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MICROPROPAGATION FOR THE CONSERVATION OF RARE *AND ENDANGERED* MALUS NIEDZWETZKYANA

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ABSTRACT

The conservation of biological diversity is one of the most important tasks at present given the strong association of biodiversity with food security and improving nutrition. Currently, 387 plant species are listed in the Red Book of Kazakhstan, including the rare, endemic, and endangered species *Malus niedzwetzkyana*, which is also included on the International Red List. Biotechnology methods are currently widely applied to preserve such rare plant species, which allows for the long-term preservation of genetic material, enabling their large-scale reproduction and propagation. One of the most effective methods for this purpose is microclonal propagation, which is similar to the vegetative method of reproduction except that the entire process proceeds with an *in vitro* culture system. The resulting clones are healthy and genetically stable. In this study, we optimized the microclonal propagation technique for *Malus niedzwetzkyana.* Axillary buds of annual shoots were used as the initial material. The mode of sterilization of the axillary buds, composition of the nutrient medium for establishment of in vitro culture, and the multiplication and rooting of microshoots were optimized. The data obtained provide an essential resource for sustaining the reproduction and propagation of this rare and endangered species of apple trees.

Keywords: *Malus niedzwetzkyana*, *in vitro* culture, micropropagation, shoot multiplication, rooting

INTRODUCTION

Biodiversity plays a significant role in life-supporting biosphere systems and affects the survival of other species [1]. Thus, the government is responsible for the conservation of biological diversity and the sustainable use of natural resources, including plant diversity. The scope and use of plants is very extensive. Many species of plants are valuable from the point of view of medicine, agriculture, food industry, cosmetology, pulp, paper and furniture production, and decorative landscaping [1, 2, 3]. However, due to climate change and human activities, many plant species are threatened with extinction. Moreover, 50% of the plants of the world are endemic and non-rational using can lead to the complete extinction of the species [4].

The international convention on conservation of biodiversity, signed by 145 countries, emphasizes the need to anticipate, prevent and eliminate the causes of reduction or loss of biological diversity. And also develop national strategies, plans or programs for conservation, maintenance and restoration of natural resources in natural habitats (*in situ*) and outside their natural habitat (*ex situ*) [5, 6]. So, around the world, various methods are using to preserve and restore plant material [7].

To conserve plants biodiversity by *in situ* approaches, special setting (natural parks, reserves and botanical gardens) are organized. Thus, it creates favorable conditions for the growth and reproduction of plants in natural places of origin [8, 9, 10].

Conservation components of biodiversity by *ex situ* approaches include the conservation of biologi-

cal resources outside natural habitats. This process is carried out by using the introduction of rare and endangered species beyond the natural habitat or using biotechnological methods. However, using the introduction method have risks when plants are acclimatized in new natural conditions (climate, soil, environmental factors). Moreover, natural reproduction takes a long time, and getting a new plant population will take years. Thus, the conservation, reproduction and propagation of rare and endangered plant species under natural conditions is a laborious and risky process [11, 12].

However, today, scientists use biotechnology methods to preserve and restore plants. Such as the creation of seed banks, cryopreservation, the creation of *in vitro* collections of cells, tissues, organs and micropropagation. All these methods are carried out under aseptic conditions, which makes it possible to heal the material from infection and diseases [10, 13].

So, the scientists of Malaysia use *in vitro* methods of conservation of biological diversity. For this, artificial conditions are created in which plant growth is very slow. Also, liquid nitrogen is used to store plant material and seeds [14]. In Brazil, scientists use several methods to preserve plant biodiversity. To preserve endemic and endangered plant species, they use methods of creating seed banks and cryobanks for long-term storage of genetic material. Also, the method of micropropagation is used to obtain seedlings for planting in the natural habitat. Such an integrated approach allows to save genetic material for a long time and reproduce identical clones to restore natural populations in nature [15].

