https://10.31489/2025FEB3/89-98

UDC 602.6:58; 634.1/.7 Received: 24.04.2025 |Accepted: 16.06.2025 | Published online: 30 September 2025

N.K. Rymkhanova[1*], U.A. Manapkanova[2], N.V. Mikhailenko[3], S.V. Kushnarenko[4]

[1, 2, 3, 4]*Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan;*
[1, 2]*Al-Farabi Kazakh National University, Almaty, Kazakhstan*
[*]*Author for correspondence: nazka_0993@mail.ru*

Development of micropropagation protocol for production of elite blueberry planting material in Kazakhstan

This study presents an optimized protocol for micropropagation of commercially valuable highbush blueberry (*Vaccinium corymbosum* L.) varieties adapted to climatic conditions of Kazakhstan. An effective explant surface disinfection technique was established, providing a high percentage of aseptic plants. Various nutrient media with auxins (IBA, IAA) and cytokinins (zeatin, BAP) were evaluated for shoot proliferation and rooting. The optimal medium for micropropagation was identified as Woodt Plant Medium (WPM) with a double concentration of $Ca(NO_3)_2$, 2 mg/L zeatin, 30 g/L sucrose, 3.5 g/L agar, 1.5 g/L gelzan, pH 5.2. The most effective shoot rooting was achieved on half-strength WPM medium with 0.5 mg/L IBA, 30 g/L sucrose, and vermiculite (pH 5.2). *In vitro* rooted plants were successfully acclimatized to soil conditions by gradually reducing humidity in a controlled greenhouse environment at 20–23 °C. The developed micropropagation protocol enables rapid and mass propagation of elite blueberry varieties, ensuring genetic stability and minimizing the risk of pathogen infection. The results obtained contribute to the development of industrial blueberry cultivation in Kazakhstan and promote of modern biotechnological approaches in berry crop production.

Keywords: blueberry, *Vaccinium corymbosum* L., micropropagation, *in vitro* rooting.

Introduction

Highbush blueberry (*Vaccinium corymbosum* L.) belongs to the *Ericaceae* family and is a promising berry crop showing a steady growth in global consumption and expansion of cultivation areas [1-2]. In recent years, global blueberry production has been increasing at an average annual rate of 10 %, reaching nearly 2 million tons in 2023, more than double the five-year average. The high economic and biological value of blueberries is due to the rich content of biologically active substances, including polyphenols, combined with low caloric content and excellent taste [1]. Blueberry is used to strengthen blood capillaries, its positive effect on the thyroid gland has been revealed; berries have antisclerotic, anti-inflammatory and antitumor effects [3].

Based on information from Kazakhstan's Ministry of Agriculture, the current production of fruits and berries in the country is about 420 thousand tons, but more than 2.5 million tons are required to fully meet the needs of the population. In this regard, about 70 % of fruit and berry products are imported, which emphasizes the need to develop domestic production of berry crops, including blueberries.

Traditional methods of vegetative propagation (green and woody cuttings) are widely used in nursery farming, but their efficiency is limited by low rooting rate and accumulation of intracellular pathogens, which negatively affects the quality of berry products and leads to significant yield losses. In this regard, micropropagation is the most effective method that allows to quickly and massively obtain healthy and high-quality plants, reduce the risks of pathogen spread, maintain genetic homogeneity and obtain high-quality planting material.

Scientific research in the field of micropropagation of blueberry is actively carried out in the USA [4, 5], Russia [6], Belarus [3, 7], EU countries [8, 9]. In Kazakhstan, studies on micropropagation of blueberry were initiated at the Kazakh National Agrarian University, but they included only the development of the mode of sterilization of explants and selection of nutrient medium at the step of *in vitro* culture initiation, while the issues of propagation and *in vitro* shoots rooting, as well as obtaining planting material with a closed root system were not considered in this article [10].

The purpose of this work is to develop biotechnology of accelerated micropropagation of blueberry and obtaining elite planting material of promising for Kazakhstan berry culture for further implementation in the practice of nursery farming.

Materials and Research Methods

Plant Material and Conditions of In Vitro Culture. Commercially valuable blueberry varieties were used as research objects: Bluecrop, Blue Gold, Chandler, Duke, Legacy, Meader, Spartan (USA).

The *in vitro* culture method was used to initiation *in vitro* plant material and micropropagation of berry plants [11].

To initiate *in vitro* culture of blueberry varieties, shoots (15–20 cm long) from container-grown plants were washed with soapy water, rinsed, cut into 1.0–1.5 cm pieces, disinfected in 0.1 % mercuric chloride for 3–10 minutes, and rinsed three times with sterile distilled water. Microcuttings were placed in tubes with various variants of Woody Plant Medium (WPM) supplemented with growth regulators: zeatin, 6-benzylaminopurine (BAP), indole-3-butyric acid (IBA) and gibberellic acid (GA) (Table 1).Composition of WPM medium (mg/L): $NH_4NO_3$400, H_3BO_3 6.2, $CaCl_2$ 72.5, $Ca(NO_3)_2$386, $CuSO_4$•$5H_2O$ 0.25, Na_2 EDTA•$2H_2O$ 37.3, $FeSO_4$•$7H_2O$ 27.85, $MgSO_4$180.7, $MnSO_4$•H_2O 22.3, Na_2MoO_4•$2H_2O$ 0,25, $KH_2PO_4$170, $K_2SO_4$990, $ZnSO_4$•$7H_2O$ 8.6, Gelzan^{TM}1.5, Agar 3.5, pH 5.2 [12].

