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Research Article

IN VITRO PROPAGATION OF *VACCINIUM ULIGINOSUM* L. IN MONGOLIA

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ABSTRACT. Mongolia harbors valuable populations of bog blueberry (*Vaccinium uliginosum* L.), known as blueberry in Mongolia, which is ecologically and medicinally significant and nutritionally rich. Blueberries are considered a "superfood" worldwide due to their numerous health benefits beyond their vitamin and mineral content. They possess strong antioxidant properties that neutralize free radicals, slow down aging, protect cells from damage, and enhance memory, concentration, and the nervous system, and they contain high levels of flavonoids and anthocyanins that contribute to vascular health. Nonetheless, growing public knowledge of blueberries' health advantages has fueled a greater demand for their production and everyday consumption in recent years. However, compared to other countries located at similar latitudes, Mongolia has a relatively cold climate, which limits the successful cultivation of imported blueberry varieties under such harsh environmental conditions. Although the country has abundant bog blueberry resources, climate change has led to periodic declines in yield, changing the amount available for harvest. Therefore, consumers have shown a growing interest in cultivating blueberry seedlings to meet their needs. This study aimed to investigate the possibility of producing disease-free Mongolian bog blueberry plantlets suitable for Mongolian climatic conditions through plant biotechnology methods and to establish an in vitro propagation protocol. The results demonstrated that viable micropropagated plant materials could be obtained from bog blueberry seeds and successfully cultured via nodal culture on a ¼ MS medium supplemented with 0.6 mg·L^{-1} BA and 1.5 mg·L^{-1} NAA. The resulting plantlets were then acclimatized in a sphagnum peat moss substrate (pH 5.5), confirming the feasibility of producing seedlings with a 75.0% survival rate. Thus, this study may provide valuable knowledge for enhancing and conserving bog blueberry biological resources and support the potential propagation and cultivation of blueberry varieties in Mongolia.

Keywords: *Bog blueberry, in vitro propagation, nodal culture, acclimatization*

INTRODUCTION

Three species of *Vaccinium* spp. (*V. uliginosum* L., *V. myrthillus* L., and *V. vitis-idaea* L.) grow in Mongolia [1]. Blueberry is considered a "superfood" because it is highly beneficial for health in addition to providing basic nutrition. It is particularly rich in anthocyanins, which are antioxidants and antiglycooxidants, and thus form a significant group of functional foods very beneficial for human health [2]. However, the comparative study suggested that *V. uliginosum* exceeded a "lowbush blueberry" sample in anthocyanin/phenolic content [3]. Furthermore, another study suggested that *bog blueberry* had strong antioxidant activity (DPPH, superoxide radical) with IC_{50} values around 33 µg/mL for the anthocyanin-rich fraction [4]. In Mongolia, bog blueberry grows in the taiga zones of the Khentii, Khuvsgul, Khangai, and Mongolian Dagur regions, in mixed forests, forest openings, and shrublands. They form 0.5–1-meter-high shrubs

with oval-shaped leaves [1]. Mongolians have long used bog blueberry in food and drinks to stop diarrhea, treat stomach and intestinal inflammation, and lower fever. Bog blueberry is also known to improve appetite, enhance gastric and pancreatic secretions, stimulate gastric peristalsis, strengthen capillary and blood vessel walls, and act as bile stimulants, diuretics, anti-inflammatories, and ulcer-healing agents [5]. They contribute to immune support, allergy relief, vision improvement, and overall health enhancement. Additionally, bog blueberries are low in calories, rich in antioxidants and phytonutrients, and have a slightly stronger and more sour taste compared to true blueberries. Currently, blueberries are cultivated commercially in more than 30 countries across various climatic zones, and global production of fresh and processed blueberries has doubled over the past decade [6]. Mongolia's climate, with mean annual temperatures ranging from -6.2°C in the North to around +4°C in the Gobi Desert, is often colder than that of other countries at similar latitudes due to high altitude. Plant communities in Mongolia have evolved and adapted to sustained grazing pressure from wild and domesticated animals [7]. There are sufficient biological resources of bog blueberry in Mongolia, but commercial cultivation or breeding into improved varieties has not yet been conducted. Since the natural yield of blueberries varies from year to year, this study was conducted to determine whether it was possible to rapidly produce seedlings of bog blueberry using a plant biotechnological method, regardless of the season.