Thus, for reproduction and propagation of plant material, micropropagation is an urgent method. This method allows to propagate the material on a large scale throughout the year and obtained clones are identical to the original material. Moreover, using artificial conditions becomes possible to control the process of plant development, depending on the tasks. For this, cultivation conditions are optimized, such as: the composition of the nutrient medium, exogenous hormones, temperature, lighting and humidity [16, 17].

Many researches use the method of micropropagation to preserve and reproduce rare and endangered species of woody plants [18, 19, 20, 21, 22]. One of such rare, endangered and endemic plants in Kazakhstan is the *Malus niedzwetzkyana.*

Malus niedzwetzkyana is a wild type of apple tree, which is considered the ancestor of many cultivated species of fruit apple trees and is resistant to high and low temperatures, diseases, gas contamination and dusty air [23, 24]*Malus niedzwetzkyana* is a decorative species of small-fronted apple tree belonging to the genus*Malus* of the family*Rosaceae* [25]. The height of the tree is about 6 meters, the crown is wide, raised, with a diameter of up to 4 meters. The flowers are collected in a small-flowered umbrella, the buds are purple-red. Autumn color of leaves brown-red with purple [26].

The chemical composition of the fruits of the *Malus niedzwetzkyana* is rich for vitamins. A distinctive feature is the formation of anthocyanin pigment in many parts of the plant, which is synthesized throughout the growing season: fruits with red coloration of the skin, pulp and seeds, flowers - red (pink), leaves (especially young) - purple-reddish, even young bark and wood have a reddish tint [27]. In China*Malus niedzwetzkyana* is propagated for commercial purposes, in order to obtain large quantities of anthocyanins. This natural antioxidant is used to treat cardiovascular and oncological diseases [28, 29, 30].

Under natural conditions, the apple tree propagates by seeds and root shoots [31]. In orchards, the apple tree is propagated by rootstocks. They are seedling (grown from seeds) and clonal (obtained vegetatively from layering) [32]. However, when growing from seeds, the parental traits are split, so only some part of the seedlings retain the characteristic purple color of the leaves [33]. Nowadays, *Malus niedzwetzkyana* is a species listed in the Red Book of Kazakhstan [34] and the International Red List in the status of endangered species [35].

Micropropagation of apple in*in vitro* culture is carried out through the growth of the main microshoot from axillary buds [36]. For this, artificial cultivation conditions for each species are optimized individually. A group of scientists from Hungary, Japan and New Zealand describe the effectiveness of adding cytokinins to the nutrition media at the microshoot multiplication stage. The optimal composition of the medium provides getting a large number of additional microshoots per explant [37].

An important stage of micropropagation is obtaining adapted plants in the soil after *in vitro* culture. But, transferring plants from an*in vitro* culture to*ex vitro* conditions is a complex process. Plants undergo severe stress, adapting to new non-sterile conditions. At this stage, success depends on the quality of the roots. Thus, a developed root system is a key factor for the adaptation of microshoots for further growth and

development in a greenhouse and open ground [38].

Researchers from Japan and India describe in their work that active root growth is achieved by reducing the composition of macrosalts to 50% and 75% and adding auxins IAA or IBA to nutrition media, which allowed to increase the survival of clones up to 85% [7]. Thus, micropropagation is an actual method for propagation, preservation, and reproduction of tree crops.

Materials and methods

The annual shoots of *Malus niedzwetzkyana* were used as the object of study (figure 1). The investigated microshoots were obtained from one initial line (line 1). Samples were collected in the dendrological garden in Nur-Sultan. For micropropagation of the rare and endangered *Malus niedzwetzkyana* establishment of *in vitro* culture, multiplication, and rhizogenesis of microshoots methods were used.

