J. Appl. Biol. Sci., 20 (1): 17-23, January 2026
https://dx.doi.org/10.71336/jabs.1545
RESEARCH ARTICLE

Journal of Applied Biological Sciences
EISSN: 2146-0108; License: CC BY 4.0
Anatolia Academy of Sciences Open Access

Studies on the *in vitro* proliferation stage of blueberry (*Vaccinium corymbosum* L. cv. O'Neal and Duke)

Evrim Okutan[1], Ebru Akyüz Çağdaş[1]*, Mehmet Polat[2], Okan Sarıtoprak[1], Hakan Aktaş[2], and Ş. Şebnem Ellialtıoğlu[3]

[1]*Has Biotech Araştırma Geliştirme Tarım Sanayi ve Ticaret A.Ş., Antalya, Türkiye*

[2]*Isparta Uygulamalı Bilimler Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümü, Isparta, Türkiye*

[3]*Ankara Üniversitesi Teknokent, Doqutech Academy Tarım Arge Eğitim ve Danışmanlık Ltd. Şti., Ankara, Türkiye*

*Correspondence:
Ebru Akyüz Çağdaş
Email: ebrucagdas@has-biotec.com.tr

Received: November 26, 2025
Revised: December 24, 2025
Accepted: January 05, 2026

ABSTRACT: Blueberry (*Vaccinium corymbosum*), a berry fruit whose cultivation is widespread in the world especially in the mainland of North America and also in Peru, Mexico and European countries, is one of the members of the Ericaceae family. The source of the purple color is delphinidin derivative pigments and antioxidant polyphenols increase the importance of this fruit in terms of health. Blueberry, whose annual production in the world has exceeded 2 million tons, is produced in Turkey as 6620 tons in 5454 decares of area. Propagation by tissue culture is preferred in the production of healthy plant material for the increasing need for sapling. Shoot tips cultured in media containing MS basic nutrient medium + 2 mg·L^{-1} zeatin + 30 g·L^{-1} sucrose + 6.5 g·L^{-1} agar in the first development stage were transferred to media with different contents after 4 weeks and proliferation experiments were established. Propagation rates were determined in Duke and O'Neal varieties by adding 2 mg·L^{-1} zeatin to MS, DKW and WPM nutrient media. The highest number of shoots was obtained from O'Neal variety 16.44 shoots per explant in the 3rd subculture in the medium containing DKW + 2 mg·L^{-1} zeatin + 30 g·L^{-1} sucrose + 6.5 g·L^{-1} agar and pH 5.5.

KEYWORDS: Tissue culture, Blueberry, Micropropagation, Zeatin

INTRODUCTION

Blueberry (*Vaccinium corymbosum*), a perennial deciduous shrub belonging to the *Ericaceae* family, is widely cultivated in the Northern Hemisphere [1, 2]. Compared to other fruit crops, its cultivation has a relatively short history of approximately one century [3]. Blueberries are rich in polyphenols with antioxidant activity, and the delphinidin derivatives responsible for their purple color exhibit strong antioxidant properties, making them an important resource for human health [4, 5]. Consequently, blueberries have emerged as one of the most promising crops worldwide, attracting increasing interest from fruit growers [6]. Global production more than doubled between 2010 and 2019, rising from 439,000 metric tons to approximately 1 million tons. In 2010, the leading producers were the United States (224,000 tons), Canada (84,000 tons), Chile (76,000 tons), and France (11,000 tons). This number began to increase from 2012 onwards, with at least 11 countries exceeding the 10,000-ton threshold by 2019. Peru showed the most dramatic growth, increasing from 50 tons to approximately 125,000 tons, becoming the fourth-largest producer after the United States, Canada, and Chile. Today, Peru is a leading global exporter. Global production has approached approximately 2 million tons, with the United States, Canada, and Peru being the leading producers [7]. In Turkey, commercial production is primarily concentrated in the Black Sea region, particularly in Rize, although cultivation has expanded to other regions using varieties adapted to different ecological conditions. Despite efforts to establish orchards through domestic propagation of foreign cultivars or direct importation of seedlings, meeting the growing demand remains a challenge. As of the end of 2019; 443 tons of blueberries were produced on 1,055 decares, increasing to 6,620 tons by 2024 [8]. Limited domestic production and reliance on imports contribute to the relatively high market price of this fruit.

