm
MM 106	2: 0.5	4.3	0.8
M 26	1: 0-0.5	3.8	1.2
54-118	0.5: 0	4.8	1.0
62-396	1.5: 0.5	5	1.0

The adaptation of rooted shoots to non-sterile conditions is the final and very responsible stage of microclonal propagation technology. Its complexity is due to the restructuring of respiratory and transpiration mechanisms, the transition from heterotrophic to autotrophic nutrition. Success at this stage directly depends on the availability of temperature and air humidity control mechanisms, as well as control over the infection load on plants during the first three weeks of acclimatization. The acclimatization methods developed by virology laboratory specialists based on years of experience allow for the successful adaptation of 65-75% of apple rootstocks to *ex vitro* conditions. The best planting times in non-sterile conditions were April and May. By the end of the vegetation period, the plants are fully developed and reach a length of 20-25 cm.

Conclusions

The method of propagation and cultivation under *in vitro* conditions is an effective way to solve the problems of preserving and maintaining a healthy gene pool of clonal pome rootstocks. It allows preserving a large number of apple rootstocks in a small area *in vitro* and quickly

multiplying high-quality planting material when necessary. An additional method for maintaining apple rootstock genotypes is their preservation with a few plants under depository conditions.

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