To induce root formation, microshoots 1.5ć2.0 cm high with several leaves were used. *QL* nutrition medium (*Quoirin&Lepoivre*) [39] containing 30 grams of sucrose and 7 grams of agar per liter was used as basal medium.

a - Annual shoots b - Axillary buds

Fig. 1. The initial material of the *Malus niedzwetzkyana* for micropropagation

Establishment of *in vitro* culture. Sterilization conditions were optimized for introducing axillary buds of the *Malus niedzwetzkyana* into *in vitro* culture. To do this, axillary buds were cut 1.0ć1.5 cm long and thoroughly washed on a magnetic stirrer in a solution of commercial washing powder. This procedure was repeated until complete removal of external dust and dirt. Further, for sterilization using detergents, 6 sterilization modes were studied, which differed in sterilizing reagents and their concentration (table 1).

Modes 1, 2, 3, 4 consisted of a 2-step sterilization. For this, at the 1st stage, the axillary buds of the *Malus niedzwetzkyana* were washed in a solution of 0.1%, 0.5%, 0.7% and 1.0% flDomestosffi (5% sodium hypochlorite) with the addition of 2 drops of flTwin 20ffi (Tween 20, viscous liquid, monolaurate polyoxyethylene sorbitan, Sigma-Aldrich) for 8-10 minutes, followed by washing in running water for at least 20 minutes. The 2nd stage of sterilization was carried out in a laminar box by a solution of 0.1%, 0.5%, 0.8% and 1.0% flBleachffi (5.0% chlorine) for 8-10 minutes.

Also, the effectiveness of using hydrogen peroxide for sterilizing the axillary buds of *Malus niedzwetzkyana* was studied. Since hydrogen peroxide least damages the material and does not require long-term washing [40]. For this, 1 step sterilization was used. That is, a preliminary washing in running water and further sterilization in a laminar box using a solution of hydrogen peroxide (H_2O_2) 6.0% and 12% for 4 minutes.

Next, the axillary buds were washed several times with sterile distilled water and dried on sterile filter paper. Sterilized axillary buds were cultivated on nutrient medium, but previously updated the slice and cleaned of external scales without damaging the apical meristem.

Table 1. Modes of sterilization of axillary buds of *Malus niedzwetzkyana*

Stage	Mode 1	Mode 2	Mode 3	Mode 4	Mode 5	Mode 6
1 stage	flDomestosffi 0,1% with flTwin 20ffi (10 min.)	flDomestosffi 0,5% with flTwin 20ffi (10 min.)	flDomestosffi 0,7% with flTwin 20ffi (10 min.)	flDomestosffi 1,0% with flTwin 20ffi (10 min.)	H_2O_2 6,0% (4 min.)	H_2O_2 12% (4 min.)
2 stage	flBleachffi 0,1% (10 min.)	flBleachffi 0,5% (10 min.)	flBleachffi 0,8% (10 min.)	flBleachffi 1,0% (10 min.)		

To obtain the main microshoot in *in vitro* culture, the sterile axillary buds of *Malus niedzwetzkyana* were cultured on *QL* medium. To select the composition of the nutrient medium, the effects of the hormones BAP (6-benzylaminopurin), GA (gibberelic acid), IBA (indolylbutyric acid) in 3 variants were studied: Variant 1 is *QL* with the addition of BAP 1.0 mg/l and IBA 0, 2 mg/l; Variant 2 is *QL* with the addition of a BAP of 0.5 mg/l, GA 1.0 mg/l and a IBA of 0.01 mg/l; Variant 3 is *QL* with the addition of BAP 1.0 mg/l and kinetin 3.0 mg/l.

Microshoot multiplication. The next step after obtaining the main microshoot is the multiplication of microshoot, which allows you to propagate plant material on a large scale. For this purpose, the apex buds were cut off from the formed main shoots to remove apical dominance, which stimulates the reproduction of additional buds.

In order to optimize a nutrient medium that induces the multiplication of microshoots of *Malus niedzwetzkyana* in *in vitro* culture, *QL* medium with the addition of hormones BAP, GA, IBA and kinetin were used. The following variants of nutrition media were studied: I-BAP 0.5 mg/l, GA 1.0 mg/l and IBA 0.01 mg/l; II - BAP 0.5 mg/l; III - BAP 1.0 mg/l, kinetin 3.0 mg/l and GA 0.5 mg/l.

