



Review

Advances in Blueberry (*Vaccinium* spp.) In Vitro Culture: A Review

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Abstract: The demand for *Vaccinium* fruits has skyrocketed due to their nutritional and medicinal properties, notably their high content of phenolic compounds and excellent sensory evaluation. The use of *Vaccinium* plants and its components as dietary supplements and health ingredients has been on the rise across the world. The attractive fruits contain a high content of anthocyanins and antioxidant compounds. In some popular and valuable cultivars, the conventional propagation methods, exploiting hard or soft wood cuttings, are inefficient. The demand for nursery plants can be fulfilled by micropropagation. This review aims to explore advances in the in vitro culture of *Vaccinium* plants, focusing on effective disinfection, optimized culture media, and the role of growth regulators in plant development and multiplication. By providing a controlled environment, micropropagation allows the large-scale production of these plants in a short time, ensuring availability throughout the year. Additionally, this technique offers the advantage of studying the effects of abiotic stresses on plants, as well as facilitating research on the production of relevant metabolites. This review seeks to provide an updated overview of the most promising methods and techniques for micropropagation of *Vaccinium*, thereby contributing to the ongoing development of the blueberry production industry and derivative products.

Keywords: blueberry; micropropagation; medium culture; growth regulators



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1. Introduction

Vaccinium consists of approximately 450 species, among which highbush blueberry (*Vaccinium corymbosum*) stands out as a highly heterozygous, polyploid crop and one of the primary *Vaccinium* fruit crops cultivated in the twentieth century [1]. Indigenous to North America and East Asia, highbush blueberry has risen in cultivation over the past century, now ranking second only to strawberries in annual yield [2]. Predominantly thriving in the midwestern, eastern, and central United States, as well as in Europe, Australia, and New Zealand, most highbush blueberry cultivars fall into two types: “highbush” (requiring 800–1000 h of cold weather) and “lowbush” (requiring 150–800 h of cold weather). Noteworthy cultivars include ‘Bluecrop’, ‘Chandler’, ‘Bluegold’, ‘Liberty’, ‘Toro’, ‘Spartan’, ‘Patriot’, ‘Aurora’, and ‘Duke’, which are all highbush varieties [3]. Blueberries have an extensive history of culinary and medicinal use [4].

These plants serve diverse purposes, from medicinal applications to ornamental landscaping [5]. They are characterized by their fleshy, small to medium-sized fruits containing high levels of antioxidant compounds such as phenolics, flavonoids, and tannins, as well as fruit colorants like anthocyanins and carotenoids, vitamins such as ascorbic acid, and various minerals [5]. From a nutritional perspective, blueberries are rich in water and sugars, particularly glucose and fructose, though other sugars like galactose and rhamnose

may also be present, often as sugar moieties associated with phenolic compounds [6]. The demand for *Vaccinium corymbosum* L. (and hybrids) plants has increased in recent decades due to market expansion [7], with blueberries being recognized as one of the richest sources of antioxidant phytonutrients among the fresh fruits and vegetables that have been studied [8]. Notably, blueberries rank high in anthocyanin content among red fruits, with levels ranging from 387 to 487 mg/100 g fresh [9].

Blueberries exemplify fruits strongly associated with health, playing roles in blood sugar regulation, oxidative stress reduction, anti-inflammatory effects, and cardiovascular disease prevention [9]. They also have antimicrobial and antitumor properties [10]. Phenolic and flavonoid compounds, important in plant defense mechanisms, fruit development, and seed dispersal, accumulate to protect plants against several stresses [5]. Anthocyanins offer cellular antioxidant protection and inhibit inflammation and cytotoxicity [11–14]. Mounting evidence suggests that blueberry consumption can reduce biomarkers associated with major diseases like cardiovascular disease, type 2 diabetes, and neurological decline [9]. Berries within the *Vaccinium* genus are renowned for their antioxidant richness, showing potential to reduce the incidence of chronic and degenerative diseases, including cancer [5]. Studies have demonstrated the inhibitory effects of blueberry extracts on the proliferation of various tumor cell lines [15,16], thus enhancing their commercial value when incorporated into products [10].

Concerning *Vaccinium* species, in vitro techniques are quite effective for rapid mass production of high-quality planting material for large-scale cultivation, germplasm improvement, gene conservation, and research purposes (genetic transformation and gene editing) [17]. Blueberries are traditionally propagated by stem cuttings [18]. Cutting propagation may not be suitable for all cultivars due to low rooting percentages and may not be an effective method for quickly increasing numbers of starting materials or propagules for commercial introduction of new cultivars [19]. Traditional methods of propagating highbush blueberry plants through semi-woody cuttings have been a common practice, but they come with limitations [20]. These traditional methods are known for producing genetically identical seedlings to the mother plants, ensuring consistency in desirable traits [20]. However, the study points out that traditional propagation methods are slow, labor-intensive, and highly dependent on weather conditions and seasons [20]. This reliance on external factors can hinder the year-round production of seedlings and limit the scalability of plant propagation [20]. However, micropropagation has a higher cost of production [21]. Mazurek's study analysis elucidates the effects of different propagation methods on highbush blueberry plants and their fruits [20]. Remarkably, the study reveals substantial differences between in vitro and conventionally propagated plants across various parameters, including growth vigor, branching, chlorophyll content, fluorescence, and DNA methylation levels [20]. In vitro propagation results in plants with enhanced growth vigor and branching, while conventionally propagated plants yielded fruits with higher antioxidant compound levels [20,21]. Micropropagation is an excellent technique for root formation [22]. The choice of the best propagation method between in vitro and conventional methods depends on specific cultivation goals and desired plant and fruit characteristics.

Micropropagation is one of the best methods for the rapid propagation of elite plants [8] and has been considered the most effective method for rapid increase in propagules on a year-round basis [20]. Micropropagation via axillary shoot production has been documented in lowbush, highbush, and rabbiteye blueberries [6,7,19,23]. Studies have shown that blueberry plants propagated through micropropagation not only yield increased fruit production but also exhibit enhanced rooting ability in cuttings compared to traditionally propagated plants [24]. One key aspect driving these advantages is the ability of micropropagation to support the production of high-health plants [24]. Diseases induced by plant pathogens like viruses, viroids, and phytoplasmas significantly impact agricultural productivity and product quality while also restricting the safe movement of plant materials across borders [25]. Thus, ensuring the use of pathogen-free planting

materials becomes crucial for effectively managing these diseases and facilitating the global exchange of genetic resources [25]. The efficacy of virus eradication techniques, used individually or together, depends on factors such as virus type, the presence of single or mixed infections, virus concentration, and the interaction between the virus and its host [26]. Choosing an appropriate method for virus elimination requires a deep understanding of plant–virus dynamics and the distribution patterns within the plant [26]. For instance, eliminating viruses and viroids that target the meristematic cells of shoot tips poses greater challenges compared to those that are restricted to the phloem [26]. Through tissue culture methods, micropropagation enables the propagation of plants in a controlled environment, minimizing the risk of pathogen transmission and facilitating the production of virus-free plant material [6–8]. This high-health status translates into improved vigor, disease resistance, and ultimately, enhanced productivity. Therefore, while the primary mechanism driving the observed benefits may involve the production of virus-free plants, additional factors such as improved nutrient uptake, uniformity in plant growth, and genetic stability may also contribute to the overall performance of micropropagated blueberry plants [27]. Efficient propagation depends on multiple factors: genotype; type and concentration of plant growth hormones in the media; concentration of media components such as vitamins, salts, and elements; and incubation conditions such as temperature and light [7].