The number of green shoots, as well as shoots with bacterial and/or fungal contamination and necrosis were counted. After 4 weeks, *in vitro* green plants, without visible signs of contamination, were tested on selective medium 523 for the presence of endophytic bacteria [13]. Composition of the 523 medium: sucrose 10.0, casein hydrolysate 8.0, yeast extract 4.0, KH_2PO_4 2.0, $MgSO_4$•$7H_2O$ 0.15, Gelzan^{TM} 6.0, pH 6.9. Only aseptic plants were used for subsequent micropropagation. *In vitro* plants were cultured at 24 °C, 25 μmol•м$^{-2}$•с$^{-1}$light intensity, 16-h photoperiod, and transferred to fresh medium every 4 weeks.

Table 1

Variants of media used for blueberry micropropagation

Nutrientmedia	Growth regulators concentration(mg/L)	pH	Reference
Modified WPM with doubled concentration of Ca $(NO_3)_2$	Zeatin2.0	5.2	[14]
Standard WPM	Zeatin 2.0.+ IBA0.2	5.8	[15]
Standard WPM	BAP 0.1	5.2	-
Standard WPM	BAP 0.1 + GA 0.4	5.2	-
Standard WPM	BAP 0.05	5.2	-
Standard WPM	BAP 0.05 + GA 0.4	5.2	-

The multiplication rate was calculated by the formula (1):

$$Mr = a/b \bullet c, \quad (1)$$

a — the number of newly formed shoots

b — the number of shoots transferred for micropropagation

c — number of subcultures

Rooting of *in vitro* plants was carried out using WPM medium with vermiculite, with half the mineral salts, and with 0.5 mg/L IBA, pH 5.2. 10 g of agrotechnical vermiculite (BioMaster) were placed in culture vessels (Magenta boxes) and 85 ml of liquid WPM medium were poured in. The Magenta boxes prepared in this way were autoclaved at 0.8-1.0 atm for 20 min.

To obtain planting material, the *in vitro* plants with roots were transferred into soil substrate consisting of peat, chernozem and perlite in various combinations and transferred to the greenhouse. The effect of temperature and humidity in the greenhouse on plant establishment and adaptation was recorded.

The experiments were repeated in three replicates (n = 25–30). Data presented are means and standard deviations. Statistical analysis was performed according to generally accepted methods [16].

Results and Discussion

In vitro culture initiation and propagation of aseptic plants

The initiation of in vitro culture and obtaining aseptic plants represents the initial and essential step in developing micropropagation techniques. One of the important issues of this stage is the selection of effective disinfection of plant material introduced into in vitro culture, since the donor plants were grown in containers, i.e. in non-sterile conditions (Fig. 1).

Figure 1. Donor blueberry plants used for *in vitro* culture initiation

For disinfecting plant material, a 0.1 % solution of mercuric chloride was used. During the initiation of *in vitro* culture of blueberry varieties, it was observed that shoot apices were sensitive to this disinfectant. A 10-minute exposure resulted in complete necrosis of all explants. Reducing the treatment time to 7 minutes led to necrosis in 40–50 % of the apices. However, shortening the duration to 4 minutes significantly improved outcomes, promoting the development of green shoots. Under this treatment, bacterial and fungal contamination occurred in only 3.2–10.3 % of explants, necrosis ranged from 0 to 34.2 %, and green shoot formation was achieved in 39.4–93.7 % of explants, depending on the variety. The Duke variety showed the highest necrosis rate (34.2 %), while the Meader variety produced the highest number of green plants (93.7 %) (Fig. 2).