Traditional propagation methods for blueberries are labor-intensive, time-consuming, and limited by seasonal growth cycles, which significantly constrain large-scale production. In contrast, micropropagation offers an efficient approach for the uniform and large-scale production of plants [9]. Compared to conventional seedling cultivation, *in vitro* culture not only reduces propagation time but also provides precise control over environmental conditions, minimizing the risk of variability. Vegetative propagation through traditional methods can facilitate the transfer of microbial

pathogens and is generally inefficient in terms of plant multiplication. These limitations can be effectively addressed using *in vitro* techniques, which ensure a consistent supply of healthy, pathogen-free planting material [10, 11, 12]. Tissue culture methods are particularly advantageous for producing virus-free plants [13] and have found widespread applications across numerous plant species [14, 15].

Micropropagation has proven to be the most effective system for the rapid production of new blueberry cultivars, enabling the generation of large numbers of uniform plants. Over the past three decades, significant advances have been made in blueberry micropropagation techniques [16-21]. Furthermore, studies have shown that micro-propagated blueberries often exhibit higher fruit yields and enhanced rooting ability compared to conventionally propagated plants [22, 23].

Different basal media have been employed for *in vitro* culture of blueberries, including MS, Anderson, and WPM [24-27]. In our previous research on the establishment phase of blueberry *in vitro* culture, a range of basal media and growth regulators was evaluated, and plant development under different pH conditions was investigated. The study incorporated MS, DKW, AN, OM, and WPM media, with MS, WPM, and DKW identified as the most suitable for blueberry propagation [28]. While subsequent investigations utilizing advanced systems, such as the SETIS® bioreactor, have demonstrated that liquid media can significantly enhance proliferation rates [29], classical semi-solid culture remains the fundamental standard for establishing baseline protocol parameters and is often more accessible for small-scale commercial laboratories due to lower initial investment and simplified handling. Therefore, the primary objective of the present study was to assess the proliferation potential of two blueberry cultivars using three different basal media under classical semi-solid *in vitro* conditions.

MATERIALS AND METHODS

Materials

Two-year-old shoots of *Vaccinium corymbosum* cultivars ‘Duke’ and ‘O’Neal’ were used as explant sources. Following the appearance of fresh shoots, *in vitro* proliferation experiments were conducted using 2 cm-long shoots developed from shoot-tip explants previously cultured in tissue culture [28].

Experiments were conducted according to a Randomized Complete Block Design with 3 replicates and 15 explants in each replicate. The data was subjected to Variance analysis using the MINITAB 18.0 software package and mean separations were performed using Duncan’s multiple range test at $p \leq 0.05$.

Methods

Healthy shoots grown in tissue culture and having completed the establishment phase were used for multiplication experiments once they reached 2 cm in length. Semi-solid media in glass jars were employed for all experiments, which were conducted under sterile conditions in a laminar flow cabinet. Entire 2 cm explant pieces were initially cultured in jars containing agar-based media. Contaminated or poorly developing explants were discarded, and the remaining explants were transferred to fresh agar media for the first subculture. The subculture process was repeated two more times, and data recorded up to the third subculture were used to evaluate the experimental results.

Preparation and Sterilization of Media

The basal media used in this study were Murashige and Skoog [24], Driver and Kuniyuki (DKW) [30], and McCown’s Woody Plant Medium (WPM) [27]. Zeatin (2 $mg \cdot L^{-1}$), sucrose (3% w/v), and agar (6.5 $g \cdot L^{-1}$) were added to the media, and the pH was adjusted to 5.5. Agar-containing media were dispensed into glass jars at 40 mL per jar and autoclaved at 121 °C and 1 atm pressure for 15 minutes, then placed in a laminar flow cabinet.