This review explores the advancements, challenges, and potential applications of tissue culture techniques in blueberry cultivation. It covers various aspects that contribute for the success of plant micropropagation.

2. Mother Plant Factors in Plant Propagation

The success of plant propagation hinges on different mother plants factors including genotype, explant type, disinfection procedure, age, and physiological state [28–30]. The age of the mother plants source, along with their physiological and growth stage, significantly influence the quality and quantity of the offspring, affecting antioxidant activity and DNA methylation patterns in regenerants [31].

Younger mother plants tend to produce more vigorous and uniform progeny, while older plants may exhibit declines in reproductive capacity and genetic stability [32]. Moreover, the physiological condition of the mother plants, including nutrient status, hormone levels, and overall health, can directly impact the success of propagation efforts [33].

Considering these factors and implementing proper techniques, the propagation of these plants can be successfully carried out, resulting in healthy and productive blueberry crops. In conclusion, when propagating blueberry plants, it is important to consider factors related to the mother plant such as the selection of a suitable variety, consistent management practices, and proper pruning techniques [34]. While *in vitro* cultures can be initiated at any time throughout the year, their success is contingent upon the season for collecting explants [30]. It was observed that contamination of explants was less prevalent when collected during spring or summer, as opposed to explants gathered in autumn or winter, and gathering explants from stock plants cultivated in controlled environments, such as greenhouses, can effectively reduce microbial contamination [30,35].

Additionally, the genetic composition of the mother plants plays a crucial role in determining the traits and characteristics of the propagated plants. Selecting mother plants with desirable traits, such as disease resistance, fruit quality, and yield potential, can significantly enhance the overall success and productivity of the propagation process [36,37]. Furthermore, advancements in molecular breeding techniques have enabled researchers to identify and select superior genotypes for use as mother plants, further optimizing the propagation process and resulting in improved crop performance [34].

3. Effective Disinfection Methods for Micropropagation

The next step involves the collection of plant material followed by disinfection. It is essential to avoid harvesting during or after rainy days, as the plant material is highly susceptible to contamination by various bacteria and fungi [38].

Disinfecting field- or ex vitro-derived plant material for use as explants in micro-propagation requires careful consideration of numerous factors: genotype, explant type, disinfection procedure, age and physiological state of the mother plant, and the physical and chemical conditions applied in vitro are particularly significant [39]. It is important to select plant materials for introduction into tissue culture that are most likely to be free of endophytes and surface contamination [35]. Disinfection methods for blueberries vary across different studies (Table 1). Nonetheless, they commonly involve the use of 70% ethanol, a solution containing Tween, or sodium hypochlorite [6–8,19,40–43]. These disinfectants are effective in minimizing microbial contamination and ensuring the success of subsequent tissue culture procedures [40]. After disinfection of the explants, they are rinsed in distilled and sterilized water to remove any residues of the disinfectant agents [40].

Table 1. Disinfection agents and corresponding treatment durations (minutes).

Disinfection Agent	Time of Operation (minutes)	References
Ethanol 70%	0.5	[6]
0.5% NaClO ¹ with 0.1% Tween 20	5	
Ethanol 70%	1	[7]
Clorox 10%	6	
Ethanol 70%	1	[8]
NaClO diluted 1% with tap water	20	
20% Clorox (1.2% NaClO)	20	[19]
Ethanol 70%	0.2	
Ethanol 70%	2	[41,42]
Mercuric chloride 0.1% with Tween	6	
Ethanol 75%	0.5	[40]
4% NaClO	15	
Tween 20	30	[43]
Ethanol 70%	0.2	
0.1% HgCl ₂ ²	8	

¹ NaClO—sodium hypochlorite; ² HgCl₂—mercuric chloride.

Bleach, or sodium hypochlorite, is known to be more effective against spores due to its ability to disrupt cellular structures and DNA, rendering microorganisms inactive [44]. When bleach encounters microorganisms, it releases hypochlorous acid, a powerful oxidizing agent [44]. Hypochlorous acid reacts with cellular components such as proteins and DNA, causing irreversible damage and leading to the inactivation of microorganisms [43,44]. This disruption of cellular structures and DNA prevents spores from germinating and bacteria from reproducing, ultimately rendering them inactive.

On the other hand, ethanol, or ethyl alcohol, is a commonly used disinfectant with broad-spectrum antimicrobial properties [45]. When ethanol is applied to microorganisms, it permeates their cell membranes and disrupts the interactions between proteins and lipids, leading to the denaturation of proteins [46]. This denaturation process alters the structure of essential proteins within the microorganism, rendering them non-functional [46].

Both agents are rinsed off with sterile water to minimize prolonged exposure to toxic compounds like ethanol or bleach. The explants are inserted in the culture media and then transferred to a growth chamber, maintained at a temperature of 23–25 ± 2 °C [23,40,47], a climatic chamber specifically designed for plants, seeds, and small living organisms. Wang et al. observed in 2023 that the southern highbush blueberry ‘ZY09’ presented the lowest contamination rate of 0.00% when the ethanol treatment time was 30 s and the NaClO treatment time was 15 min [40]. Conversely, the highest induction rate of 93.33% was achieved when the ethanol treatment time was 60 s and the NaClO treatment time was 5 min [40]. Contamination levels exhibited an inverse correlation with in vitro shoot

development or viability, whereas infection levels declined with increasing exposure time or concentration of HgCl_2 [48].

Disinfection is the initial step of in vitro propagation, and inadequate process can lead to the contamination and explants death [40]. To ensure thorough decontamination and optimize the induction rate, it is imperative to tailor the type, concentration, and duration of disinfection, based on the specific characteristics of the explant [40].

4. In Vitro Blueberry Germination

In vitro germination of blueberry seeds plays a crucial role in producing healthy seedlings and rapidly expanding commercial crops [49]. However, blueberry seed germination in the field poses significant challenges due to seed dormancy and the influence of variable environmental factors [50]. Blueberry seeds often require specific conditions of temperature, humidity, and light to successfully germinate, factors that are not always easy to replicate in natural settings [50]. Additionally, seed dormancy can be an additional obstacle, prolonging the time needed for seeds to germinate and establish viable plants [50]. Therefore, in vitro germination offers a controlled and efficient approach to overcoming these challenges, allowing for the creation of optimal conditions for the initial development of blueberry seedlings before transplanting to the field [49]. This technique not only accelerates the germination process but also increases the success rate in seedling production, thus contributing to the sustainability and productivity of blueberry crops [49].