Rooting of microshoots. The final step in micropropagation *in vitro* culture is the rooting of microshoots. For this, the mineral composition of the nutrient medium is reduced, in particular the content of macrosalts, and auxins (IBA, IAA) are added. At this stage, microshoots with a height of 1.5-2.0 cm., without additional microshoots and with several leaves, were cultivated on a nutrient medium inducing root formation.

Thus, the culture medium ½*QL* was studied without and with the addition of hormones. The following nutrient medium variants were studied for inducing root growth in *Malus niedzwetzkyana* microshoots: I - ½*QL* with IBA of 1.5 mg/l; I - ½*QL* with IBA of 1.0 mg/l; III - *QL* without hormones.

At all stages of micropropagation, the pH of the nutrient medium was adjusted to 5.8 before autoclaving, hormones were added after autoclaving. The hormones were sterilized using syringe sterilizers from polyethersulfone with a hydrophilic membrane (pore size 0.20 um). All sterile work was carried out in a laminar box. Cultured microshoots were grown in a light room with a 16-hour light regime, a temperature of 24-26°C, a humidity of 60-70%. During the study, we observed the quantitative changes in plant development over 50 days.

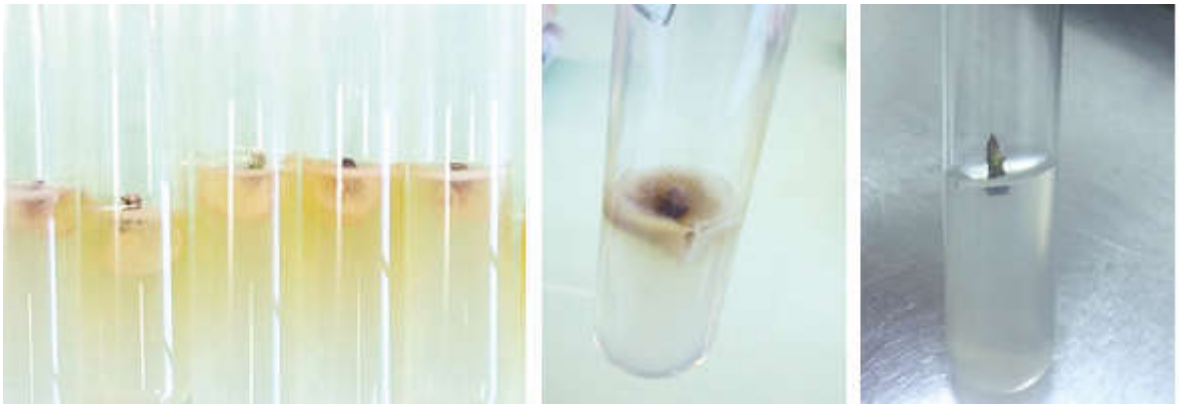
RESULTS AND DISCUSSION

Sterilization is an important step; it depends on the plant species. For example, for micropropagation researchers from Hungary in their review work about *Malus* micropropagation described effective sterilization modes for various species of *Malus*, where explant viability ranged from 70% to 90%. To sterilize the axillary buds of dwarf rootstocks of apple trees, a solution of sodium hypochlorite (NaOCl) (15%) and a solution of mercury chloride ($HgCl_2$) were effectively used. Sterilization with a NaOCl solution (10%) with the addition of Tween 20 for 20 minutes was effective for stock MM111 and M9 [36]. Also, researchers used 70% ethanol and 0.1% mercury chloride, 5.0% and 10% commercial flBleachffi solution, 6.0% and 12% hydrogen peroxide (H_2O_2) to sterilize the axillary buds of apple trees. Depending on the species of apple tree, the use of these sterilizers made it possible to obtain sterile and viable explants [41, 42, 43]. Thus, individual selection of sterilization for each species of plant is a necessary task.

