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РАЗРАБОТКА ПРОТОКОЛА МИКРОКЛОНАЛЬНОГО РАЗМНОЖЕНИЯ ФИСТАШКИ НАСТОЯЩЕЙ (*PISTACIA VERA* L.)

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ЧЫНЫГЫ МИСТЕ (PISTACIA VERA L.) МИКРОКЛОНАЛДЫК КӨБӨЙТҮҮ ПРОТОКОЛУН ИШТЕП ЧЫГУУ

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DEVELOPMENT OF A PROTOCOL FOR MICROCLONAL PROPAGATION OF *PISTACIA VERA* L.

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Аннотация. Кыргызстан и Казахстан характеризуются преобладанием аридных земель, где особенно актуально выращивание засухоустойчивых культур. К таким культурам относится произрастающая на их территории фисташка настоящая (*Pistacia vera* L.), обладающая высокой эколого-хозяйственной значимостью. Наиболее эффективным для массового получения растительного посадочного материала является микроклональное размножение. Первичным материалом для разработки протокола микроклонального размножения являлись зеленые, активно растущие черенки с 2-3 почками. Всего было введено 20 форм фисташки настоящей. Стерилизация от сапрофитной микрофлоры проводилась раствором «Белизна» (1:1) в течение 10 минут и 0,1% раствором $HgCl_2$ в течение 5 минут, после чего материал трижды промывали стерильной водой. Наилучшие результаты микроклонального размножения были достигнуты на среде MS с добавлением 1,0 мг/л 6-бензиаминопурина (БАП), 0,02 мг/л

индолил-масляной кислоты (ИМК). Полученные данные имеют важное значение для сохранения генофонда и получения качественного посадочного материала фисташки настоящей.

Ключевые слова: фисташка настоящая, асептические растения, микроклональное размножение, *in vitro*, зеленые побеги, стерилизация.

Аннотация. Кыргызстан жана Казакстан кургакчылыкка чыдамдуу өсүмдүктөрдү өстүрүү өзгөчө актуалдуу болгон ариддик жерлердин басымдуу болушу менен мүнөздөлөт. Мындай өсүмдүктөргө алардын аймагында өскөн, экологиялык-чарбалык мааниси жогору чыныгы мисте кирет. Өсүмдүк отургузуучу материалды массалык түрдө алуу үчүн эң натыйжалуусу микроклоналдык көбөйтүү болуп саналат. Микроклоналдык көбөйтүү протоколун иштеп чыгуу үчүн негизги материал 2-3 бүчүр менен жашыл, Активдүү өсүп жаткан кыюулар болгон. Бардыгы болуп чыныгы мистенин 20 формасы киргизилген. Сапрофиттик микрофлорадан Стерилизация «актык» (1:1) эритмеси менен 10 мүнөт жана 0,1% $HgCl_2$ эритмеси менен 5 мүнөт жүргүзүлгөн, андан кийин материалды стерилдүү суу менен үч жолу жууган. Микроклоналдык көбөйүүнүн эң жакшы натыйжалары 1,0 мг/л 6-бензиаминопурин (БАП), 0,02 мг/л индолил-бутир кислотасы (ИМК) кошуу менен МС чөйрөсүндө жетишилген. Табылгалар генофондду сактоо жана сапаттуу мисте отургузуучу материалды чыныгы алуу үчүн маанилүү мааниге ээ.

Негизги сөздөр: чыныгы мисте, асептикалык өсүмдүктөр, микроклоналдык көбөйтүү, скот, жашыл бутактар, стерилдөө.

Abstract. Kyrgyzstan and Kazakhstan are characterised by a predominance of arid lands. In such regions, the cultivation of drought-resistant crops is particularly important. One such crop is the pistachio (*Pistacia vera* L.). It grows in these territories and is of high ecological and economic importance. Microclonal propagation is the most effective method for large-scale production of planting material. The primary material for developing the microclonal propagation protocol was green, actively growing pistachio cuttings with 2-3 buds. A total of 20 forms of true pistachio were introduced. Sterilisation from saprophytic microflora was carried out with a solution of "Bleach" (1:1) for 10 minutes and a 0.1% solution of $HgCl_2$ for 5 minutes, after which the material was rinsed three times with sterile distilled water. The best results of microclonal propagation were achieved on Murashige and Skoog medium supplemented with 1.0 mg/L 6-benzylaminopurine (BAP) and 0.02 mg/L indole-3-butyric acid (IBA). The data obtained are important for preserving the gene pool and obtaining high-quality planting material of *Pistacia vera*.

Keywords: pistachio, aseptic plants, microclonal propagation, *in vitro*, green shoots, sterilisation.