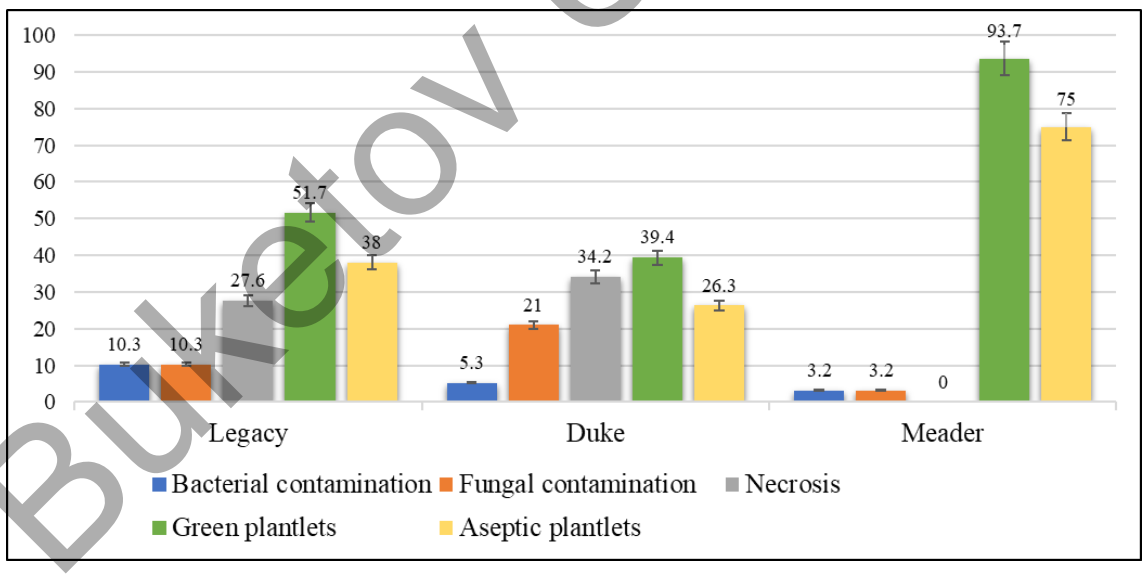
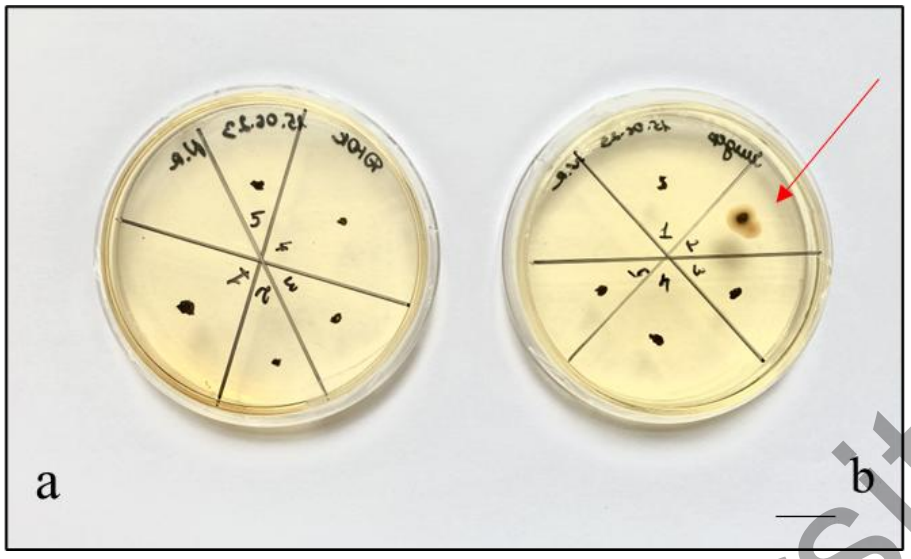


Figure 2. *In vitro* culture initiation results for the blueberry cultivars Legacy, Duke, and Meader

In vitro plant testing for endophytic contamination

An important stage of this study was to test plants for endophytic bacterial contamination. Four weeks after *in vitro* culture initiation, green plants without visible signs of contamination were tested on selective medium 523 to detect endophytic bacteria [13]. During passaging, basal parts of shoots were placed in Petri dishes with 523 medium. The development of bacterial contamination on selective medium 523 indicated endophytic contamination of in vitroplants (Fig. 3).



Arrows indicate bacterial contamination on the basal parts of in vitro shoots
Scale 1 cm.

Figure 3. Detection of endophytic bacterial contamination in tissues of Duke (a) and Meader (b) blueberry varieties on 523 selective medium

In vitro plants showing endophytic bacterial contamination were discarded. Following contamination screening, aseptic plants of seven blueberry varieties were successfully obtained. The proportion of aseptic plants ranged from 26.3 % to 75 %, depending on the variety, and these plants were suitable for further micropropagation (Fig. 4).

Scale 1 cm.

Figure 4. Aseptic shoots of Meader blueberry variety suitable for further micropropagation

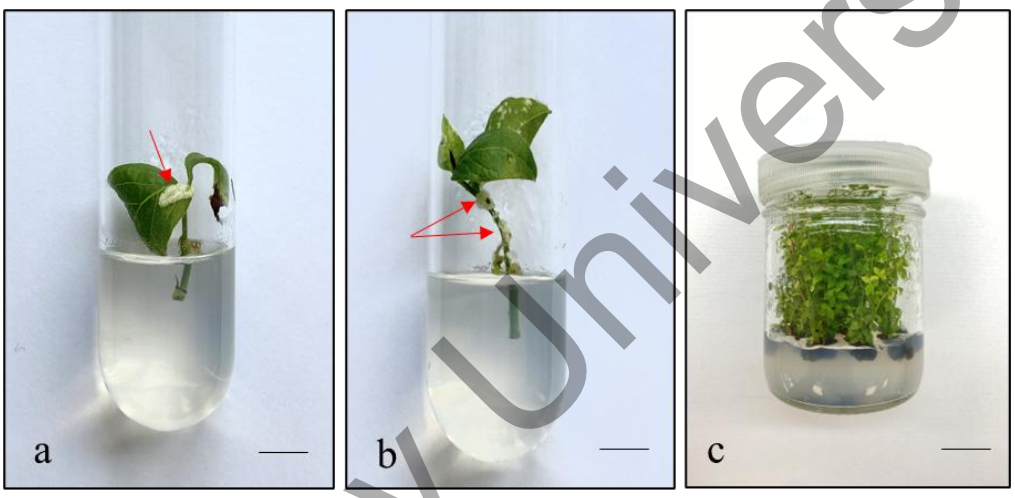
It is noteworthy that this method of testing plant material for endophytic contamination is not consistently applied in other studies [10, 14, 17–19]. However, this step is crucial for ensuring the production of healthy planting material and is also essential for successful cryopreservation and long-term storage of plant genetic resources in cryobanks.