As a result of a comparative analysis on the sterilization of the axillary buds of an apple tree, 6 modes were studied that differed from each other (Table 1). The results of the study showed that one-step sterilization with hydrogen peroxide is optimal for obtaining sterile viable axillary buds of *Malus niedzwetzkyana*. Sterilization with a 6.0% hydrogen peroxide solution showed 60% of viable axillary buds while using a 12% hydrogen peroxide solution provided 80% (Table 2). In variants 3 and 4, a burn was observed, as a result of which, the axillary buds completely lost viability. Tissue necrosis reached 80% (Mode 3) when flDomestosffi 0.7% and flBleachffi 0.8% were used. The increase in the concentration of flDomestosffi and flBleachffi to 1.0% led to almost complete death of the explant. At a low concentration of detergents, plant material became infected with infections (table 2, figure 2).

Table 2. Optimization of the sterilization conditions of axillary buds of *Malus niedzwetzkyana*

Variant	Sterile viable explants	Explant necrosis	Infected explants
1	-	40%	60%
2	-	60%	40%
3	-	80%	20%
4	-	90%	10%
5	60%	-	40%
6	80%	10%	10%



a - Axillary buds sterilized by variant 1 (flDomestosffi 0.1% with flTwin 20ffi and flBleachffi 0.1%)

b - Axillary buds sterilized by variant 2 (flDomestosffi 0.5% with flTwin 20ffi and flBleachffi 0.5%)

c - Axillary buds sterilized by variant 6 (12% H2O2)

Fig. 2. Optimization of the conditions for sterilization of axillary buds of *Malus niedzwetzkyana*

Thus, the best option for sterilizing the axillary buds of *Malus niedzwetzkyana* is a 12% hydrogen peroxide solution for 4 minutes (Variant 6). Using this sterilization provides 80% of sterile and viable explants.

After receiving sterile and viable axillary buds, it is necessary to select a nutrient medium for their cultivation in order to get the main shoot in *in vitro* culture. The literature contains data about nutrient media used for tree crops (*Murashige-Skoog, Quoirin&Lepoivre (QL), Woody Plant Medium, DKW*). They differ in the content and concentration of macrosalts, microsalts and vitamins [44].

Analysis of studies about micropropagation of apple trees showed that the culture medium *QL* is most effective at all stages of micropropagation for different species of apple trees [36, 45]. And also, to achieve a more effective result, the researchers add hormones IBA, BAP to the nutrient medium, which made it possible to obtain up to 97-100% regeneration of the main microshoot from callus in the Fuji, Gala, low apple and domestic apple [37, 46].

Based on the above results, for introducing the axillary buds of *Malus niedzwetzkyana* into the *in vitro* culture, *Q&L* with the addition of the hormones BAP, GA, and IBA in three variants were used. So, on *QL* nutrient medium (Variant 1) with the addition of a BAP of 1.0 mg/l and IBA of 0.2 mg/l from 20 axillary buds 5 microshoots were formed and at the variant 2 (*QL* with a BAP of 0.5 mg/l and GA 1.0 mg/l) 12 axillary buds formed main microshoots from 20 buds. As studies showed, the most optimal composition of the nutrient medium is *QL* with the addition of BAP 1.0 mg/l and kinetin 3.0 mg/l. On this nutrient medium, 18 explants formed the main microshoots, it was highest rate (table 3, figure 3).

Table 3. Optimization of the QL medium composition for introduction into the culture in vitro of axillary buds of *Malus niedzwetzkyana*

Variant	Nutrition media	Initial explants, pcs	Regenerated explants, pcs	Regeneration in %
1	*QL* with BAP 1,0 mg/l and IBA 0,2 mg/l	20	5	25
2	*QL* with BAP 0,5 mg/l and GA 1,0 mg/l	20	12	60
3	*QL* with BAP 1,0 mg/l and kinetin 3,0 mg/l	20	18	90

Fig.3. Regenerated main microshoots of *Malus niedzwetzkyana* on nutrient medium *QL* with BAP 1.0 mg/l and kinetin 3.0 mg/l

Thus, *QL* with the addition of BAP 1.0 mg/l and kinetin 3.0 mg/l, where the percentage of regeneration was 90%, is an effective nutrient medium for the growth of the main microshoots from *Malus niedzwetzkyana* axillary buds.