Subculture and Observations

Shoot tips initially cultured on MS medium supplemented with 2 $mg \cdot L^{-1}$ zeatin, 30 $g \cdot L^{-1}$ sucrose, 6.5 $g \cdot L^{-1}$ agar at pH 5.5 were transferred to different media compositions after four weeks to establish proliferation experiments. During subculture, the number of axillary shoots produced per explant and the length of each shoot were recorded. Care was taken to ensure that explants in the first subculture were at least 2 cm long before being transferred to jars containing the same medium composition. Each jar contained 15 explants, and three replicates were used. Micropropagation experiments involved three subculture cycles and shoot measurements from the subcultures were averaged. Shoots were sub-cultured every four weeks.

RESULTS AND DISCUSSION

During the proliferation phase, three subcultures were conducted using three different basal media compositions, and the number and length of the shoots were recorded. After the third subculture, statistically significant differences were observed among the media in terms of the number of axillary shoots and their lengths produced per explant within four weeks (Tables 1 and 2).

According to the results, in the 'Duke' cultivar, the differences in shoot number among media composition and subculture numbers were found to be statistically significant (p-value 0.002, 0.000 respectively), whereas the 'medium × subculture number' interaction was not statistically significant (p-value 0.230). The average number of shoots across all three subcultures in the 'Duke' cultivar was 6.48 adventitious shoots in MS medium, 8.19 in DKW medium, and 6.13 in WPM medium. When the subcultures were examined individually, considering the average of each medium at each subculture, the lowest shoot number was obtained from the 1st subculture, followed by the 2nd subculture, while the highest adventitious shoot production was achieved in the 3rd subculture (4.50, 7.42 and 8.78 adventitious shoots, respectively). The DKW medium produced the most favorable results for shoot proliferation in the 'Duke' cultivar, yielding 9.36 shoots per explant in the 2nd subculture and 10.10 shoots per explant in the 3rd subculture. Since there was no statistical difference between these two values, considering the time efficiency, it can be concluded that the highest shoot number compared to the other two media was achieved in the 2nd subculture of DKW medium (Table 1).

Table 1. Number of shoots per explant and shoot lengths in 'Duke' blueberry cultivar across different media during the 1st–3rd subcultures.

Medium	Subculture number	cv. Duke	
		Adventitious shoot number	Shoot length (mm)
MS	1	4.27 c	31.2 b
	2	6.19 bc	30.7 b
	3	8.99 ab	20.0 cd
DKW	1	5.12 c	29.7 b
	2	9.36 ab	28.3 b
	3	10.10 a	21.7 c
WPM	1	4.11 c	36.4 a
	2	6.72 bc	29.8 b
	3	7.25 a-c	17.6 d

Means followed by the same letter or sharing common letters within a column are not significantly different according to Duncan's multiple range test at $p \leq 0.05$.

The interaction between medium and subculture was found to be significant for shoot length ($p = 0.000$) in 'Duke' variety. The highest shoot length was observed in the explants of the 1st subculture in WPM medium (36.4 mm). Except for the shoots in the 3rd subculture, the lengths of the other shoots generally fell within the same statistical group, ranging from 28.3 to 31.2 mm. In the 3rd subculture, shoot lengths decreased across all media, with an average shoot length of 19.77 mm.

In the 'O'Neal' cultivar, shoot number differed significantly among media compositions and subculture numbers ($p = 0.029$ and $p = 0.000$, respectively), while the interaction between medium and subculture number was not significant ($p = 0.436$). Across all three subcultures, the average number of adventitious shoots per explant was 9.92 in MS medium, 11.57 in DKW medium, and 9.53 in WPM medium. When the subcultures were analyzed individually, the lowest shoot number was recorded in the 1st subculture, followed by the 2nd, with the highest adventitious shoot production occurring in the 3rd subculture (6.24, 10.32 and 14.46 shoots, respectively). Among the tested media, DKW yielded the most favorable proliferation results, producing 16.44 shoots per explant in the 3rd subculture, compared to 14.20 in MS and 12.73 in WPM. As no statistically significant differences were found among these three media in the 3rd subculture, all were considered suitable for promoting shoot proliferation in the 'O'Neal' cultivar (Table 2) (Figure 1). Images of adventitious shoots developed in the third subculture of the 'O'Neal' blueberry cultivar on DKW medium are shown in Figure 2.