Introduction

Pistacia vera L. is one of the most valuable nut crops due to its high nutritional, economic, and environmental significance, as well as its resistance to arid climates, sudden temperature changes, and ability to take root and grow in poor, rocky soils. This makes it particularly promising for cultivation in Kazakhstan, where about 60% of the land is classified as arid or semi-arid, as well as in Kyrgyzstan, with its mountainous terrain and limited access to water resources [1,2].

The natural habitats of the true pistachio are Central Asia (Kyrgyzstan, Kazakhstan, Uzbekistan, Tajikistan, and Turkmenistan), Afghanistan and Iran. Due to anthropogenic pressures and climate change, the area of natural pistachio forests shrinks each year. In Kazakhstan, this species is even included in the Red Book [3,4].

Pistachio fruits are rich in proteins, unsaturated fatty acids, vitamins and minerals. They also contain antioxidant compounds that reduce the risk of cardiovascular disease and improve metabolism. As a result, global demand for pistachios grows steadily. This crop is attractive from an economic point of view [5].

Scaling up pistachio cultivation in Kazakhstan and Kyrgyzstan is an opportunity to strengthen food security and create new export opportunities. Therefore, the development of pistachio plantations is of strategic importance [6,7].

However, traditional methods of pistachio propagation have serious limitations. Seed propagation does not preserve economically valuable

traits, while cuttings and grafting produce only a small amount of planting material. Therefore, biotechnological methods are becoming increasingly important, especially microclonal propagation, which provides opportunities for the mass and accelerated production of uniform, healthy plants with the preservation of the characteristics of the original forms, regardless of the season, under laboratory conditions [3,8].

This work is aimed at developing a protocol for the microclonal propagation of pistachio. This will make it possible to obtain healthy planting material for the creation of industrial plantations, as well as for the maintenance and restoration of wild populations.

Materials and methods

The object of the study was wild forms of pistachio (*Pistacia vera* L.) growing in southern Kyrgyzstan, in the Toskool-Ata Forestry, and in southern Kazakhstan, in the Sairam-Ugam State National Nature Park. As a result of the expedition, plus trees were selected and described according to the "Descriptor of pistachio (*Pistacia vera* L.)". The selection of plus forms was carried out according to the following economically valuable characteristics: ripening time, yield, absence of diseases and pests, shape and size of nuts, hardness and degree of shell opening [9]. One-year-old woody cuttings about 20 cm long were taken from the selected forms and then stratified at 4°C for 4-5 weeks. To obtain aseptic plants, the following were used: 1) one-year-old woody cuttings 3-4 cm long with 2-3 buds; 2) green actively growing shoots with 2-3 buds obtained from one-year-old woody cuttings in laboratory conditions. To sterilise the cuttings and green shoots, they were treated with a solution of bleach (1:1) for 10 minutes and 0.1% $HgCl_2$ for 5 minutes, followed by three rinses with sterile distilled water [10]. To detect latent infections, the bases of the shoots were planted and cultivated for 1-3 weeks at 23-25°C on a special VISS medium [11].

Optimisation of the concentration of phytohormones, 6-benzylaminopurine (BAP), indole-3-butyric acid (IBA) and gibberellic acid (GA) in the nutrient medium for introduction into *in vitro* culture and microclonal propagation was carried out on the basis of the Murashige and Skoog (MS) medium. The nutrient media were sterilised by autoclaving (UT KBS-150LV) for 25 minutes at 0.8-1.0 atm. Aseptic plants were cultivated at a temperature of 23-25°C, light intensity of 40 μmol m^{-2} s^{-1} and a photoperiod of 16 hours [12].

Results

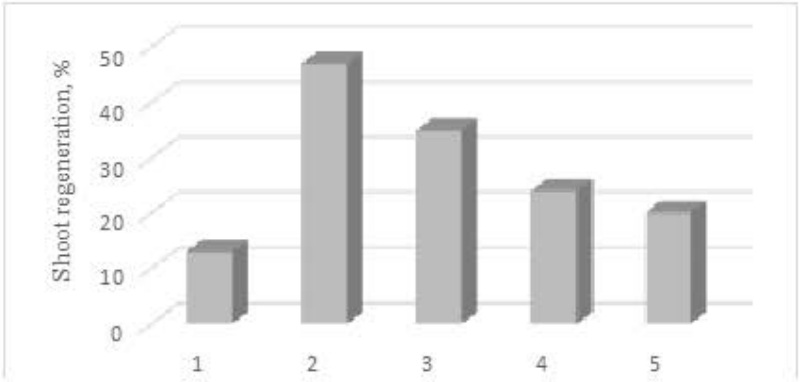
As a result of expeditions to the habitats of wild pistachio populations in southern Kyrgyzstan and Kazakhstan, 20 pistachio forms with valuable economic characteristics were selected. The coordinates and locations of sample collection are presented in Table 1.