Cutting propagation of blueberry may not be an efficient way to rapidly increase the quantity of starting materials or propagules for the commercial cultivars and may not be appropriate for all cultivars due to low rooting percentages [8]. In addition to having a major negative influence on agricultural production and product quality, diseases caused by plant pathogens, including viruses, viroids, and phytoplasmas, also make it more difficult for plant materials to be transported safely over international boundaries [9]. One of the greatest techniques for rapid propagation of disease-free, strong, healthy, and genetically identical planting materials is micropropagation [10], which enables multiplying propagules quickly all year long [11]. The study noted that conventional propagation techniques are labor-intensive, slow, and heavily reliant on the seasons and weather. There were significant variations between normally propagated and in vitro plants in a number of metrics, such as growth vigor, branching, fluorescence, chlorophyll content, and DNA methylation levels, and the results indicated that conventionally grown plants produced fruits with higher levels of antioxidant compounds. However, in vitro propagation produced plants with increased growth vigor and branching, higher chlorophyll content and different photosynthetic efficiency compared to conventionally propagated plants [8]. Genotype, the amount of plant growth hormones in the medium, the concentration of media components such as vitamins, salts, and minerals, and incubation conditions, including temperature and light, all affect effective propagation [13]. Therefore, this study aimed to assess the feasibility of propagating Mongolian bog blueberry and to establish an effective plant tissue culture protocol for this species, which would facilitate the production of disease-free planting material suitable for commercial cultivation.

MATERIALS AND METHODS

Plant Sample

Bog blueberry seeds were collected from Tosontsengel soum, Zavkhan aimag and used in this study. (Fig. 1). Seeds were kept frozen at -20 °C until use in the experiment. The blueberry fruits were spherical, measuring 10 ± 1.6 mm in diameter. They were juicy, strongly sweet but slightly sour, and exhibited a deep reddish-purple coloration with a soft, smooth epidermis. All

experiments were carried out from March. of 2025 to Nov. of 2025 at Laboratory for Virus-Free Potato Minituber Production, Food, Agriculture and Light Industry Research and Development Center.

Fig. 1. *Seed of Bog blueberry*

Sterilization protocol

Seeds were separated from the fruit flesh, washed thoroughly, sterilized with 70% ethanol for 1 min, then with 20% NaOCl for 5 min, and rinsed with sterile distilled water three times. Sterilized seeds were then transferred to medium or soil substrates. The sphagnum peat moss soil and watered seed-starting sponge (SSS) were applied as soil substrates for the evaluation of seed germination rate.

Medium preparation and cultivation conditions

In this study, we applied 2-4-fold diluted MS (Murashige Skoog, 1962) medium containing 2% sucrose, 0.8% agar, and pH was adjusted to 5.5 with the addition of a combination of two hormones: 6-benzylaminopurine (BA) and 1-Naphthaleneacetic acid (NAA), as shown in Table 1. In vitro plants were maintained under a PPFD of 40 µmol photons m^{-2} s^{-1} with cycles of 16 h light at 24.0 °C and 8 h dark at 20.0 °C in a growth room.

Acclimatization of in vitro plants

The sphagnum peat moss soil substrate (Klasmann Deilmann GMBH, Germany) with pH 5.5, NPK (nitrogen, phosphorus, and potassium) fertilizer (14:10:18) 1.0 kg/m^3, and electrical conductivity of 35 mSm (+/-25%) was applied for plantlet acclimatization with a comparison of its mixture with sterilized sand (1:1). Regardless of the nutrient level and hormonal combination in the medium in which the in vitro plants were grown separately, 200 in vitro plants were transferred into the soil substrates of equal size twice and measured for survival rate. The survival rate in ex vitro conditions was determined after 4 weeks. Plantlets were watered twice a week and grown under a PPFD of 40 µmol photons with cycles of 16 h light at 24.0°C and 8 h dark at 20.0°C in a growth room.

Table 1. *Medium treatments for plant proliferation*

Medium treatments		No phytohormone	Phytohormones							
			BA (mg L^{-1})				NAA (mg L^{-1})			
			0.3	0.6	1.0	1.5	0.1	0.5	1.0	1.5
1/2 MS	1									
1/3 MS	2									
1/4 MS	3									
1/4 MS	4									
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Statistical analyses

An independent samples T-test was used to compare the mean values between the experimental and control groups at the 0.05 level of significance.