Shoot length in the 'O'Neal' cultivar was significantly affected by the interaction between medium and subculture ($p = 0.006$). The longest shoots in the 1st subculture were observed in explants grown on MS, DKW, and WPM media, measuring 34.6 mm, 33.0 mm, and 32.8 mm, respectively. The shortest shoot lengths were recorded in the 3rd subculture on DKW and WPM media (21.6 mm and 19.3 mm).

Across all media and subcultures, 'O'Neal' consistently produced a higher number of adventitious shoots than 'Duke', with a markedly greater magnitude of increase across successive subcultures. Conversely, shoot length declined significantly in both cultivars regardless of the basal medium. This reduction was more pronounced in 'O'Neal', particularly under high-proliferation conditions (e.g., DKW in the third subculture).

These contrasting trends reflect a well-documented trade-off during repeated *in vitro* subculturing. Enhanced shoot initiation increases competition for assimilates and growth regulators, thereby limiting internode elongation. The stronger proliferative response of 'O'Neal' suggests a higher sensitivity to cytokinin-mediated induction, whereas 'Duke' allocates more resources to structural elongation during early stages.

Table 2. Number of shoots per explant and shoot lengths in O'Neal blueberry cultivar across different media during the 1st–3rd subcultures.

Medium	Subculture number	cv. O'Neal	
		Adventitious shoot number	Shoot length (mm)
MS	1	6.26 ^{ef}	34.6 ^{a}
	2	9.31 $^{c-f}$	29.7 ^{cd}
	3	14.2 ^{ab}	17.5 ^{f}
DKW	1	7.08 $^{d-f}$	33.0 ^{ab}
	2	11.18 $^{b-d}$	30.1 $^{b-d}$
	3	16.44 ^{a}	21.6 ^{e}
WPM	1	5.37 ^{f}	32.8 $^{a-c}$
	2	10.48 $^{b-e}$	29.6 ^{d}
	3	12.73 $^{a-c}$	19.3 ^{ef}

Means followed by the same letter or sharing common letters within a column are not significantly different according to Duncan's multiple range test at $p \leq 0.05$.

Figure 1. Images from the shoot proliferation stage of O'Neal variety in MS and DKW basic nutrient medium and pH: 5.5 conditions, in a composition containing 2 mg·L^{-1} zeatin.

Figure 2. **A**dventitious shoots developed in the third subculture on DKW medium in the 'O'Neal' blueberry cultivar.

In this study, the collected data were evaluated collectively and statistical analysis of shoot number and shoot length revealed a significant 'genotype × medium × subculture number' interaction. However, since the objective of this study was not to determine at which medium or subculture stage the highest or lowest values were obtained for each cultivar, both genotypes were analyzed separately through statistical procedures. The results confirm that genotype is a primary determinant in *Vaccinium*

micropropagation. 'O'Neal' consistently demonstrated a higher proliferative capacity than 'Duke', suggesting a higher sensitivity to cytokinin-mediated axillary bud release. The superiority of DKW medium for multiplication in both cultivars likely stems from its nutrient density. Compared to WPM and MS, DKW contains higher concentrations of calcium and magnesium, which are essential for cell wall integrity and enzymatic processes during rapid biomass accumulation [31].

The observed reduction in shoot length during successive subcultures indicates a physiological "trade-off." This can be explained through several mechanisms:

1. **Cytokinin Accumulation:** Repeated exposure to 2 mg·L^{-1} Zeatin promotes high multiplication by breaking apical dominance. However, over-saturation of cytokinins in tissues often inhibits internode elongation, a phenomenon documented by George et al. [32].
2. **Sink Competition:** As the number of shoots per explant increases (e.g., doubling in 'O'Neal' from the 1st to 3rd subculture), the competition for limited carbohydrates and mineral nutrients within the culture vessel intensifies. This reduces the resource allocation available for the elongation of individual shoots.
3. ***In Vitro* Aging:** Repeated subculturing may lead to a gradual loss of vigor in terms of vertical growth, shifting the plant's metabolic energy toward axillary branching rather than primary axis extension.