The next stage of micropropagation after receiving the main shoot is the propagation of additional shoots in *in vitro* culture. This allows propagating plants in large volumes and the success of propagation depends on the composition of the nutrient medium. Optimized nutrient medium makes it possible to obtain additional shoots from the main microshoots and cultivate them on a fresh nutrient medium, having previously divided each microshoots separately. According to the published data, the addition of the hormones BAP and IBA to the *QL* nutrient medium is effective for multiplication of microshoots of an apple tree. Thus, the authors studied the effect of BAP at a concentration of 0.5 mg/l, where 13 additional microshoots were formed to explant for Zumi apple tree [40]. Also, a high coefficient of microshoots propagation was achieved (up to 5.5 additional microshoots per explant) with the addition of BAP and IBA hormones for Domestic apple [41, 47].

Based on these results, the effect of the hormones BAP, IBA, and GA in 3 variants for the multiplication of microshoots of *Malus niedzwetzkyana in vitro* culture was studied. As shown by the results of phenological observations, the optimal variant for increasing the number of microshoots is *QL* with the addition of BAP 0, 5 mg/l, GA 1.0 mg/l, IBA 0.01 mg/l. So, on the 50th day of cultivation, the number of microshoots per explant averaged 24.8 pcs. Microshoots were green and had developed leaves. At variants 2 and 3, the number of microshoots was the same (on average 4.4 pcs per explant). The microshoots were weak and had a pale green color; moreover, on variants 2 and 3, the microshoots height was significantly lower than at variant 1. On the 50th day of cultivation, blackening of the leaves was observed and the leaves fell off after repeated cultivation (table 4, figure 4).

Table 4. Optimization of the nutrient medium for the multiplication of *Malus niedzwetzkyana* microshoots in *in vitro* culture

Variants	The number of microshoots, pieces				
	Day 1	Day 10	Day 25	Day 40	Day 50
1 - *QL* with BAP 0,5 mg/l, GA 1,0 mg/l, IBA 0,01 mg/l	1,0	4,6±1,14	9,0±4,58	16,2±8,34	24,8±7,94
2 - *QL* with BAP 1,0 mg/l, IBA 0,2 mg/l	1,0	1,0	2,6±1,14	3,6±1,14	4,4±1,14
3 - *QL* with BAP 1,0 mg/l, kinetin 3,0 mg/l	1,0	1,0	2,6±1,14	2,8±1,78	4,4±0,54

a ć Variant 1 (QL with BAP 0,5 mg/l, GA 1,0 mg/l, IBA 0,01 mg/l)

b ć Variant 2 (QL with BAP 1,0 mg/l, IBA 0,2 mg/l)

c ć Variant 3 (QL with BAP 1,0 mg/l, kinetin 3,0 mg/l)

Fig. 4. Microshoots of *Malus niedzwetzkyana* on the 50th day of cultivation on 3 variants of culture media for the multiplication of microshoots

Thus, as shown study results, the optimal nutrient medium for the multiplication of microshoots shoots of *Malus niedzwetzkyana* is *QL* with the addition of a BAP of 0.5 mg/l, GA 1.0 mg/l, and IBA 0.01 mg/l. On this nutrient medium, the 50th day of cultivation is observed in the propagation of microshoots (an average of 24.8 per explant).

The last stage of micropropagation in *in vitro* culture is to obtain microshoots with a developed root system. As the analysis of the literature about rooting of apple shoots showed, scientists use the hormones IBA and NAA (Alpha-Naphthylacetic Acid) based on *QL* nutrient medium with a reduced content of macrosalts (½QL) to induce root growth in the Jork 9 apple tree. Thus, the authors obtained, on average, 15 roots per explant when IBA was added and 8 roots when the NAA was added [48].