RESULTS AND DISCUSSION

Seed Germination

Seeds, kept at -20 °C, were transferred to 4 °C and kept for 7 days to protect from sudden heat shock before use in the experiment. Firstly, seeds were treated with a concentration of 500 ppm GA_3 (Gibberellin A3) solution for 24 hours and then sterilized, transferred into prepared 1/4 MS media, and grown for 4 weeks. Also, seeds were planted in the soil substrates to compare its germination. Seeds were germinated with a rate of 78.1% in ¼ MS medium, and the hormone concentration in the medium did not have a significant effect on germination rate (Table 1).

Table 1. *Germination of Blueberry Seeds*

Substrate	No treatment	Treated with GA_3
Soil	0%	5.0 %
SSS	2.0 %	10.0 %
1/4 MS	2.0 %	78.1%

Research studies showing that plant hormones, especially gibberellic acid (GA_3), can help break seed dormancy in combination with cold stratification in blueberry and related species**.** For example, applying GA_3 together with cold stratification significantly increased seed emergence for sparkleberry seeds. Seeds treated with combinations of 500–1000 mg L^{-1} GA_3 and 3–9 weeks cold stratification reached 61–70 % emergence, whereas untreated seeds did not germinate over the same period. Harutyunyan et al. reported germination rates 74.2-88.3% for wild blueberry species (*V. myrtillus* L. and *V. uliginosum* L.) after 8 weeks of cold stratification followed by in vitro culture [14]. Correia et al. (2024) observed that in vitro seed germination of *V. myrtillus* L. reached 87.5% after optimizing growth condition (22.5 °C) [15].

In our study, frozen seeds (-20 °C) were stratified at 4 °C for one week prior to use. Although one week of stratification represented a relatively short period for breaking dormancy, we observed that treatment with hormones for 24 hours markedly enhanced seed germination. This indicated that combining cold stratification with hormonal treatment can effectively accelerate seed germination within a short period. In contrast, seeds treated similarly and sown in soil substrate exhibited very low germination rates (10.0 %), further confirming that in vitro culture provided optimal conditions for bog blueberry seed germination (78.1 %).

In vitro plantlets and nodal culture

When the growth of in vitro plants from seed material was evaluated, the results confirmed that half-strength MS medium was suboptimal for their growth. Conversely, media with 4-fold diluted mineral content supported markedly enhanced growth and development.

During in vitro seed germination, no abnormalities such as delayed germination, morphological variations, or callus formation were observed, and all in vitro plants exhibited uniform growth and well-developed roots. Therefore, instead of using nodal segments excised from the in vitro seedlings, the sterilized seed material itself was grown on hormone-supplemented media for four weeks to evaluate the effects of plant growth regulators on the growth of blueberry in vitro plants. The medium containing 0.3 mg·L^{-1} BA in medium (4^{th} medium treatment) showed the highest growth of plants in terms of height (4.3±0.1 cm) and number of leaves (8.0±2.1). In comparison, the medium containing 0.6 mg·L^{-1} of BA and 1.5 mg·L^{-1} of NAA (17^{th} medium treatment) showed the greatest number of roots (8.6±1.6), indicating strong root development (Fig. 2). Subsequently, nodal explants of in vitro plants were cultured on media supplemented with 0.6 mg·L^{-1} BA and 1.5 mg·L^{-1} NAA. After six weeks, the explants regenerated in vitro plants with a height of 5.4±0.4 cm and well-developed roots with additional shoots, rendering them suitable for acclimatization under ex vitro conditions (Fig. 3 and Fig. 4). No abnormal growth or morphological variations were observed in the microshoots grown on any of the culture media. All in vitro plants exhibited 100.0 % root formation and showed vigorous growth with a healthy, bright green coloration.

According to the literature review, recent studies have been conducted on the propagation of bog blueberry. Blueberry green seedling cuttings with the treatment of phytohormones were applied to grow bog blueberry in Tashkent province, Uzbekistan [16]. Generally, in blueberry

biotechnology research, researchers have mainly used Woody Plant Medium (WPM) and MS media. For example, Georgieva et al. successfully used Woody Plant Medium (WPM) with half-reduced salt concentration enriched with 1.0 mg/L IAA for in vitro propagation of *Vaccinium corymbosum* L. [17]. Also, it was reported that WPM was the most suitable medium for in vitro propagation of *Vaccinium uliginosum* L. [18].