Studies on tissue culture have demonstrated that genotype plays a crucial role in determining the outcomes of *in vitro* propagation, as different *Vaccinium* cultivars often exhibit distinct responses even under identical medium compositions and light conditions. Scalzo et al. [33] reported that among five *Vaccinium* genotypes cultured under four proliferation media and two light intensities, the composition of the medium had a significant effect on shoot length and number, while certain traits, such as callus formation, were primarily influenced by genotype. Similarly, the comprehensive review by Correia et al. [34] emphasized that effective *in vitro* propagation protocols require genotype-specific optimization. The authors highlighted that interactions among growth regulators, medium composition, and environmental conditions substantially affect propagation efficiency, rooting success, and acclimatization outcomes.

Considerable variability among blueberry (*Vaccinium*) cultivars in terms of both shoot proliferation and root development has been reported by Georgieva and Kondakova [35] and Fan et al. [36] highlighting the importance of developing cultivar-specific micropropagation protocols to optimize regeneration and rooting efficiency in *Vaccinium* species. Several studies have explored the influence of medium composition and plant growth regulators on shoot proliferation and elongation in different blueberry cultivars. Cao and Hammerschlag [37] reported that in 'Duke', 'Georgiagem', 'Sierra', and 'Jersey' cultivars, the highest shoot numbers per explant—13.0, 13.0, 12.6 and 4.6 respectively—were obtained using a modified WPM regeneration medium (pH 5.2) supplemented with zeatin. Similarly, Ostrolucká et al. [13] demonstrated that when 'Bluecrop', 'Berkeley', and 'Duke' cultivars were cultured on AN medium containing 2 mg·L^{-1} zeatin, the corresponding shoot proliferation rates reached 3.94, 3.78 and 5.28 respectively, with 'Duke' exhibiting the most favorable response. Ružić et al. [38] further investigated the propagation of 'Berkeley', 'Bluecrop', and 'Goldtraube' using MS and mAN (modified Anderson's Rhododendron) media, reporting that shoot length varied depending on both cultivar and medium combination, with the longest shoots obtained in 'Goldtraube' grown on mAN medium supplemented with 0.5 mg·L^{-1} zeatin and 1 mg·L^{-1}IBA. Frett and Smagula [39], utilizing the Zimmerman and Broome [26], basal medium, examined the effects of various 2-iP and IAA combinations on lowbush blueberry and found that increasing 2-iP concentration enhanced shoot elongation, with a maximum length of 20.4 mm estimated at 12 mg·L^{-1} 2-iP through regression analysis. Likewise, Debnath and McRae [40] reported that in *Vaccinium vitis-idaea*, the longest shoots were produced on modified MS medium supplemented with either 5.7 µM zeatin or 12.3 µM 2-iP, noting that while higher cytokinin concentrations promoted shoot multiplication, they concurrently reduced shoot elongation. Overall, shoot proliferation on MS medium is generally slow [38], whereas WPM has been found more suitable for micropropagation compared to Anderson's medium [25].

CONCLUSION

In this study, the effects of different basal medium compositions on the proliferation stage of two highbush blueberry (*Vaccinium corymbosum*) cultivars under *in vitro* conditions were investigated. The aim was to determine the number and length of shoots across successive subcultures. This study demonstrates that while DKW medium supplemented with 2.0 mg·L^{-1} Zeatin is the superior basal medium for maximizing shoot proliferation in both 'Duke' and 'O'Neal' cultivars, the morphogenic response is highly genotype dependent. 'O'Neal' represents a high-multiplication variety, whereas 'Duke' requires a more balanced approach to maintain shoot elongation. Consequently, a staged protocol—utilizing WPM for initial

elongation followed by DKW for multiplication—is recommended for 'Duke' to ensure high-quality explant production.