The successful in vitro germination of seeds is critical for ensuring optimal growth and development in blueberry cultivation. Meneses et al. obtained seeds (Figure 1) from harvested ripe fruits, followed by careful washing, sieving, and selection to ensure seed quality [49]. Disinfection procedures were then meticulously carried out to prevent contamination and promote healthy seedlings [49]. The study aimed to determine the best conditions for germination of *V. floribundum* seeds by investigating the effects of different photoperiods and temperatures on seed germination and growth [49]. Additionally, it aimed to evaluate the influence of culture media on seed germination rates and to identify the optimal conditions for maximizing germination percentage and reducing germination time [49]. For in vitro seed germination, the study investigated the impact of two culture media (McCown Woody Plant medium (WPM) and Murashige and Skoog medium (MS)), two photoperiods (16 and 24 h of light), and two temperatures (18 and 28 °C) [49]. The optimal treatment identified was WPM supplemented with 24 h of light at 18 °C with germination percentages above 62% [49,51]. However, seeds of *V. corymbosum* hybrids were germinated on 0.6% water agar at 24 ± 1 °C, under a 16 h photoperiod, resulting in germination percentages of 42% for *V. uliginosum* × (*V. corymbosum* × *V. angustifolium*), 'Northcountry', and 88% for *V. uliginosum* × (*V. corymbosum* × *V. angustifolium*), 'SC 5-8' [52].



Figure 1. In vitro seed germination process of *V. floribundum*. Adapted from Meneses et al. [49].

The utilization of in vitro techniques enables the production of viable hybrids from seeds with underdeveloped embryos, thus overcoming the adverse effects of various factors on seed germination and seedling viability during the initial stages of development [52]. Subsequently, the hybrid seeds are germinated either after pretreatment with gibberellin in in vitro culture or following stratification in nurseries [52].

Furthermore, cold stratification is a well-established technique for overcoming seed dormancy and facilitating germination [18]. It is noted that shorter durations of cold stratification at 4–5 °C lead to decreased germination rates [18]. The percentage of germinated seeds increases with longer periods of cold stratification, with the highest germination rates achieved after 8 weeks of cold treatment (88% for *V. myrtillus* and 74% for *V. uliginosum*) [18]. These results emphasize the significance of cold stratification in improving germination rates, especially for species such as *V. myrtillus* and *V. uliginosum* [18]. These findings highlight the importance of controlled environments and specialized techniques in overcoming the challenges associated with blueberry seed germination, ultimately contributing to the successful propagation and cultivation of blueberry crops.

5. In Vitro Multiplication and Rooting

In vitro multiplication and rooting represent pivotal stages in the process of micropropagation. The micropropagation stage involves the controlled proliferation of plant cells or tissues in a nutrient-rich, sterile environment, followed by the induction of root formation to facilitate acclimatization and subsequent transplantation to the field.

The multiplication stage typically involves the proliferation of shoot tips, nodal segments, or other explants derived from elite blueberry cultivars or selected genotypes in a controlled laboratory setting [6]. Shoot proliferation is initiated through the formation of axillary buds or adventitious shoots from the explant's meristematic tissues [7,19,40,53]. Cytokinins, such as zeatin, 2-isopentenyladenine (2-iP), and 6-benzylaminopurine (BAP), are often used in the culture medium to promote shoot proliferation and inhibit apical dominance, resulting in the formation of multiple shoots from a single explant [1,6,7,54,55]. This plant hormone can affect plant meristem and leaf proliferation based on its concentration and genotype used [7,8].

Blueberry shoot multiplication is enhanced by using zeatin (18 µM) in the culture medium and white light [56]. Cappai et al. showed that zeatin increases leaf proliferation and the number of shoots in blueberry genotypes [7]. Litwińczuk et al. reported that reducing the concentration of 2-iP, in combination with a low concentration of the auxin IBA (indole-3-butyric acid), increased the number of healthy shoots with well-developed internodes and rigid leaves in WPM medium [8]. Regarding the presence or absence of auxin, Fan and colleagues [19] reported that auxins, along with cytokinins, promoted plant growth. Although most authors prefer WPM medium, Trejgell et al. observed better shoot multiplication results using MS medium supplemented with BAP (1 mg/L) [57].

The technique of adventitious shoot formation from leaf explants in blueberries can produce clonal material [27]. When shoots regenerate from leaf explants, they are genetically identical to the parent plant from which the explants were taken [27]. Shoots can develop from blueberry leaves cultured on regeneration medium, regardless of whether the abaxial or adaxial surface is in contact with the medium [27]. Shoot formation from blueberry leaves in tissue culture occurs in response to stimuli from the culture medium, such as thidiazuron (TDZ) [27]. This process involves a series of anatomical events, including the proliferation of parenchyma cells around the major and minor veins of the leaf, leading to the formation of shoot meristems [27]. These meristems develop into shoots, establishing vascular continuity with the leaf's original veins [27].

Recent advancements in tissue culture techniques, such as the use of temporary immersion systems or bioreactors, have facilitated the scaling-up of blueberry micropropagation, allowing for the efficient production of large quantities of uniform plantlets in a shorter time period [58,59]. Additionally, the integration of genetic engineering approaches, such as the overexpression of key genes involved in shoot development or stress tolerance,

holds promise for enhancing the efficiency and productivity of in vitro multiplication in blueberries [18,60].

Several factors influence the rooting process in vitro, including the choice of rooting medium, the concentration and type of auxins used, the physiological state of the shoots, and environmental conditions such as temperature, light, and humidity [61]. Auxins, particularly IBA and indole-3-acetic acid (IAA), are commonly employed to stimulate root initiation and development [62]. The concentration of auxins in the rooting medium needs to be carefully optimized to achieve the desired rooting response while avoiding adverse effects such as excessive callus formation or root malformation [62]. Ex vitro rooting can be conducted without auxin treatment or by preliminary immersion in IBA solution [63]. Rooting in ex vitro conditions could reduce costs, although the process is often slower than in vitro rooting, which has been found to reduce disease risk and overcome environmental stress [63]. The utilization of IBA (0.5 mg/L) was observed to result in higher rates of root formation (75% and 65%) and superior root development (0.97 and 1 cm) when compared to other tested auxins in laboratory settings for *V. corymbosum* 'Bluejay' and *V. ashei* 'Pink Lemonade' [19]. The authors mentioned the best root formation was established on WPM culture medium with half-reduced salt concentration, supplemented with 0.1 mg/L IBA for *V. corymbosum* 'Ozarkblue' (97.7%), 0.5 mg/L IBA for *V. corymbosum* 'Weima Kuahuat' (84.0%), and 1.0 mg/L IBA for *V. corymbosum* 'Sierra' (100%) [64]. In vitro rooting of *V. myrtillus* and *V. floribundum* was successful (100%) using WPM medium with 1.0 mg/L of IBA and 2.0 mg/L of IBA, respectively. (Figure 2) [49].



Figure 2. Micropropagated *V. floribundum* plant after 8 weeks of culture with IBA for root induction. Culture conditions: 5 mL of WPM culture medium (3% sucrose, 0.6% agar, pH 5) under growth room conditions at 18 ± 2 °C and 16 h light. Adapted from Meneses et al. [49].