Thus, we studied non-hormonal nutrient medium *1/2QL* and supplemented with the IBA hormone to induce root growth in microshoots of *Malus niedzwetzkyana* in *in vitro* culture. As shown by the results (Table 5), on the 1st variant of the nutrient medium (½QL with IBA 1, 5 mg/l) the number of roots, on average, was 11.6 pcs. for microshoots on the 50th day of cultivation. The length of the roots was 2.88 cm, which is significantly more than the other 2 variants. On variant 2 (½QL with IBA 1.0 mg/l), the number of roots was 3.0 pcs. per explant, root length on average 0.42 cm. Variant 3 (½QL without hormones) showed the worst result. On this medium, on average, 2.0 pcs. root on the explant, whose length was 0.24 cm (table 5, figure 5).

Table 5. Optimization of the nutrient medium for the induction of rhizogenesis of *Malus niedzwetzkyana* in *in vitro* culture

Variant	The number of formed roots of microshoots and their length					
	Day 1		Day 25		Day 50	
	Roots, pcs	Length, cm	Roots, pcs	Length, cm	Roots, pcs	Length, cm
1 - ½QL with IBA 1,5 mg/l	-	-	7,2±4,26	0,66±0,04	11,6±7,60	2,88±0,92
2 - ½QL with IBA 1,0 mg/l	-	-	-	-	3,0±1,41	0,42±0,16
3 - ½QL without hormones	-	-	-	-	2,0±0,70	0,24±0,11

a ć Variant 1 (½QL with IBA 1,5 mg/l) b ć Variant 2 (½QL with IBA 1,0 mg/l) c ć Variant 3 (½QL without hormones)

Fig. 5. *Microshoots of Malus niedzwetzkyana* on the 50th day of cultivation on 3 variants of culture media for the induction of rhizogenesis

Thus, from the studied variants of the nutrient medium for inducing root growth, the optimal medium is ½*QL* with IBA of 1.5 mg/l. On this variant, a positive dynamics of root growth is observed throughout the entire cultivation period. Thus, a sufficient number of roots will increase the percentage of survival of microshoots during adaptation in ex vitro conditions.

CONCLUSION

The results of the studies made it possible to conclude that, an individual approach to optimizing cultivation conditions of micropropagation is necessary for each crop separately. Thus, as a result of experiments on the micropropagation of *Malus niedzwetzkyana*, the conditions for the sterilization of axillary buds, the composition of nutrient media for inducing the growth of the main shoot, the multiplication and rooting of the microshoots of the *Malus niedzwetzkyana* in *in vitro* culture were optimized.

Thus, to sterilize the axillary buds of *Malus niedzwetzkyana*, it is effective to use a 12% hydrogen peroxide solution for 4 minutes. In this mode, 80% of explants are viable for further propagation. To obtain the main microshoot in *in vitro* culture,

we recommend culturing the axillary buds on a *QL* culture medium with the addition of BAP 1.0 mg/l and kinetin 3.0 mg/l, where the percentage of regeneration reaches 90%. For the multiplication of microshoots, it is effective to transfer the main shoots to *QL* nutrient medium with the addition of a BAP of 0.5 mg/l, GA 1.0 mg/l, and IBA 0.01 mg/l. On this nutrient medium, on the 50th day of cultivation, the number of microshoots per explant is 24.8 pcs. The rooting of *Malus niedzwetzkyana* microshoots takes place best of all on nutrient medium ½*QL* with IBA of 1.5 mg/l, where the number of roots was 11.6 pcs. per explant.