While the present study identifies DKW as a superior basal medium for the proliferation of 'Duke' and 'O'Neal' cultivars, further research is required to refine the micropropagation protocol for commercial-scale applications. Future investigations should focus on the following parameters:

- **Optimization of Growth Regulators:** Beyond the fixed concentration of zeatin used here, a response surface methodology could be employed to determine the ideal balance between different cytokinins (e.g., meta-topolin or 2-iP) and low concentrations of auxins (e.g., IBA) to further enhance shoot quality and spontaneous rooting.
- **pH Stability and Nutrient Uptake:** Considering the decline in shoot length over subcultures, studies monitoring the pH fluctuations of the medium during the 28-day growth cycle are necessary, as *Vaccinium* species are highly sensitive to media acidification, which affects nutrient bioavailability.
- **Light Conditions:** The transition from traditional fluorescent lighting to specific LED spectra (e.g., varying red/blue light ratios) should be explored to evaluate its effects on internode elongation.

DECLARATION

Acknowledgement

This study was prepared as part of the TÜBİTAK TEYDEB Project No. 7210364. We would like to express our gratitude to TÜBİTAK for their support.

Authorship Contributions

Concept: Ş. Ş. E., M. P., Design: H .A., E. A. Ç., Data Collection or Processing: M. P., E. A. Ç., Analysis or Interpretation: M.P., Ş. Ş. E., Literature Search: E. O., O. S., Writing: Ş. Ş. E., E. A. Ç., Control & Photo: H. A.

Financial Disclosure

This research was funded by the Has Biotech tissue culture company's own resources. It was also supported by the TÜBİTAK TEYDEB project.

Conflict of Interest

The authors declared that there is no conflict of interest.

REFERENCES

[1] Zhao, X., Zhan, L., Zou, X. (2011): *In vitro* high-frequency regeneration of half-highbush 'Northland' blueberry. New Zealand Journal of Crop and Horticultural Science 39(1): 51-59. https://doi.org/10.1080/01140671.2010.526620

[2] Retamales, J. B. (2012): Blueberries crop production and utilization. In: Retamales J.B., Hancock J.F (Eds.), Blueberries. CABI 8 p. DOI: 10.1079/9781845938260.0000

[3] Stribley, D., Read, D. (1976): The biology of mycorrhiza in the *Ericaceae*. New Phytologist 77: 63–72. https://doi.org/10.1111/j.1469-8137.1976.tb01501.x

[4] You, Q., Wang, B., Chen, F., Huang, Z., Wang, X., Luo, P.G. (2011): Comparison of anthocyanins and phenolics in organically and conventionally grown blueberries in selected cultivars. Food Chemistry 125(1): 201–208. https://doi.org/10.1016/j.foodchem.2010.08.063

[5] Zheng, X., Zhang, Z., Jin, C., Mu, Y., Liu, C., Chen, Z., Liu, H., Lin, Z. (2015): Purification characteristics and parameters optimization of anthocyanin extracted from blueberry. International Journal of Agricultural and Biological Engineering 8(2): 135–144. https://doi.org/10.3965/j.ijabe.20150802.1158

[6] Brazelton, C. (2013): World blueberry acreage & production. World Blueberry Acreage & Production Report, p. 77. North American Blueberry Council. http://www.chilealimentos.com/2013/phocadownload-Aprocesados_congelados/nabc_2012-world-blueberry-acreage-production.pdf

[7] Anonymous (2025): Blueberries around the globe: past, present, and future. United States Department of Agriculture Foreign Agricultural Service. https://www.fas.usda.gov/data/blueberries-around-globe-past-present-and-future (Access: 25.12.2025)

[8] Anonim (2025): TÜİK (2024): Maviyemiş- Blueberry üretim miktarları. Türkiye İstatistik Kurumu. tuikweb.tuik.gov.tr (Access: 11.10.2025)

[9] Meiners, J., Schwab, M., Szankowski, I. (2007): Efficient in vitro regeneration systems for *Vaccinium* species. Plant Cell, Tissue and Organ Culture 89(2): 169–176. https://doi.org/10.1007/s11240-007-9230-7