 ISSN 3080-6836 (Print) ISSN 3080-6844 (Online)

Micropropagation of blueberries

An equally important stage in the micropropagation process is optimizing the composition of the nutrient medium. For blueberries, the goal of nutrient medium selection was to maximize the multiplication rate (Mr) and ensure optimal plant quality—characterized by bright green leaves, absence of pigmentation or necrosis on leaves and shoots, and no callus formation. Phytohormones as BAP, IBA, GA are the main growth regulators used in *in vitro* blueberry propagation [14, 17]. In studies conducted by Reed B.M., it was demonstrated that the use of modified WPM medium with double concentration of $Ca(NO_3)_2$ and supplemented with zeatin significantly enhanced plant quality and Mr compared to media containing BAP as cytokinin [14].

Six variants of nutrient media based on the WPM with various plant regulators—zeatin, BAP, IBA, and GA were tested for blueberry micropropagation (Table 1). The results showed that excessive callus formation on leaves and shoots was the main limiting factor in the micropropagation process. This issue was especially evident when using WPM medium supplemented with BAP, even at a low concentration of 0.1 mg/L (Fig. 5). Replacing BAP with zeatin at 2.0 mg/L significantly improved shoot quality, eliminated callus formation, and increased the multiplication rate (Mr) (Fig. 5).



Arrows indicate callus formation on leaves and shoots.
Scale1 cm.

Figure 5. Blueberry shoots of Blue Gold variety on WPM medium with 0.1 mg/L BAP (a, b), shoots of Meader variety on WPM medium with 2.0 mg/L zeatin (c)

The Mr of blueberry varieties on the modified WPM medium varied from 6.3 for the Spartan variety to 8.5 for the Meader variety, and on average for all varieties was 7.5 (Table 2).

Table 2

Multiplication rate, rooting percentage and adaptation percentage of blueberry *in vitro* plants

Cultivar	Mr*	Rooting**, %	Adaptation***, %
Bluecrop	6.6±0,89	96.3	92.0
Blue Gold	8.1±0.76	92.6	100
Chandler	7.2±0.53	100	96.6
Duke	8.1±0.31	96.7	96.6
Legacy	7.5±0.23	92.6	92.0
Meader	8.5±0.31	100	100
Spartan	6.3±0.31	88.9	88.0
Mean	7.5±0.82	95.3±3.37	95.0±3.74

*Note**. n = 30 (3 x 10)
*Note***. n = 27–30 (3 x 9-10)
*Note****. All rooted plants were transferred for adaptation, n = 25–30

In the article by V. Litwińczuk [17], micropropagation of blueberry was carried out in two stages, differing in the content of phytohormones. At the first stage, cytokinin zeatin (0.5-1.0 mg/L) was used during *in vitro* culture in 2-3 passages, which was replaced by cytokinin 2-isopentyladenine (2-iP) (5–10 mg/L) in subsequent propagation. This approach was used by the author to reduce the cost of purchasing the expensive hormone zeatin, although some authors [16] have noted that zeatin is a more suitable phytohormone for *in vitro* culture initiation and micropropagation of blueberry. In this study, a high Mr of blueberry cultivars was also achieved on medium with zeatin (Fig. 5, c).

In vitro rooted plants and adaptation to soil substrate

The aseptic blueberry plants obtained were propagated in sufficient numbers to carry out the subsequent shoot rooting step. The in vitro rooting process is a difficult step, especially for hard-to-root species. Several variants of nutrient medium with auxins: IBA and indole-3-acetic acid (IAA) were tested for rooting blueberries.

Earlier foreign studies have shown that *ex vitro* rooting can be carried out both without pretreatment with auxins and after a short-term immersion in the IBA solution [18]. This method helps lower the cost of plant propagation; however, its effectiveness is limited by a reduced root formation rate compared to in vitro rooting. Previous studies have shown that in vitro rooting reduces the risk of disease and enhances resistance to environmental stress factors [19].

In the present work, an improved method of rooting blueberry shoots was applied based on the use of vermiculite added to the medium instead of agar, which ensured a high percentage of rooting of blueberry *in vitro* shoots (Table 2), as well as higher adaptability of plants when transferred to a soil substrate (Fig. 6, a). The optimal medium for shoot rooting was determined to be half-strength WPM containing 0.5 mg/L IBA, 30 g/L sucrose and vermiculite at pH 5.2. The rooting percentage varied from 88.9 % for the Spartan variety to 100 % (Meader, Chandler), and the average for varieties was 95.3 % (Table 2).

The rooted plants in *in vitro* culture were transplanted into soil substrate consisting of mixture of black and brown peat: perlite (9:1). Within a week, plants were adapted to reduced humidity by removing the lids from the cultivation containers. Temperature and humidity were monitored daily in the greenhouse where the plants were adapted. The optimal temperature for transferring aseptic plants to soil is 20–23°C.