In recent years, advancements in tissue culture technology have led to the development of novel approaches to enhance rooting efficiency in vitro. These include the use of root-promoting rhizobacteria [65,66], application of biostimulants and growth enhancers [59], and manipulation of the microenvironment around the explants to optimize root induction and development [65]. The application of glutamic acid and BAP as biostimulants positively affects flower bud sprouting, fruit quality, and antioxidant activity in blueberries, enhancing yield and quality [67]. The blueberry root-associated microbiome plays an essential role in its adaptation to acidic soils and in limiting the uptake of soil nutrients by its poor root system [65]. Specifically, symbiotic interactions with mycorrhizal fungi facilitate the acquisition of essential nutrients such as phosphorus, which is often limited in acidic soils [65]. Additionally, rhizospheric bacteria contribute to the enhancement of plant growth and stress tolerance through mechanisms such as nitrogen fixation, production of phytohormones, and suppression of soil-borne pathogens [65].

6. Medium Formulations and Conditions for Micropropagation

Tissue culture in plants is a broad term referring to the cultivation of any part of a plant (cells, tissues, or organs) in artificial media, under aseptic conditions, and in controlled environments [68]. The ability of plant cells, when in culture, to give rise to new plants without the intervention of a sexual reproduction process is referred to as totipotency and forms the basis of asexual reproduction in plants. Therefore, already-differentiated tissues can recover genetic potential and behave like a zygote (totipotent cell) [69]. The success of plant cell culture relies on the use of appropriate culture media. These consist of an aqueous solution containing various macro and micronutrients, as well as essential vitamins and sucrose for the development of the plant [70].

The initial step in establishing *in vitro* culture involves the preparation of the culture medium and adjustment of its pH. The choice of the culture medium is crucial as it directly influences the growth and development of plants. Several studies indicate that the most effective culture medium for blueberry *in vitro* culture is the WPM medium (Figure 3) [4,6,8,19,53,63]. The effectiveness of the WPM medium can be attributed to its balanced nutrient composition, empirical optimization, and suitability for woody plant species [71]. WPM medium contains essential macro- and micronutrients, vitamins, and growth regulators that support cell division, elongation, and differentiation, crucial for the development of healthy blueberry plantlets [71]. It was specifically developed for woody plants, as the name suggests. Its formulation has been optimized to meet the growth and regeneration needs of these plants, distinguishing it from more generic media like MS or DKW (Driver and Kuniyuki Woody plant medium) [71].

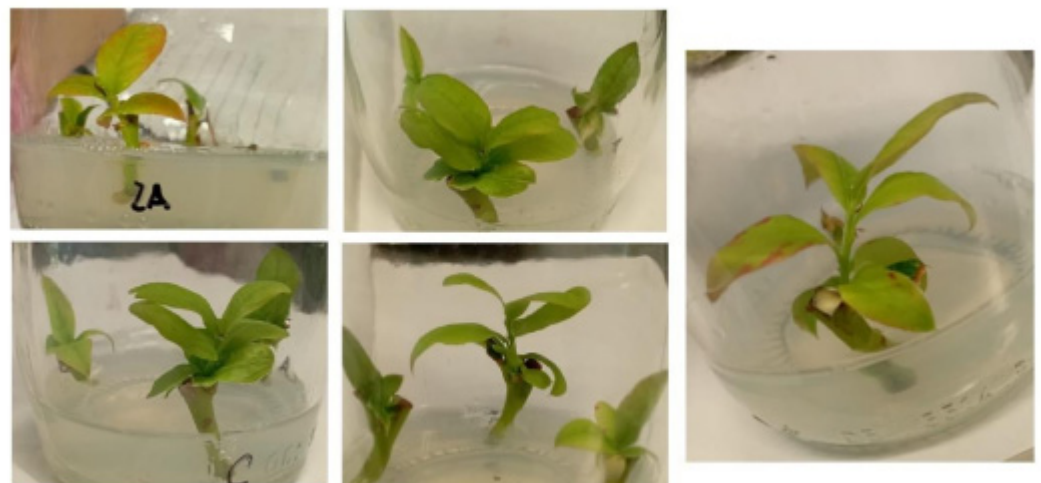


Figure 3. Micropropagated blueberry plants (*V. corymbosum* 'Duke') in a laboratory environment (WPM medium with 1 mg/L 2-iP).

When comparing WPM medium to MS or DKW, one major difference lies in the nitrogen source [71]. In WPM medium, nitrogen is typically provided in the form of ammonium sulfate $((\text{NH}_4)_2\text{SO}_4)$ which is incorporated into the medium [72]. In contrast, in MS and DKW media, $(\text{NH}_4)_2\text{SO}_4$ is typically provided as a separate component, which is added to the medium as a concentrated nitrogen solution [72]. While these media share common components such as macronutrients, micronutrients, and growth regulators essential for plant growth and development, they also exhibit distinct differences in their formulations [73]. Macronutrient and micronutrient concentrations, as well as the types and concentrations of growth regulators, vary between WPM, MS, and DKW media formulations [73]. These subtle differences in nutrient composition and growth regulator concentrations make each medium uniquely suited for specific plant species or tissue culture applications, thereby contributing to the success and efficiency of *in vitro* cultivation and micropropagation techniques [71].

However, alternative approaches have been explored, such as supplementing half of the WPM medium with MS or utilizing Anderson's Rhododendron medium (AN) as the sole culture medium [19,41,74]. During the establishment of the in vitro culture, Wang et al. discovered that the modified WPM was effective: substituting NH_4NO_3 from $(\text{NH}_4)_2\text{SO}_4$ promoted the proliferation of blueberry microshoots [40]. To provide a comprehensive overview of micropropagation methodologies, Table 2 compiles data on sucrose quantity, pH values, base culture media, and agar concentrations from several works.

Table 2. Comparison of different culture media, pH values and concentrations of sucrose and agar for in vitro culture of blueberry.

Media Culture	Sucrose (g/L)	Agar (g/L)	pH	References
WPM ¹	20	6	5.2	[4]
WPM	30	6	5.2	[6]
WPM	15	5–6	5.0	[8]
AN ² or WPM	30	8	5.0	[19]
MW (MS ³ and WPM)	30	8	5.0	[19]
WPM	20	7.5	4.9	[53]
WPM	-	-	4.2	[62]
AN	30	8	3.8	[41]
M-WPM	20	6	5.4	[40]
AN	30	8	4.5–5.5	[42]

¹ McCown Woody Plant; ² Anderson's Rhododendron; ³ Murashige and Skoog.

Selecting an appropriate growth medium, pH, and concentrations of sucrose and agar are critical for the successful establishment of cell and organ cultures. Research comparing different media compositions has revealed that AN medium is particularly suitable for blueberry shoot multiplication and in vitro rooting [19,53]. Furthermore, the combination of WPM and MS media, as well as AN medium, has shown promising results in promoting shoot growth and development [17,19]. Sedlak et al. concluded that WPM medium was more appropriate for the micropropagation of the 'Spartan', 'Bluecrop', and 'Berkeley' blueberry cultivars compared to AN medium [75]. However, Tetsumura et al. showed that during the multiplication phase, shoots in WPM medium exhibited inferior growth compared to those cultivated in MS medium or a mixture of equal parts of MS and WPM media [76]. Shoots in MS medium grew well but tended towards hyperhydration, likely due to a high concentration of ammonium ions in this medium [76].