Table 1 – Coordinates and locations of pistachio sample collection in Kyrgyzstan and Kazakhstan

Form	Toskool-Ata Forestry Enterprise, Kyrgyzstan	Sairam-Ugam National Nature Park, Kazakhstan
F1	41°05'35.78 072°35'33.46"	42° 40'04.91" 070 14'55.94"
F2	41°06'35.55" 072°36'09.02"	42° 40'04.87" 070 14'56.11"
F3	41°08'02.37" 072°37'37.46"	42° 40'07.40" 070 14'59.38"
F4	41°08'02.37" 072°37'37.46"	42° 40'07.45" 070 14'59.45"
F5	41°10'46.61" 072°29'34.93"	42° 40'09.53" 070 15'00.40"

F6	41°07'54.11" 072°37'55.37"	42° 40'09.52" 070 15'00.48"
F7	41°10'48.43" 072°29'35.25"	42° 40'04.89" 070 15'02.04"
F8	41°07'07.85" 072°38'15.88"	42° 40'09.81" 070 15'01.94"
F9	41°08'01.59" 072°40'35.34"	42° 40'12.99" 070 15'04.41"
F10	41°08'01.59" 072°40'35.34"	42° 40'12.93" 070 15'04.45"

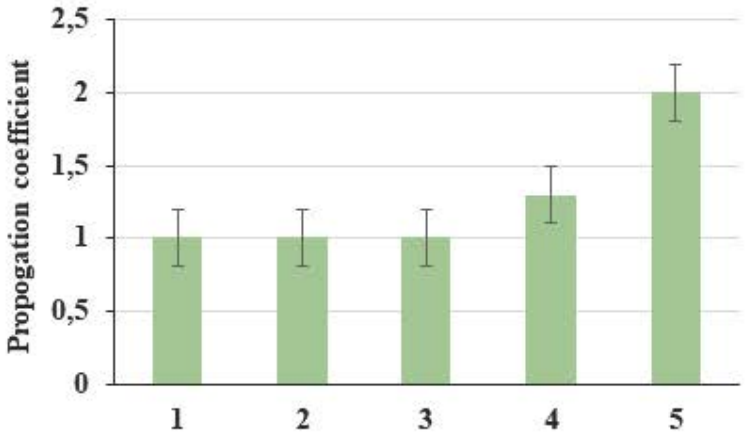
The method of obtaining aseptic plants using lignified annual cuttings did not yield positive results. The optimal method for obtaining aseptic plants was the use of green shoots with 2-3 buds, germinated after winter dormancy in laboratory conditions from woody cuttings. The optimal nutrient medium was MS without the addition of phytohormones, sucrose-30 g/l; pH-5.7 (Figure 1).



1. MS½, sucrose-30 g/l; pH-5.7
2. MS, sucrose 30 g/l; pH 5.7
3. MS, BAP-0.1 mg/l; GA-0.01 mg/l; sucrose-30 g/l; pH-5.7
4. MS, BAP-0.2 mg/l; GA-0.02 mg/l; sucrose-30 g/l; pH-5.7
5. MS, BAP-0.1 mg/l; GA-0.01 mg/l; sucrose-20 g/l; pH-5.7

Figure 1 – Effect of nutrient medium composition on the production of aseptic plants

Plants in which a hidden latent infection was detected on a special VISS medium were disposed of, and those free from bacterial and fungal contamination were transplanted to various nutrient media in order to select the optimal composition for microclonal propagation (Figure 2).



1. MS, BAP-0.1 mg/l; GA-0.01 mg/l; sucrose – 30 g/l; pH-5,
2. MS, BAP-0.5 mg/l; GA-0.01 mg/l; sucrose – 30 g/l; pH-5.7
3. MS, BAP-1.0 mg/l; GA-0.01 mg/l; sucrose – 30 g/l; pH-5.7
4. MS, BAP-1.0 mg/l; IBA-0.01 mg/l; sucrose – 30 g/l; pH-5.7
5. MS, BAP-1.0 mg/l; IBA-0.02 mg/l; sucrose – 30 g/l; pH-5.7

Figure 2 – Effect of nutrient medium composition on microclonal propagation of pistachio (average)

The optimal composition for microclonal propagation was MS medium, BAP 1.0 mg/l and IBA 0.02 mg/l, with a propagation coefficient of 2.0. The plants were bright green in colour and active shoot formation was observed.

CONCLUSION

As a result of the study, aseptic plants were obtained and propagated from 20 forms of the true pistachio. For sterilisation, it is optimal to use treatment with a solution of "Bleach" (1:1) for 10 minutes and 0.1% $HgCl_2$ for 5 minutes, followed by three rinses with sterile distilled water. The optimal medium for propagation is MS with the addition of 1.0 mg/l BAP, 0.02 mg/l IBA and 30 g/l sucrose.

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