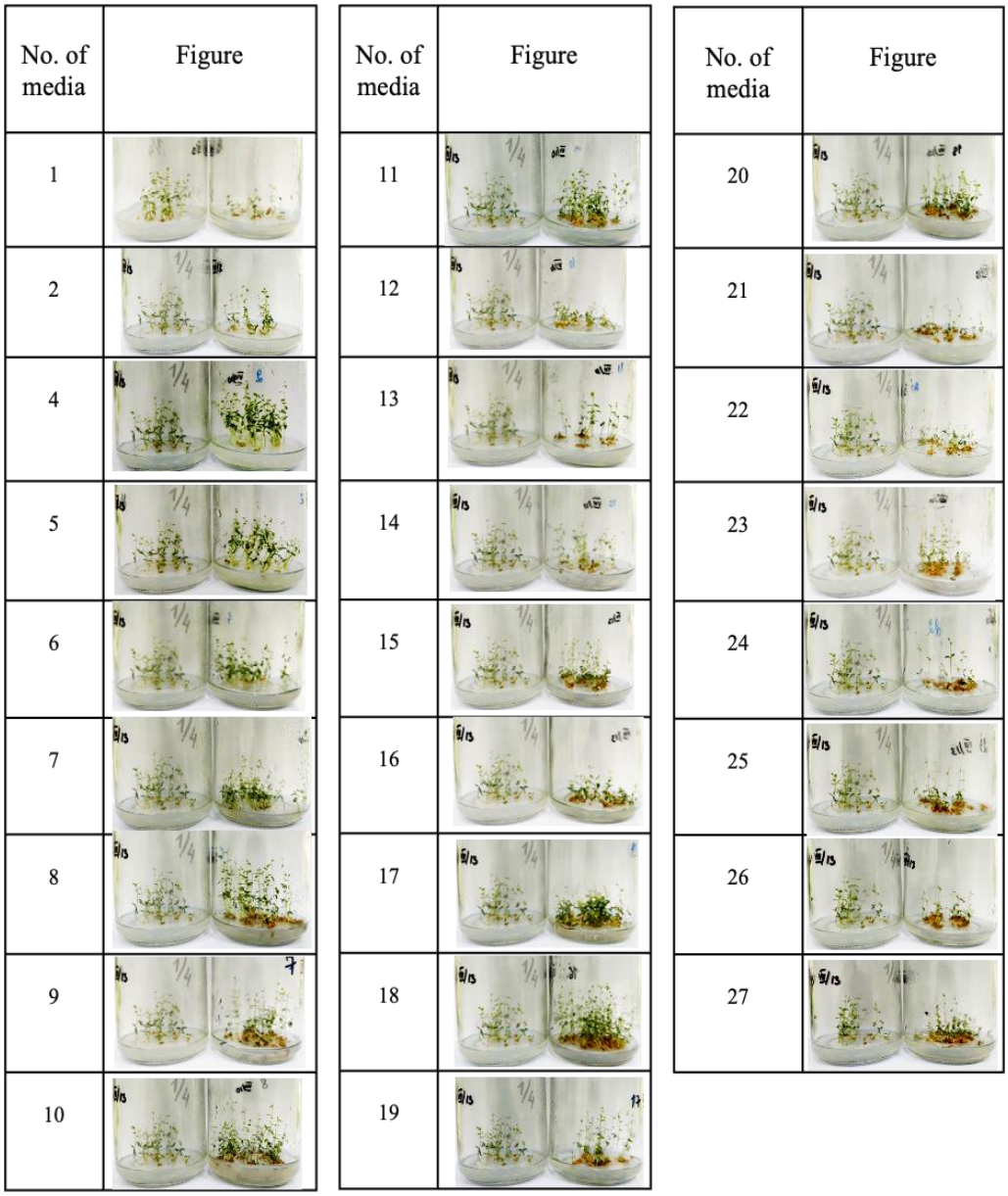

Fig. 2. *Growth of bog blueberry in vitro plants. Note: In vitro plantlets grown on 4-fold diluted MS nutrient medium or 3rd medium (right) compared to plants grown on other media differing in hormone and nutrient level (left).*