To facilitate the growth of cell cultures, it is crucial to maintain a balanced and stable pH level. Each plant species has its own recommended pH range due to its specific nutrient requirements, as pH directly influences nutrient availability in the soil. Blueberry plants thrive in acidic soils with a pH range of 4.0 to 5.5, provided they are well-drained, moist, and rich in organic matter (exceeding 5%) [4,6–8,17,19,63]. Adjusting the pH of the medium is typically achieved by adding diluted solutions of HCl or NaOH [24]. It is important to note that the addition of these chemicals alters ion concentrations, which can potentially have adverse effects on plant growth [24]. The impact of pH levels on shoot growth has also been investigated, with varying results depending on blueberry varieties and medium composition [19]. Fan et al. conducted a study with two blueberry cultivars ('Bluejay' and 'Pink Lemonade'), concluding that the shoot length of the 'Bluejay' variety obtained at pH 4.5 was significantly greater than those obtained at pH 5.0 [19]. However, pH levels had no effect on the shoot length of the 'Pink Lemonade' variety [19]. Additionally, when using AN medium with different pH values ranging from 4.5 to 5.0, no significant differences were observed in shoot formation in both cultivars [19]. However, the average number of shoots per explant was higher in the medium with pH 5.0 compared to pH 6.0 [19].

Light, temperature, and relative humidity are crucial parameters influencing culture growth. While photosynthetic activity may not be pivotal during initial in vitro stages, subsequent phases induce a degree of autotrophy [77]. Light plays a pivotal role in morpho-

genetic processes, such as shoot initiation, root formation, and somatic embryogenesis. Light quality, intensity, and photoperiod significantly impact in vitro culture success [78]. Recommended light exposure ranges from 12 to 16 h daily, at 35–50 $\mu\text{mol m}^{-2}\text{s}^{-1}$, provided by cool fluorescent lamps, preferably white [78].

7. Maximizing Growth: Using Growth Regulators for Micropropagation

Growth regulators play pivotal roles in plant tissue culture, influencing various aspects of plant growth and development (Table 3); for instance, they regulate stem elongation, controlling the length and structure of stems [79]. Additionally, growth regulators contribute to tropism, guiding the direction of plant growth in response to environmental stimuli such as light and gravity [79]. Moreover, they are involved in maintaining apical dominance, where the terminal bud at the top of the plant inhibits the growth of lateral buds [79]. Besides these functions, growth regulators also regulate processes such as cell division, differentiation, and organogenesis, ensuring the successful propagation and manipulation of plants in tissue culture systems [79].

Table 3. Growth regulators and their concentrations used in micropropagation of blueberry.

Growth Regulators	Concentrations (mg/L)	Stage of Micropropagation	References
Zeatin	0.5–4.0	In vitro proliferation	
TDZ ¹	1	In vitro rooting	[4]
NAA ²	0.5		
Zeatin 2-iP ³ BAP ⁴	0.6; 1.1 2.0; 4.1; 6.1; 8.1; 10.2 6.8; 9.0; 11.3	In vitro initiation and proliferation	[6]
Zeatin	1–5	Leaf, meristem, and shoot proliferation	[7]
Zeatin 2-iP IBA ⁵	0.5–1 4–10 1	In vitro initiation In vitro proliferation In vitro rooting	[8]
Zeatin 2-iP 2-iP riboside IAA ⁶	1–2 2.5–5 2.5–5 0.2–0.5	In vitro initiation and proliferation	[41]
IBA	0.8	In vitro rooting	
Zeatin TDZ IBA	2.5–5 0.0005 0.1	In vitro initiation and proliferation	[40]
Zeatin 2-iP TDZ	2 15 0.2–0.5	In vitro regeneration and proliferation	[43]
Zeatin 2-iP	3 2	In vitro proliferation	[63]
IAA	1	In vitro rooting	

¹ Thidiazuron, ² α -Naphthaleneacetic acid, ³ 2-Isopentenyladenine, ⁴ 6-Benzylaminopurine, ⁵ Indole-3-butyric acid, ⁶ Indole-3-acetic acid.

When developing in vitro regeneration protocols, the role of exogenous plant growth is widely recognized as the most significant factor [54]. These substances, introduced from external sources, exert profound effects on the growth and development of cultured plant tissues [54]. Their precise concentrations and combinations are meticulously optimized to elicit specific responses, such as shoot or root formation, organogenesis, and callus induction [54]. Auxin and cytokinin play crucial roles in regulating organ regeneration, with the concentration ratio between these hormones being critical in determining specific organo-

genesis processes from different somatic tissues (such as leaves, roots, etc.) [54]. Among the growth regulators, zeatin and 2-iP are the most used for shoot proliferation. A superior overall culture appearance, characterized by robust growth and healthy morphology, is typically attained when both these regulators are incorporated into the medium [41,43,63]. These growth regulators synergistically promote various aspects of plant development, including shoot proliferation, leaf expansion, and root elongation, leading to improved vigor and quality of the cultured plants [4,6–8]. Zeatin promotes the growth of lateral buds, stimulates cell division (lateral dominance), initiates callus formation, and facilitates seed germination [63]. It also promotes the generation of multiple shoots from internodes and the development of high-frequency, thick-spread roots [63]. Zeatin is particularly effective for the multiplication of *Vaccinium* species [63].

Additionally, other cytokinins such as BAP, kinetin (KIN), and TDZ can also be used for shoot proliferation and regeneration (Figure 4). BAP promotes shoot elongation and branching, while KIN is involved in chloroplast development and senescence delay [80]. TDZ is known for its strong cytokinin activity, promoting shoot regeneration and multiplication [80]. Each of these cytokinins may exert specific effects on tissue culture, such as promoting shoot proliferation, callus formation, or root development, depending on their concentration and interaction with other growth regulators in the medium [80]. Sun et al. observed that zeatin (1.5 mg/L) exhibited a superior effect compared to other cytokinins (KIN (2–4 mg/L), BAP (1–3 mg/L), and TDZ (0.5–1.5 mg/L)) during the shoot proliferation stage of *V. dunalianum* [81]. Zeatin has emerged as a preferred cytokinin for initiating and proliferating *Vaccinium* cultures, including blueberries [6,19,81]. Schuchovski et al., using zeatin, 2-iP, and other cytokinins such as BAP and KIN, reported that cytokinin 2-iP showed positive results in explant growth, while KIN did not yield results in blueberry micropropagation [6]. Regarding BAP, blueberries developed only in the presence of high concentrations of this regulator [6].