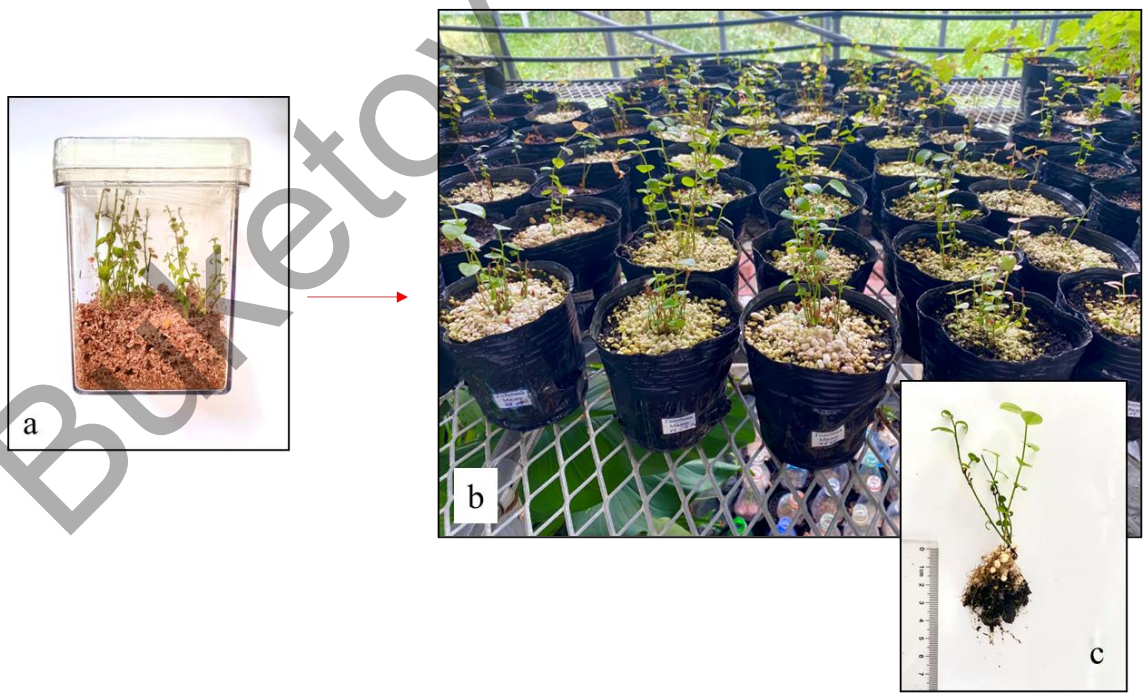


Figure 6. a) Shoot rooting on half-strength WPM medium containing 0.5 mg/L IBA, 30 g/L sucrose and vermiculite, pH 5.2. b) Blueberry plants in container culture in the greenhouse c) rooted blueberry plant (Meader variety)

 ISSN 3080-6836 (Print) ISSN 3080-6844 (Online)

Due to the fact that blueberry grows best in peat soils, the following composition of soil substrate was used: a mixture of black and brown peat: perlite (9:1), a small depression was made in it for the volume of the root system and filled with perlite, where the plant was transplanted. As a result, on average 95.0 % of blueberry plants adapted to the soil substrate (Table 2).

Based on the conducted research, a protocol for accelerated *in vitro* propagation of plants of commercially valuable blueberry varieties was developed (Fig. 7)

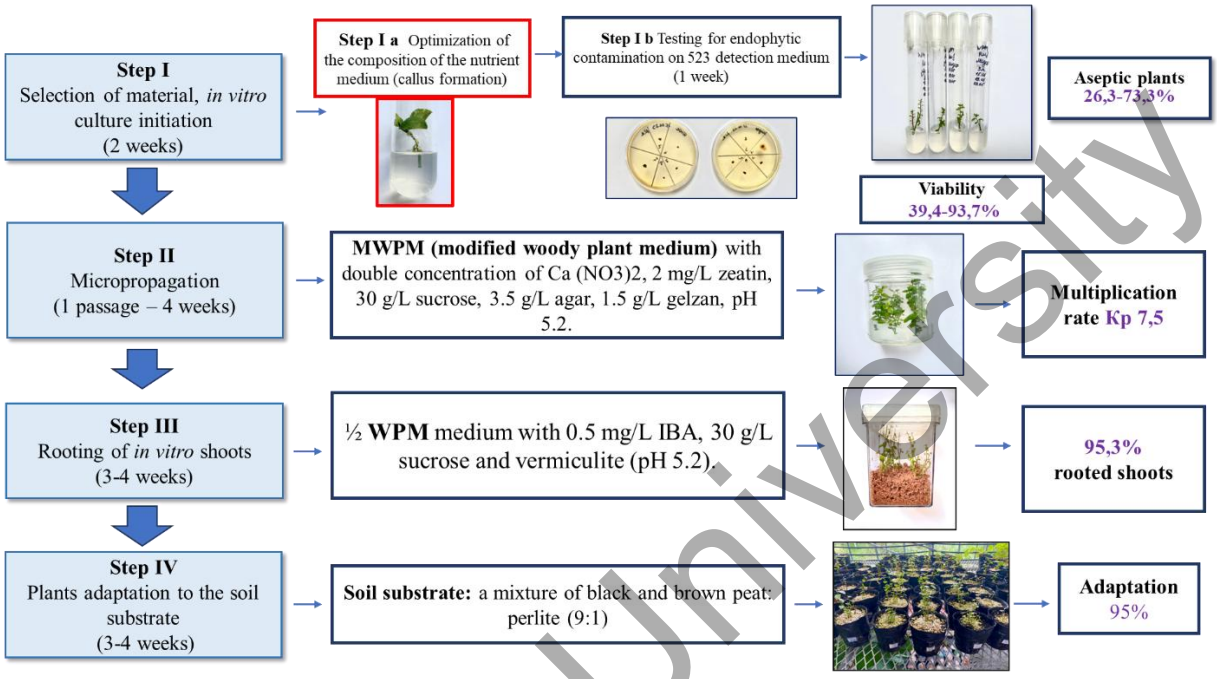


Figure 7. Protocol for micropropagation of blueberry varieties

The developed protocol for accelerated *in vitro* propagation of commercially valuable blueberry varieties includes a number of consecutive stages: selection of plant material, *in vitro* culture initiation of shoot apices, disinfection from epiphytic and endophytic fungal and bacterial contamination, micropropagation and rooting of *in vitro* shoots, *in vitro* plants adaptation to soil substrate

Conclusion

As a result of this research, an effective method of micropropagation of highbush blueberry (*Vaccinium corymbosum* L.) has been developed. Disinfection treatment for explants were optimized, providing a high percentage of aseptic plants. The most effective nutrient medium for micropropagation and rooting was established, which allowed to obtain healthy planting material with a high degree of rooting.