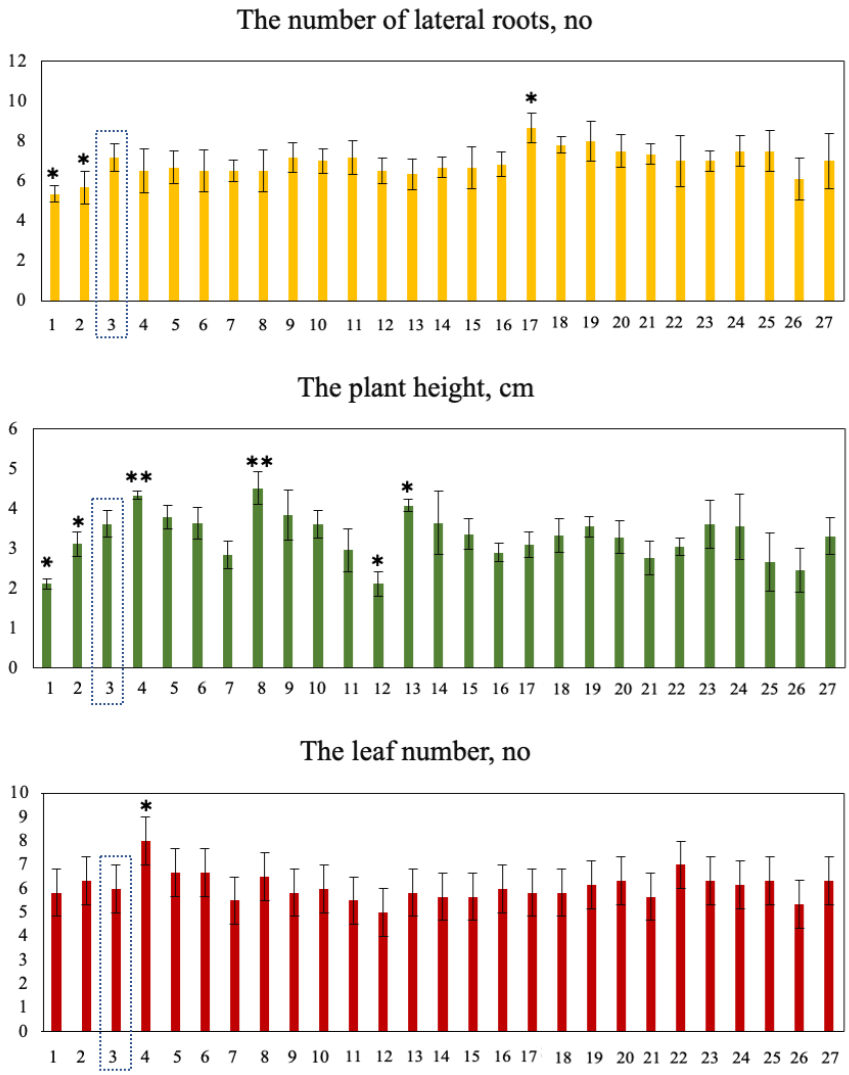

Fig. 3. *Growth response of bog blueberry in vitro plants to phytohormones and the strength of MS medium. Note: The mean values of the groups were compared with the mean value of the control group (3rd medium treatment) statistically.*

Fig. 4. *In vitro culture of bog blueberry a). In vitro plantlets grown in control medium or ¼ MS medium (left) and in medium containing 0.1 mg·L⁻¹ BA (right); b). In vitro plantlets grown in control medium or ¼ MS medium (left) and in medium containing 0.6 mg·L⁻¹ BA and 1.5 mg·L⁻¹ NAA (right), c). In vitro plantlets before transferration into ex vitro condition*

Acclimatization of in vitro plantlets

In vitro plantlets grown for 4 weeks were transferred into (i) pure peat moss soil and (ii) a mixture of peat moss and sand (1:1). According to the result, the plantlets successfully acclimatized on peat moss soil with a 75.0% survival rate, while 5.5% of plantlets survived on soil of sand mixture (Figure 5).