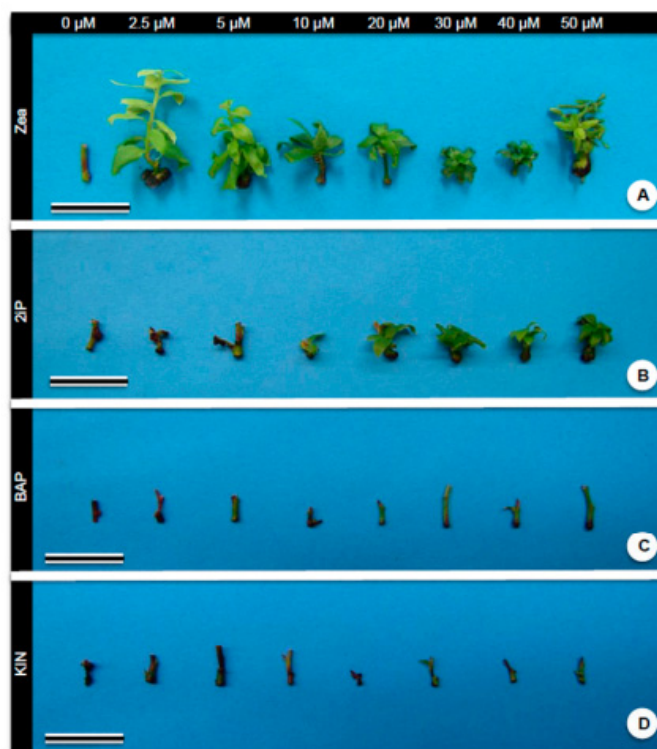


Figure 4. Initial in vitro shoot culture of D'elite' rabbiteye blueberry was conducted using eight different concentrations (0, 2.5, 10, 20, 30, 40, and 50 μ M) of four different cytokinins: zeatin (ZEA) (A), 6-(γ - γ -dimethylallylamino)-purine (2iP) (B), 6-benzylaminopurine (BAP) (C), and kinetin (KIN) (D). The bars in the figures represent 2 cm. Adapted from Schuchovski et al. [6].

Additionally, culture media can be supplemented with NAA, IAA, or IBA. NAA and IAA, belonging to the auxin family, are commonly used for root initiation [62]. However, excessively high concentrations of auxin can lead to the reverse, where auxins become inhibitors instead [62]. IBA was described as a ‘synthetic auxin’ that induces auxin-like effects such as root initiation, stem bending, and leaf epinasty [82]. Subsequently, it was identified in maize plants and is a precursor of IAA, indicating that it is a natural auxin [57].

Research in the rooting process have revealed that both IBA and NAA are commonly utilized auxins for promoting root development in plant tissue culture [83]. In blueberry tissue culture, the effects of IBA, NAA, and IAA can significantly influence various developmental processes. IBA is often utilized to stimulate the formation and elongation of adventitious roots, thereby enhancing the rooting efficiency of blueberry plantlets [82]. Conversely, NAA plays a crucial role in promoting root initiation and may also contribute to shoot proliferation under certain conditions [83]. Additionally, IAA influences callus formation and regulates shoot development and branching in blueberry cultures [83]. The inclusion of IBA as an auxin has demonstrated superior efficacy in stimulating higher rooting percentages and fostering root development when compared to other auxins tested in vitro [19]. Other studies referenced in this study suggest that optimal outcomes were achieved through the utilization of NAA in conjunction with other cytokinins [19]. However, the specific effects of these auxins depend on factors such as concentration, culture conditions, and blueberry genotype [83]. Thus, optimizing the auxin treatments is essential for achieving successful blueberry micropropagation.

8. Acclimatization of *Vaccinium* spp.

Acclimatization is a critical stage in the propagation process of blueberry plants. This stage involves acclimating the plantlets from the controlled environment of the laboratory to the natural conditions of the field or greenhouse. During acclimatization, plantlets are exposed gradually to ambient light, temperature, humidity, and other environmental factors to help them adapt and thrive in their new surroundings [49]. Careful monitoring and management during this phase are essential to ensure the successful establishment of the plants. Factors such as watering frequency, nutrient levels, and pest control measures may need to be adjusted to support optimal growth and development [40,49]. Additionally, providing adequate shade and protection from harsh weather conditions can help minimize stress on the newly propagated plants [40,49,83].

The acclimatization of *V. floribundum* seedlings revealed significant outcomes during greenhouse experiments (Figure 5). Seedlings grown in peat substrate exhibited the highest survival rate (100%), followed by black páramo soil (70%) [49]. Furthermore, seedlings grown in peat substrate showed more than double the length compared to those in páramo soil [49,84].



Figure 5. *V. floribundum* under ex vitro conditions at 1 day (A) and 6 months (B), depicting the development over time of the same plant. Adapted from Meneses et al. [49].

The physical and chemical attributes of peat, such as its fine structure, lightweight nature, porosity, and neutral pH, likely contribute to optimal root system growth and development [49,63,84]. Treatment in sphagnum moss produced the highest average root count, significantly surpassing that of the other treatments (peat, perlite, peat–perlite (vv. 2:1), sphagnum moss–peat (vv. 2:1), and perlite–peat–vermiculite (vv. 3:3:1)) [40]. Treatment in peat–perlite yielded a significantly longer average root length, and the treatment in perlite exhibited significantly higher fresh root weight and dry root weight in comparison to the other treatments [40].

Additionally, the results from fertilization experiments suggest that nutrient uptake may be limited in micropropagated plants with underdeveloped root systems, potentially hindering their growth and overall performance in tissue culture conditions [49]. This limitation underscores the importance of optimizing root development protocols and nutrient supplementation strategies to enhance the nutritional status and vigor of in vitro plants [49].

9. Exploring Diversity in *Vaccinium* Species and Varieties: Explants, Optimal Growth Regulators, and Research Outcomes

The type of culture media used, the salt strength of the medium employed, and the growth regulators, type, and concentration used are key factors that must be established for every culture. Blueberries are traditionally propagated by stem cuttings, which may not be suitable for all cultivars, as evidenced by low rooting percentages, rendering it ineffective for rapidly increasing the number of new propagules for commercial introduction of new cultivars [19].

Micropropagation stands out as one of the most effective methods for rapid propagation of elite plants [8], offering year-round propagation. Micropropagation through axillary shoot production has been identified as a method for obtaining various types of blueberries [24]. Thus, plant tissue culture emerges as an excellent tool for multiplying species that naturally reproduce asexually, such as blueberries [68].

The reviewed works revealed a variety of outcomes concerning the growth and development of blueberry plants. During micropropagation, an increase in shoot size and vigor was observed, with a more abundant production of shoots compared to traditionally propagated plants [20,24].

The assessed works encompassed a diversity of blueberry species and cultivars, including *V. corymbosum* and other *Vaccinium* spp. Remarkably, studies examined different types of explants for micropropagation, such as nodal segments, shoot tips, and leaf tissues, reflecting the diverse methodologies employed in blueberry tissue culture. Overall, the results suggest that micropropagation offers an effective method for producing blueberry plants with desirable characteristics such as larger shoots, stronger roots, and faster growth, thereby contributing to improving the productivity and quality of blueberry cultivation (Table 4).