[10] Tsao, C.W.V., Postman, J. D., Reed, B.M. (2000): Virus infections reduce in vitro multiplication of 'Malling Landmark' raspberry. *In Vitro* Cellular & Developmental Biology - Plant 36(1): 65-68. https://doi.org/10.1007/s11627-000-0015-4

[11] O'Herlihy, E. A., Croke, J. T., Cassells, A. C. (2003): Influence of in vitro factors on titre and elimination of model fruit tree viruses. Plant Cell, Tissue and Organ Culture 72(1): 33-42. https://doi.org/10.1023/A:1021235418146

[12] Gajdošová, A., Ostrolucká, M.G., Libiaková, G., Ondrušková, E., Šimala, D. (2006): Microclonal propagation of *Vaccinium* sp. and *Rubus* sp. and detection of genetic variability in culture in vitro. Journal of Fruit and Ornamental Plant Research 14(Suppl.1): 61-76.

[13] Ostrolucká, M.G., Libiaková, G., Ondrušková, E., Gajdošová, A. (2004): In vitro propagation of *Vaccinium* species. Acta Universitatis Latviensis 676: 207–215.

[14] Kuo, C.L., Agrawal, D.C., Chang, H.C., Chiu, Y.T., Huang, C.P., Chen, Y.L., Huang, S.H., Tsay, H.S. (2015): In vitro culture and production of syringin and rutin in *Saussurea involucrata* (Kar. et Kir.) – an endangered medicinal plant. Botanical Studies 56: 12. https://doi.org/10.1186/s40529-015-0092-8

[15] Zhang, J., Tian, J., Tai, D.Q., Li, K.T., Zhu, Y.J., Yao, Y.C. (2016): An optimized TRV-based virus-induced gene silencing protocol for *Malus* crabapple. Plant Cell, Tissue and Organ Culture 126: 499–509. https://doi.org/10.1007/s11240-016-1019-0

[16] Fukui, H., Murakami, Y., Harada, T., Tamura, T. (1991): Response of highbush blueberry axillary leaf bud apices to growth regulators and its seasonal changes. Memoirs of the Faculty of Agriculture, Hokkaido University 15: 1–6.

[17] Gonzalez, M. V., Lopez, M., Valdes, A. E., Ordas, R. J. (2000): Micropropagation of three berry fruit species using nodal segments from field-grown plants. Annals of Applied Biology 137(1): 73-78. https://doi.org/10.1111/j.1744-7348.2000.tb00049.x

[18] Isutsa, D. K., Pritts, M. P., Mudge, K. W. (1994): Rapid propagation of blueberry plants using *ex vitro* rooting and controlled acclimatization of micropropagules. HortScience 29(10): 1124-1126. https://doi.org/10.21273/HORTSCI.29.10.1124

[19] Lyrene, P. M. (1980): Micropropagation of rabbiteye blueberries. HortScience 15(1): 80-81. https://doi.org/10.21273/HORTSCI.15.1.80

[20] Nickerson, N. L. (1978): *In vitro* shoot formation in lowbush blueberry seedling explants. HortScience 13(6): 698. https://doi.org/10.21273/HORTSCI.13.6.698

[21] Wolfe, D., Chin, C. K., Eck, P. (1986): Relationship of the pH of medium to growth of 'Bluecrop' highbush blueberry *in vitro*. HortScience 21(2): 296-298. https://doi.org/10.21273/HORTSCI.21.2.296

[22] El-Shiek, A., Wildung, D. K., Luby, J. J., Sargent, K. L., Read, P. E. (1996): Long-term effects of propagation by tissue culture or softwood single-node cuttings on growth habit, yield, and berry weight of 'Northblue' blueberry. Journal of the American Society for Horticultural Science 121(2): 339-342. https://doi.org/10.21273/JASHS.121.2.339

[23] Lyrene, P. M. (1981): Juvenility and production of fast-rooting cuttings from blueberry shoot cultures. Journal of the American Society for Horticultural Science 106(3): 396-398. https://doi.org/10.21273/JASHS.106.3.396

[24] Murashige, T., Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

[25] Anderson, W. C. (1980): Tissue culture propagation of red and black raspberries, *Rubus idaeus* and *R. occidentalis*. Acta Horticulturae 112: 13–20. https://doi.org/10.17660/actahortic.1980.112.1

[26] Zimmerman, R.H., Broome, O.C. (1980): Blueberry micro-propagation. Proceedings of the Conference on Nursery Production of Fruit Plants Through Tissue Culture – Applications and Feasibility. USDA, ARR-NE-11: 44–47.