The developed technologies allow rapid and mass multiplication of promising blueberry varieties, reducing the risk of pathogen spread and increasing the genetic homogeneity of plants. The results obtained can be used for industrial cultivation of blueberries and introduction of modern biotechnological approaches in berry growing in Kazakhstan.

Acknowledgments

Thanks to Dr. Tadeusz Kusibab (https://in-vitro.pl/ru/) for providing blueberry source plant material.

The work was carried out with the financial support of the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan in the framework of grant financing (IRN project AR19676481)

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Rymkhanova N.K.** – Conceptualization, Methodology, Investigation, Writing – original draft; **Manapkanova U.A.** – Investigation (micropropagation); **Mikhailenko N.V.** –

Investigation (rooting and plant adaptation); **Kushnarenko S.V.** – Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

References

1 Brazelton C. World blueberry statistics and global market analysis / C. Brazelton, K. Young // Spring Preview for GBC. International Blueberry Organization, 2017.

2 Podymniak M. Zbioryborówkinafiniszu [Electronic resource] / M. Podymniak // Jagodnik. — 2015. — Access mode: https://jagodnik.pl 12.08.2021.

3 Сидоренко Т. Н. Особенности микроклонального размножения и укоренения голубики высокорослой / Сидоренко Т. Н., Левзикова Е. Г. // Плодоводство. — 2022. — Т. 32. — № . 1. — С. 154–158.

4 Uchendu E. Desiccation tolerance and cryopreservation of *in vitro* grown blueberry and cranberry shoot tips / E. Uchendu, B. M. Reed // Acta Horticulturae. — 2009. — Vol. 810. — P. 567–574.

5 Reed B. M. Propagation of blackberries and related *Rubus* species / B. M. Reed, S. Poothong, H. K. Hall // In: Blackberries. Hall H. K. & Funt R. C., Eds., CABI: Wallingford, UK, 2017. — P. 101–112.

6 Стахеева Т. С. Некоторые аспекты размножения *in vitro* перспективных сортов высокой и полувысокой голубики / Т. С. Стахеева, О. И. Молканова, Л. Н. Коновалова // Плодоводство и ягодоводство России. — 2017. — Т. 48. — С. 279–285.

7 Кухарчик Н. В. Размножение плодовых растений в культуре *in vitro* / Н. В. Кухарчик, М. С. Кастрицкая, Е. В. Семенас, С. Э. Колбанова, Т. А. Красинская, Н. Н. Волосевич, О. В. Соловей, А. А. Змушко, Т. Н. Божидай, А. П. Рундя, А. М. Малиновская. — Минск: Беларуская навука, 2016. — 208 с.

8 Ružić D. Micropropagation *in vitro* of highbush blueberry (*Vaccinium corymbosum* L.) / D. Ružić, T. Vujović, G. Libiakova, R. Cerović, A. Gajdošova // Journal of Berry Research. — 2012. — Vol. 2. — P. 97–103.

9 Ružić D. Micropropagation as means of rapid multiplication of newly developed blackberry and black currant cultivars / D. Ružić, T. Lazić // Agriculturae Conspectus Scientificus. — 2006. — Vol. 71(4). — P. 149–153.

10 Алимбекова Б. Е. Микроклональное размножение голубики высокой *Vaccinium corymbosum* L. / Б. Е. Алимбекова, Г. А. Кампитова, Л. С. Ерболова, Р. К. Даминова, Т. К. Егизбаева, М. Д. Есеналиева // Известия Национальной Академии наук Республики Казахстан. — 2016. — Vol. 2. — № 32. — С. 5–11.

11 Plant Tissue Culture, Development, and Biotechnology / Eds. R.N. Trigiano and D.J. Gray. — USA: CRC Press. Taylor & Francis Group, 2011. — 583 p.

12 Lloyd G. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture / G. Lloyd, B. Mc Cown // Comb. Proceed. Int. Plant Prop. Soc. — 1980. — Vol. 30. — P. 421–427.

13 Viss P. R. A simplified method for the control of bacterial contamination in woody plant tissue culture / P. R. Viss, E. M. Brooks, J. A. Driver // In Vitro Cellular & Developmental Biology. — 1991. — Vol. 27. — P. 42.

14 Reed, B. M. (1991). The use of zeatin to initiate *in vitro* cultures of Vaccinium species and cultivars / B. M. Reed, A. Abdelnour-Esquivel // HortScience. — Vol. 26. — No. 10. — P. 1320–1322.

15 Figiel-Kroczyńska M. Efficient micropropagation protocol of three cultivars of highbush blueberry (*Vaccinium corymbosum* L.) / M. Figiel-Kroczyńska, M. Krupa-Małkiewicz, I. Ochmian // Notulae Botanicae Horti Agrobotanici Cluj-Napoca. — 2022. — Vol. 50. — No. 4. Article 12856. DOI: https://doi.org/10.15835/nbha50412856.