Thus, optimized technology of micropropagation will allow to preserve and reproduce the rare and endangered species of *Malus niedzwetzkyana.*

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МИКРОКЛОНДЫҚ КӨБЕЙТУ АРҚЫЛЫ ЖОЙЫЛЫП БАРА ЖАТҚАН НЕДЗВЕЦКИЙ АЛМА АҒАШЫНЫҢ *(MALUS NIEDZWETZKYANA)* ТҮРЛЕРІН САҚТАП ҚАЛУ ЖӘНЕ КӨБЕЙТУ

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ТҮЙІН

Биологиялық алуантүрлілікті сақтау - табиғатты қорғаудағы маңызды міндеттердің бірі, өйткені биологиялық алуантүрлілік азық-түлік қауіпсіздігіне қол жеткізу және тамақтануды жақсартудың маңызды факторы болып табылады. Сонымен, Қазақстанның Қызыл кітабына өсімдіктердің 387 түрі енген. Осындай сирек кездесетін, эндемикалық және құрып кету қаупі төнген түрлердің бірі - Қазақстанның Қызыл кітабына және Халықаралық Қызыл тізімге енген *Malus niedzwetzkyana*. Бүгінгі күні өсімдіктердің осындай сирек кездесетін түрлерін сақтау үшін генетикалық материалды ұзақ уақыт сақтауға, сонымен қатар көбейтуге және көп көлемде көбейтуге мүмкіндік беретін биотехнологиялық әдістер белсенді қолданылады. Осындай тиімді әдістердің бірі – микроклондық көбейту. Бұл әдіс көбеюдің вегетативті әдісіне ұқсас және бүкіл процесс in vitro жағдайда жүретіндігімен ерекшеленеді. Алынған клондар аурулардан босатылған және генетикалық біртектілікті сақтайды. Ұсынылған жұмыста Недзвецкийдің алма ағашының микроклоналды көбейту технологиясын оңтайландыру нәтижесінде алынған нәтижелер сипатталған. Бастапқы материал ретінде Недзвецкий алма ағашының біржылдық өсінділерінің қолтық бүршіктері пайдаланылды. Қолтық бүршіктерді залалсыздандыру режимі, негізгі өркеннің өсуіне, микро өркендердің мультипликациясына мен тамырлануына ықпал ететін қоректік ортаның құрамы оңтайландырылды. Алынған мәліметтер сирек кездесетін және құрып кету қаупі төнген Недзвецкий алма ағашын көбейту үшін өзекті болып табылады.

Негізгі сөздер: *Malus niedzwetzkyana, in vitro* культурасы, микроклондық көбейту, өркендердің мультипликациясы, тамырландыру.

СОХРАНЕНИЕ И РАЗМНОЖЕНИЕ РЕДКОГО ИСЧЕЗАЮЩЕГО ВИДА ЯБЛОНИ НЕДЗВЕЦКОГО *(MALUS NIEDZWETZKYANA)* С ПОМОЩЬЮ МИКРОКЛОНАЛЬНОГО РАЗМНОЖЕНИЯ

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АБСТАКТ

Сохранение биологического разнообразия - одна из важнейших задач в охране природы, так как биоразнообразие является важным фактором в достижении продовольственной безопасности и улучшении питания. Так, в Красную книгу Казахстана занесено 387 видов растений. Одним из таких редких, эндемичных и исчезающих видов, является Яблоня Недзвецкого *(Malus niedzwetzkyana)*, занесенная в Красную книгу Казахстана и в Международный Красный лист.

На сегодняшний день, для сохранения таких редких видов растений активно применяются методы биотехнологии, которые позволяют сохранить генетический материал на длительный срок, а также воспроизвести и размножить в большом масштабе. Одним из таких эффективных методов - метод микроклонального размножения. Этот метод аналогичен вегетативному способу размножения и различаются лишь тем, что весь процесс протекает в условиях in vitro. Полученные клоны являются оздоровленными и сохраняют генетическую однородность. В представленной работе описываются результаты по оптимизации технологии микроклонального размножения яблони Недзвецкого. В качестве исходного материал были использованы пазушные почки однолетних побегов. Оптимизирован режим стерилизации пазушных почек, состав питательной среды для индукции роста основного побега, мультипликации и укоренения микропобегов. Полученные данные являются существенными для размножения и воспроизводства редкого и исчезающего вида яблони.

Ключевые слова: *Malus niedzwetzkyana*, культура *in vitro*, микроклональное размножение, мультипликация микропобегов, укоренение.