Micropropagation significantly influences the growth and morphology of *Vaccinium* plants, leading to faster growth with more shoots and increased height [5]. It has been reported that micropropagated blueberry plants yield more fruit and generate cuttings with higher rooting capacity compared to plants propagated by traditional cuttings [24]. Furthermore, the antioxidant activity observed in fruits from in vitro propagated plants surpasses that of fruits grown under natural conditions [5,85].

The germination of blueberry seeds plays a crucial role in various fields, from agricultural production to scientific research. This initial stage is essential for propagating new blueberry plants, allowing for the expansion of plantations and the development of new varieties through genetic improvement. Each blueberry seed has the potential to generate a unique plant, contributing to the preservation of genetic variability. However, germinating blueberry seeds can be a challenging process due to their delicate nature and specific requirements for temperature, humidity, and substrate. Therefore, it is important to optimize

in vitro studies to improve the germination rate of this notoriously difficult-to-cultivate species [18,49,52].

In addition to the benefits, there is a growing focus on studies aimed at enhancing antioxidant activity in blueberries [83]. This is particularly significant as research suggests that the in vitro culture of blueberry plants can lead to an increase in these beneficial compounds [23,55,85,86].

Micropropagation also facilitates the increase in photosynthetic pigments [23].

Most studies highlight zeatin as one of the most effective growth regulators in blueberry in vitro culture [1,6,7,19,53–55,81,87]. Numerous experiments have demonstrated the positive effects of zeatin on various aspects of blueberry tissue culture, including enhancing shoot multiplication rates and improving rooting efficiency [1,6,19,54,87]. Its ability to stimulate the production of lateral shoots and adventitious roots makes it particularly valuable for the propagation and establishment of in vitro blueberry plants [7].

Table 4. Different species and varieties of *Vaccinium*, explants used, best growth, regulators and achievements in each investigation.

Species	Explants Used	Achievements	Growth Regulators	Ref.
<i>V. corymbosum</i> L.	Leaf explants	Shoot proliferation for a further study with <i>A. tumefaciens</i>	0.1–10 mg/L IBA ¹ ; 0.5–5 mg/L KIN ² and 0.1–10 mg/L NAA ³	[5]
<i>V. corymbosum</i> “Bluejay”	Two-node stem explants	Shoot proliferation	1 mg/L IBA and 0.5–1.5 mg/L zeatin	[19]
<i>V. rabbiteye</i> ‘Delite’	Two-node segments	Shoot multiplication	0.5 mg/L zeatin and 5 mg/L IBA	[53]
<i>V. corymbosum</i> ‘Bluecrop’, ‘Bluegold’, ‘Bluejay’, ‘Spartan’, ‘Patriot’	Axillary organogenesis	Proliferation capacity, length of in vitro shoots, rooting	3 mg/L zeatin and 2 mg/L 2-iP ⁴	[63]
<i>V. corymbosum</i> ‘ZY09’	Apical shoots	Shoot proliferation and acclimatization	1 mg/L zeatin; 0.1 mg/L IBA and 0.0005 mg/L TDZ ⁵	[40]
<i>V. hybrid</i> ‘Blue Suede’	Nodal segments	Shoot elongation and proliferation	3–5 mg/L 2-iP and 2 mg/L zeatin	[43]
<i>V. corymbosum</i> ‘Farthing’, ‘Legacy’, and ‘FL11-35’	Non-lignified stems from	Growth of plants with large leaves	2 mg/L zeatin	[54]
<i>V. corymbosum</i> ‘Brigitta Blue’	Axillary and adventitious shoots	Shoots multiplication, chlorophyll and ascorbic acid content, antioxidant activity, and DNA methylation	10 mg/L 2-iP and 4 mg/L IAA ⁶	[20]
<i>V. corymbosum</i> ‘Duke’	Nodal segments	Regeneration by organogenesis	1 mg/L 2-iP	[23]
<i>V. corymbosum</i> × <i>V. angustifolium</i> ‘St. Cloud’, ‘Patriot’, ‘Northblue’, ‘Chippewa’	Leaf segments	Somatic embryogenesis	2 mg/L TDZ	[86]
<i>V. avotum</i> ‘Jewel’; <i>V. corymbosum</i> ‘DrisBlueTwo’	Semi-hardwood cuttings	Shoot rooting	3000 mg/L IBA	[88]
<i>V. uliginosum</i>	Leafless two-bud softwood cuttings	Shoot rooting and multiplication	3.5 mg/L 2-iP + 3.5 mg/L zeatin and 1 mg/L IAA	[89]
Hybrids of <i>V. uliginosum</i> × (<i>V. corymbosum</i> × <i>V. angustifolium</i>).	Seeds	Seed germination, shoot multiplication	1–3 mg/L 2-iP	[60]

Table 4. Cont.

Species	Explants Used	Achievements	Growth Regulators	Ref.
<i>V. arboreum</i>	Nodal segments	Shoot proliferation and rooting	0.5 mg/L zeatin and 0.01 mg/L IBA	[87]
<i>V. corymbosum</i> 'Elliot'	Stem cuttings	Shoot proliferation	5 mg/L 2-iP	[90]
<i>V. corymbosum</i> 'Duke', 'Hortblue Petite'	In vitro culture shoots	Shoots for biomass production as a source of phenolic compounds	0.5–1 mg/L zeatin	[55]
<i>V. corymbosum</i> 'Duke', 'Legacy', 'Brigitta', 'Elliott', 'Misty', 'Bluegold'	Apical shoots	Shoot proliferation for evaluation of antioxidant activity	2.5 mg/L 2-iP	[85]
<i>V. corymbosum</i> 'Legacy', 'Farthing'	Apical shoots	Shoot proliferation	4–8 mg/L BAP ⁷	[91]
<i>V. myrtillus</i> and <i>V. uliginosum</i>	Seeds	Germination and shoot proliferation	0.6–1.0 mg/L BAP	[18]
<i>V. floribundum</i> 'Kunth'	Seeds	Germination and shoot proliferation	0.5 mg/L trans-zeatin-riboside	[49]
<i>V. dunalianum</i>	In vitro seedlings	Shoot proliferation	1.5 mg/L zeatin	[81]

¹ Indole-3-butyric acid, ² kinetin, ³ α -Naphthaleneacetic acid, ⁴ 2-Isopentenyladenine, ⁵ Thidiazuron, ⁶ Indole-3-acetic acid, ⁷ 6-Benzylaminopurine.

10. Blueberry Tissue Culture Innovations

In recent years, the agricultural industry has witnessed remarkable advancements in the utilization of tissue culture techniques for the cultivation and enhancement of various crop species. Among these, blueberries stand out as a particularly promising candidate for exploration. Tissue culture offers a revolutionary approach to propagating blueberry plants, enabling the rapid generation of high-health plantlets from selected elite genotypes [92]. By meticulously controlling the growth conditions and nutrient compositions in vitro, tissue culture laboratories can produce disease-free plantlets with enhanced vigor and productivity [7]. Moreover, tissue culture facilitates the conservation of plant genetic resources by providing a platform for the long-term storage and propagation of rare and endangered blueberry cultivars, thus safeguarding biodiversity and promoting genetic resilience in the face of evolving agricultural landscapes [93]. Plant genetic resources are potential sources for farming, experimenting, and plant conservation [94]. The crisis imparted by genetic erosion instigated global conservation schemes to protect plants [94]. Biotechnology-based approaches like in vitro propagation, metabolic engineering, gene markers, and genome sequencing have substantially contributed to the conservation of dye-yielding plants [94].