[27] Lloyd, G., McCown, B. (1981): Commercially-feasible micro-propagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Combined Proceedings of the International Plant Propagators' Society 30: 421–427.

[28] Okutan, E., Akyüz Çağdaş, E., Polat, M., Sarıtoprak, O., Aktaş, H., Ellialtıoğlu, Ş.Ş. (2024): Maviyemiş (*Vaccinium corymbosum* L.)'in *in vitro* kültüre alınması aşamasında besin ortamı içerikleri ve genotip etkisi üzerinde çalışmalar. Bitkisel Üretimde Yeni Teknolojiler (Eds: Kasım, R., Kasım, M.U.), Bidge Yayınları, Ankara, Türkiye.

[29] Akyüz Çağdaş, E., Okutan, E., Sarıtoprak, O., Polat, M., Aktaş, H. (2024): Doku kültürüyle maviyemiş (*Vaccinium corymbosum* cv. Duke) çoğaltımında yeni nesil biyoreaktör kullanımı üzerine bir araştırma. BAHÇE 53 (Özel Sayı 1): 342–348.

[30] Driver, J.A., Kuniyuki, A.H. (1984): *In vitro* propagation of Paradox walnut rootstock. HortScience 19(4): 507-509. https://doi.org/10.21273/HORTSCI.19.4.507

[31] Debnath, S. C. (2007): Influence of ionic strength and donor plant age on *in vitro* propagation of highbush blueberry (*Vaccinium corymbosum* L.). African Journal of Biotechnology 6(14): 1653-1659.

[32] George, E. F., Hall, M. A., De Klerk, G. J. (2008): Plant Propagation by Tissue Culture 3rd Edition. Springer.

[33] Scalzo, J., Donno, D., Miller, S., Ghezzi, M., Mellano, M. G., Cerutti, A. K., Beccaro, G. L. (2016): Effect of genotype, medium and light on *in vitro* plant proliferation of *Vaccinium* spp. New Zealand Journal of Crop and Horticultural Science 44(4): 231-246. https://doi.org/10.1080/01140671.2016.1206946

[34] Correia, S., Matos, M., Leal, F. (2024): Advances in blueberry (*Vaccinium* spp.) *in vitro* culture: a review. Horticulturae 10(6): 533. https://doi.org/10.3390/horticulturae10060533

[35] Georgieva, M., Kondakova, V. (2021): *In vitro* propagation of *Vaccinium corymbosum* L. Bulgarian Journal of Agricultural Science 27(2): 323–327.

[36] Fan, S., Jian, D., Wei, X., Chen, J., Beeson, R.C., Zhou, Z., Wang, X. (2017): Micropropagation of blueberry 'Bluejay' and 'Pink Lemonade' through *in vitro* shoot culture. Scientia Horticulturae 226: 277–284. https://doi.org/10.1016/j.scienta.2017.08.052

[37] Cao, X., Hammerschlag, F. A. (2000): Improved shoot organogenesis from leaf explants of highbush blueberry. HortScience 35: 945–947. https://doi.org/10.21273/HORTSCI.35.5.945

[38] 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(2): 97–103. https://doi.org/10.3233/JBR-2012-030

[39] Frett, J. J., Smagula, J. M. (1983): *In vitro* shoot production of lowbush blueberry. Canadian Journal of Plant Science 63(2): 467-472. https://doi.org/10.4141/cjps83-055

[40] Debnath, S. C., McRae, K. B. (2001): *In vitro* culture of lingonberry (*Vaccinium vitis-idaea* L.) the influence of cytokinins and media types on propagation. Small Fruits Review 1(3): 3-19. https://doi.org/10.1300/J301v01n03_02