16 Лакин Г. Ф. Биометрия: Учебное пособие для биол. спец. вузов / 4-е изд., перераб. и доп. — М.: Высш. школа, 1990. — 352 с.

17 Litwińczuk W. Micropropagation of *Vaccinium* sp. by *in vitro* axillary shoot proliferation / W. Litwińczuk // Methods in Molecular Biology. — 2013. — Vol. 11013. — P. 63–76.

18 Georgieva M. *In vitro* propagation of Vaccinium corymbosum L. / M. Georgieva, V. Kondakova // Bulgarian Journal of Agricultural Science. — 2021. — Vol. 27. — P. 323–327.

19 Correia S. Advances in blueberry (*Vaccinium* spp.) *in vitro* culture: A review / S. Correia, M. Matos, F. Leal // Horticulturae. — 2024. — Vol. 10. — No. 533. — P. 2–21.

Н.К. Рымханова, У.А. Манапканова, Н.В. Михайленко, С.В. Кушнаренко

Қазақстанда жоғары сапалы көкжидек көшеттерін өндіру үшін микроклоналды көбейту әдісін әзірлеу

Зерттеуде Қазақстанның климаттық жағдайларына бейімделген, шаруашылықта құнды биік бұталы көкжидек (*Vaccinium corymbosum* L.) сорттарының микроклоналды көбейтуге арналған оңтайландырылған протоколы ұсынылды. Жұмыс барысында эксплантты тиімді зарарсыздандыру әдістері әзірленіп, жоғары пайыздық асептикалық өсімдіктер қамтамасыз етілді. Өскіндерді микроклоналды көбейту және олардың тамырлануын қамтамасыз ету үшін ауксиндер (ИМК, ИУК)

мен цитокининдер (зеатин, БАП) қосылған түрлі қоректік орталар сыналды. Микроклоналды көбейту үшін оңтайлы орта ретінде қосарланған мөлшердегі $Ca(NO_3)_2$, 2 мг/л зеатин, 30 г/л сахароза, 3,5 г/л агар, 1,5 г/л джелрайт және pH 5,2 болатын WPM ортасы анықталды. Өскіндерді тамырландыруда ең жақсы нәтижелер ½ WPM ортасында, 0,5 мг/л ИМК, 30 г/л сахароза және вермикулит (pH 5,2) қолданылған жағдайда алынды. *In vitro* тамыры бар өсімдіктер 20–23°C температурада бақыланатын жылыжай ортасында ылғалдылықты біртіндеп төмендету арқылы топырақ жағдайына сәтті бейімделді. Әзірленген протокол көкжидектің элиталық сұрыптарын жаппай әрі жедел көбейтуге мүмкіндік береді, генетикалық тұрақтылықты қамтамасыз етеді және патогендермен зақымдану қаупін азайтады. Бұл нәтижелер Қазақстанда көкжидекті өнеркәсіптік өсіруді дамытуға және жидек шаруашылығында заманауи биотехнологиялық әдістерді енгізуге өз үлесін қосады.

Кілт сөздер: көкжидек, *Vaccinium corymbosum* L., микроклоналды көбейту, *in vitro* тамырландыру.

Н.К. Рымханова, У.А. Манапканова, Н.В. Михайленко, С.В. Кушнаренко

Разработка способа микроклонального размножения для производства элитного посадочного материала голубики в Казахстане

В данном исследовании представлен оптимизированный протокол микроклонального размножения коммерчески ценных сортов голубики высокорослой (*Vaccinium corymbosum* L.), адаптированных к климатическим условиям Казахстана. Разработан эффективный способ дезинфекции эксплантов, обеспечивающий высокий процент асептических растений. Были протестированы различные питательные среды с добавлением ауксинов (ИМК, ИУК) и цитокининов (зеатин, БАП) для микроклонального размножения побегов и их укоренения. Оптимальная среда для микроклонального размножения оказалась среда WPM с удвоенной концентрацией $Ca(NO_3)_2$, 2 мг/л зеатина, 30 г/л сахарозы, 3,5 г/л агара, 1,5 г/л джелрайта, pH 5,2. Наилучшие результаты по укоренению побегов были получены при использовании WPM среды с половинной концентрацией минеральных компонентов, с 0,5 мг/л ИМК, 30 г/л сахарозы и вермикулитом (pH 5,2). Укоренённые *in vitro* растения были успешно акклиматизированы к почвенным условиям путем постепенного снижения влажности в контролируемой тепличной среде при температуре 20–23 °C. Разработанный протокол позволяет быстро и массово размножать элитные сорта голубики, обеспечивая генетическую стабильность и минимизируя риск заражения патогенами. Полученные результаты способствуют развитию промышленного выращивания голубики в Казахстане и внедрению современных биотехнологических методов в ягодоводство.

Ключевые слова: голубика, *Vaccinium corymbosum* L., микроклональное размножение, укоренение *invitro*.

References

1 Brazelton, C. & Young, K. (2017). *World blueberry statistics and global market analysis*. Spring Preview for GBC. International Blueberry Organization.

2 Podymniak, M. (2015). Zbioryborówkinafiniszu. *Jagodnik*. Retrieved from https://jagodnik.pl 12.08.2021

3 Sidorenko, T. N. & Levzikova, E. G. (2022). Osobennosti mikroklonalnogo razmnozheniia i ukorenenia golubiki vysokorosloi [Features of micropropagation and rooting of highbush blueberry]. *Plodovodstvo — Pomology, 32*(1), 154–158 [in Russian].