Cryopreservation emerges as the premier method for safeguarding germplasm over extended durations, especially considering the arduous process of vegetative propagation required for preserving clonal genotypes in numerous fruit crops [95,96]. This technique offers the ability to store plant material in an unaltered state indefinitely, shielding it from contamination while demanding minimal upkeep [95]. Its versatility extends to enabling crossbreeding among plants with disparate flowering schedules and bridging geographical gaps in plant populations [95]. Moreover, cryopreservation plays a pivotal role in curbing disease transmission via pollination vectors [95]. Crucially, it mitigates the risks associated with somaclonal variations, common in tissue culture and field collections, respectively [94,95]. This method facilitates the cultivation of cultures characterized by both genetic uniformity and the ability for vegetative propagation among clones [95]. Thus far, vitrification-based cryopreservation methods have predominantly been employed for preserving highbush blueberry and strawberry varieties [95].

Significant progress has been achieved for the propagation of berry crops using tissue culture techniques [97]. Although bioreactor micropropagation has been developed as a cost-effective propagation technology for berry crops, genetic stability can be a problem for commercial micropropagation that can be monitored at morphological, biochemical, and molecular levels [97]. Somaclonal variations, both genetic and epigenetic, in tissue culture regenerants are influenced by different factors, such as donor genotype, explant type and origin, chimeral tissues, culture media type, concentration and combination of plant growth regulators, and culture conditions and period [97]. During plant development, epigenetic regulations play an important role as they help the plant to maintain the stability and integrity of their gene expression profiles [97]. Mutations can be induced either physically, chemically, or by tissue culture, and techniques such as callus induction, embryo formation, and regeneration can be stressful to plant cells [97]. Epigenetic variations, although typically transient, can persist across generations, even in asexually propagated plant material [97]. These variations, which involve changes in gene expression, may endure for many generations. In multicellular organisms, coordinated gene expression is governed by a combination of genetic and epigenetic mechanisms, including DNA methylation, chromatin modification, and non-coding RNA biosynthesis [97]. Shoots of two highbush blueberry cultivars, 'Patriot' and 'Chippewa', derived from semisolid media and bioreactor systems, showed higher levels of methylation when compared with their greenhouse-grown donor plants [98].

Previous studies have shown that micropropagated berries exhibit higher levels of antioxidants compared to conventionally propagated blueberries [5,9]. Goyali et al. observed that tissue culture-regenerated lowbush blueberry plants showed increased levels of antioxidants compared to their softwood cutting counterparts [99]. Conversely, micropropagated lingonberry plants were found to have lower chlorophyll a and b contents in their leaves compared to cutting-propagated plants [98]. These differing levels of bioactive compounds during plant tissue culture may be attributed to the use of plant growth regulators in the growth media [5,100].

In addition to its role in plant propagation and conservation, tissue culture holds immense promise for accelerating the pace of blueberry breeding efforts [36]. Through techniques such as somatic embryogenesis and micropropagation, researchers can expedite the development of new cultivars with improved traits, including disease resistance, fruit quality, and shelf life [86]. Furthermore, tissue culture enables precise manipulation of plant genetics, facilitating the incorporation of desirable traits from wild relatives or exotic germplasm into cultivated blueberry varieties [101,102].

In the realm of crop improvement programs, integrating genome editing tools such as CRISPR/Cas9 is pivotal for detecting undesirable traits arising from epigenetic variation during the tissue culture process [91,101,102]. Additionally, leveraging various bioinformatic tools aids in predicting the inheritance of altered epigenetic patterns in regenerants [97].

Automation and robotization are emerging as promising areas in blueberry micropropagation, offering opportunities to improve the efficiency and precision of various stages of the process [103]. In modern micropropagation laboratories, automated systems are used for precise and standardized preparation of culture media, ensuring consistency in media composition and reducing the time and effort required for this initial step [104]. Additionally, programmable robots are being employed in the transfer of blueberry explants, manipulating plant tissues quickly and accurately to ensure a high success rate in inoculation into culture media [105]. This not only increases process efficiency but also reduces the risk of contamination during explant manipulation [105]. To monitor plant growth in culture, automated monitoring systems using sensors and computer vision technologies are being developed [106]. These systems enable precise assessment of seedling development, allowing for early identification of growth issues and corrective measures [106]. Furthermore, maintenance of optimal growth conditions for plants can also be automated. Automated systems for controlling temperature, humidity, and light-

ing ensure that blueberry plants receive the ideal environmental conditions for growth throughout the micropropagation process [107].

These automated technologies have the potential to revolutionize blueberry micropropagation, increasing efficiency, reducing costs, and enabling more scalable and consistent production of high-quality seedlings [105].

However, technical complexities, cost considerations, and regulatory hurdles pose significant barriers to entry for small-scale growers and resource-limited regions. Moreover, concerns regarding genetic stability, acclimatization of tissue-cultured plants, and socio-economic implications necessitate careful consideration and further research.

11. Conclusions and Future Perspectives

In conclusion, micropropagation emerges as a versatile and indispensable technique for various applications in blueberry tissue culture. Beyond its function in commercial plant propagation, micropropagation plays a crucial role in research endeavors aimed at genetic improvement, conservation, reforestation, and medicinal purposes. The efficient transition from field conditions to tissue culture is paramount for ensuring the availability of high-quality plant materials for downstream applications. Moreover, advances in micropropagation techniques are instrumental in optimizing protocols for the proliferation and regeneration of blueberry tissues, enabling cryopreservation, *in vitro* selection of new genotypes, interspecific and intersectional hybridization, and genetic transformation. Furthermore, tissue culture serves as a vital tool for the conservation of plant biodiversity, allowing for the preservation of valuable genetic resources and the protection of endangered plant species from extinction. This comprehensive approach not only facilitates the multiplication of selected plants but also supports molecular, antifungal, and chemical studies, thereby contributing to the advances and sustainability of blueberry cultivation and research efforts.

Future perspectives in blueberry tissue culture research include exploring somaclonal variations, which are genetic variations that arise from tissue culture processes. Understanding these variations can lead to the development of novel blueberry varieties with desirable traits. Additionally, studies focused on metabolites and molecular mechanisms hold promise for uncovering the biochemical pathways involved in blueberry growth, development, and stress responses. By elucidating these mechanisms, researchers can identify targets for genetic manipulation or breeding efforts aimed at improving blueberry yield, quality, and resilience to environmental challenges. Overall, continued advances in blueberry tissue culture research will further enhance our understanding of this valuable crop and contribute to its sustainable cultivation and utilization.

Also, exploring the integration of automation technologies in future research endeavors could enhance productivity and decrease production costs in blueberry micropropagation. Blueberries, known for their compatibility with *in vitro* propagation and substantial commercial value, stand to benefit significantly from such advancements.

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