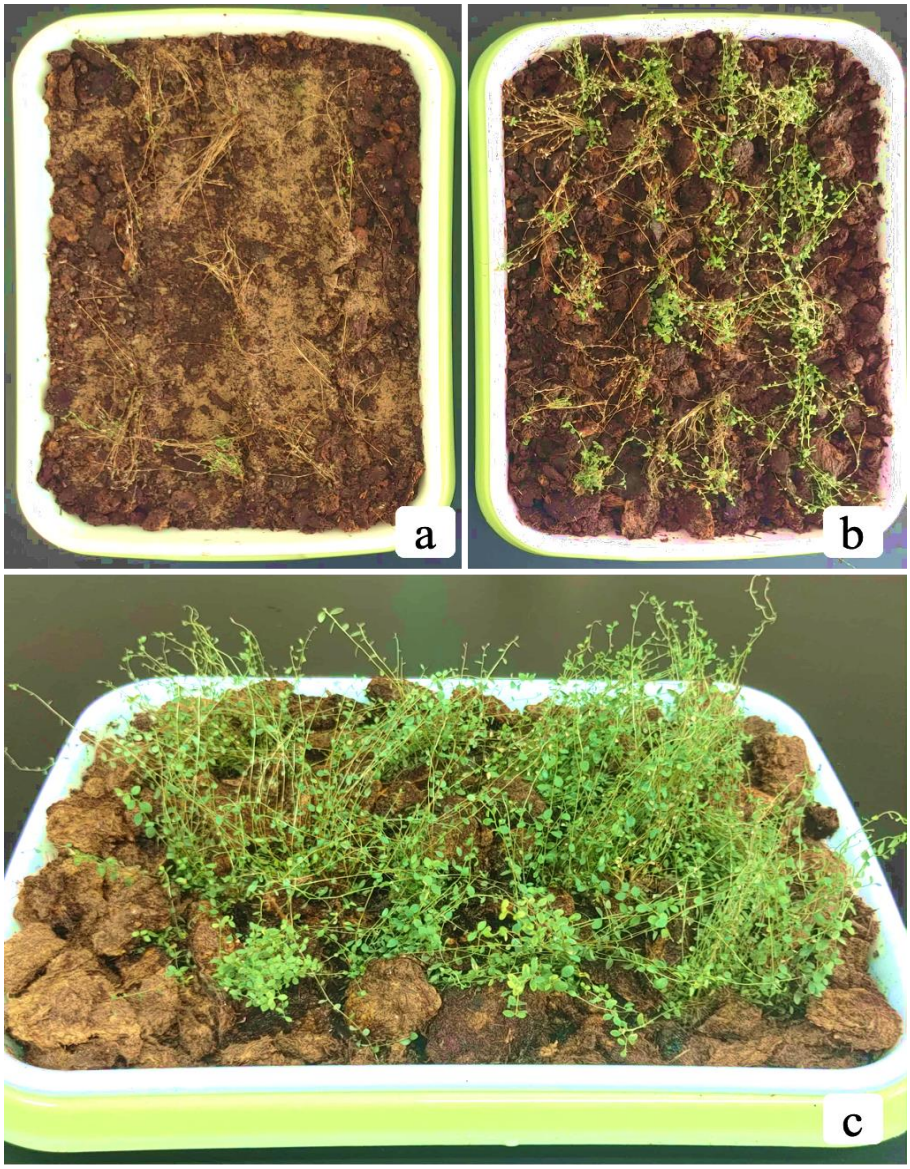

Fig. 5. *Acclimatization of blueberry plantlets. Note: Plantlets growing on peat moss soil (a); its mixture with sand (b) for 2 weeks; plantlets growing on peat moss soil for 8 weeks (c)*

These results highlighted the influence of substrate composition on ex-vitro establishment of blueberry micropropagated plants. Although there are relatively few published studies on the combination of peat moss vs peat and sand mixture for blueberry in vitro plant acclimatization, the available literature supports the general pattern that acidic and well-aerated substrates (such as peat moss) tend to yield higher survival and better establishment than those with substantial soil during the early acclimatization phase.

A study by Meneses et al. on in vitro culture of *Vaccinium* spp. reported that during acclimatization, seedlings grown in peat substrate exhibited the highest survival rate (100.0 %), compared with other substrates such as native soil [20]. Efficient micropropagation protocol of three cultivars of highbush blueberry (*V. corymbosum* L.)" by Figiel-Kroczyńska et al., rooted plantlets of cultivars were transferred to a peat: perlite (4:1) substrate and achieved an acclimatization survival rate of 70.0 % for the cultivar 'Liberty.' [21].

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