4 Uchendu, E., & Reed, B. M. (2009). Desiccation tolerance and cryopreservation of *in vitro* grown blueberry and cranberry shoot tips. *Acta Horticulturae, 810*, 567–574.

5 Reed, B. M., Poothong S., & Hall, H. K. (2017). Propagation of blackberries and related *Rubus* species. In: *Blackberries*, 101–112. Hall H.K. & Funt R.C., Eds., CABI: Wallingford, UK.

6 Stakheeva, T. S., Molkanova, O. I., & Konovalova, L. N. (2017). Nekotorye aspekty razmnozheniia *in vitro* perspektivnykh sortov vysokoi i poluvysokoi golubiki [Certain aspects of *in vitro* propagation of promising highbush and half-highbush blueberry cultivars]. *Plodovodstvo i yagodovodstvo Rossii — Fruit Growing and Berry Growing of Russia, 48*, 279–285 [in Russian].

7 Kukharchik, N. V., Kastritskaya, M. S., Semenas, S. E., Kolbanova, E. V., Krasinskaya, T. A., Volosevich, N. N., Solovey, O. V., Zmushko, A. A., Bozhidai, T. N., Rundya, A. P., & Malinovskaya, A. M. (2016). *Propagation of fruit crops in vitro culture*. Minsk: Belarusian Science [in Russian].

8 Ružić, D., Vujović, T., Libiakova, G., Cerović, R., & Gajdošova, A. (2012). Micropropagation *in vitro* of highbush blueberry (*Vaccinium corymbosum* L.). *Journal of Berry Research, 2*, 97–103.

9 Ružić, D., & Lazić, T. (2006). Micropropagation as means of rapid multiplication of newly developed blackberry and black currant cultivars. *Agriculturae Conspectus Scientificus, 71*(4), 149–153.

10 Alimbekova, B. E., Kampitova, G. A., Yerbolova, L. S., Daminova, R. K., Yegizbayeva, T. K., & Yesenaliyeva, M. D. (2016). Mikroklonalnoe razmnozhenie golubiki vysokoi *Vaccinium corymbosum* L. [Micropropagation of

Highbush Blueberry *Vaccinium corymbosum* L.]. *Izvestiia Natsionalnoi Akademii nauk Respubliki Kazakhstan — Proceedings of the National Academy of Sciences of the Republic of Kazakhstan, 2*(32), 5–11 [in Russian].

11 Trigiano, R. N., & Gray, D. J. (Eds.). (2011). *Plant Tissue Culture, Development, and Biotechnology*. USA: CRC Press. Taylor & Francis Group.

12 Lloyd, G., & McCown, B. (1980). Commercially feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture. *Comb. Proceed. Int. Plant Prop. Soc., 30*, 421–427.

13 Viss, P. R., Brooks, E. M., & Driver, J. A. (1991). A simplified method for the control of bacterial contamination in woody plant tissue culture. *In Vitro Cellular & Developmental Biology, 27*, 42.

14 Reed, B. M. & Abdelnour-Esquivel, A. (1991). The use of zeatin to initiate *in vitro* cultures of *Vaccinium* species and cultivars. *HortScience, 26*(10), 1320–1322.

15 Figiel-Kroczyńska, M., Krupa-Małkiewicz, M., & Ochmian, I. (2022). Efficient micropropagation protocol of three cultivars of highbush blueberry (*Vaccinium corymbosum* L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 50*(4), Article 12856. https://doi.org/10.15835/nbha50412856

16 Lakin, G. F. (1990). *Biometry: A Textbook for Biology Students* / 4th ed., revised and supplemented. Moscow: Vysshaia Shkola [in Russian].

17 Litwińczuk, W. (2013). Micropropagation of *Vaccinium* sp. by *in vitro* axillary shoot proliferation. In *Methods in Molecular Biology, 11013*, 63–76).

18 Georgieva, M. & Kondakova, V. (2021). *In vitro* propagation of *Vaccinium corymbosum* L. *Bulgarian Journal of Agricultural Science, 27,* 323–327.

19 Correia, S., Matos, M., & Leal, F. (2024). Advances in blueberry (*Vaccinium* spp.) *in vitro* culture: A review. *Horticulturae, 10*(533), 2–21.

Information about the authors

Rymkhanova Nazgul Kabdulakyzy *(contact person)* — PhD student, Researcher of the Laboratory of Germplasm Cryopreservation, Institute of Plant Biology and Biotechnology, the Committee of Science of the Ministry of Science and Higher Education, Almaty; e-mail: nazka_0993@mail.ru

Manapkanova Ulzhan Aitmukhametkyzy — PhD student, Junior Researcher of the Laboratory of Germplasm Cryopreservation, Institute of Plant Biology and Biotechnology, Committee of Science, Ministry of Science and Higher Education, Almaty; e-mail: ulzhan.manapkanova@mail.ru

Mikhailenko Natalya Vyacheslavovna — Laboratory Assistant of the Laboratory of Germplasm Cryopreservation, Institute of Plant Biology and Biotechnology, Committee of Science, Ministry of Science and Higher Education, Almaty; e-mail: georgi-nata@mail.ru

Kushnarenko Svetlana Veniaminovna — Professor, Candidate of Biological Sciences, Head of the Laboratory of Germplasm Cryopreservation, Institute of Plant Biology and Biotechnology, Committee of Science, Ministry of Science and Higher Education, Almaty; e-mail: svetlana_bio@mail.ru