

EFFECT OF AUXINS AND ACTIVATED CARBON ON *IN VITRO* ROOTING OF TWO BLUEBERRY CULTIVARS (*VACCINIUM CORYMBOSUM*)

Short communication

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ABSTRACT

The optimization of tissue culture protocols for blueberry propagation is crucial to meet the increasing demand for disease-free planting materials. This study aimed to determine the effects of three auxins (IBA, IAA, and NAA) at different concentrations to enhance root and shoot development of two blueberry cultivars with different abilities to root, ‘Lanmei #1’ and ‘Brightwell’. Results showed that NAA at 0.5 mg·L⁻¹ significantly promoted shoot number and length in ‘Lanmei #1’ while ‘Brightwell’ showed a limited response. IAA had a variable impact on both shoot and root parameters, with ‘Lanmei #1’ showing more pronounced positive responses. IBA showed a differential response on root and shoot parameters in both cultivars, with ‘Lanmei #1’ showing a superior response for shoot number, length, root length, and overall plant vigor. Activated carbon had minimal effect on growth parameters across both cultivars. ‘Lanmei #1’ demonstrated a robust response to auxin treatments, suggesting it is more suitable for *in vitro* rooting. The present results confirm previous data on the different abilities of blueberry genotypes to root and the necessity of optimizing the rooting protocol depending on the cultivar.

Key words: *in vitro* culture, growth regulators, micropropagation, rooting potential

INTRODUCTION

Blueberries (*Vaccinium*) are the members of the family Ericaceae. The genus *Vaccinium* comprises about 450–500 species, most of which are widespread on mountain slopes in tropical regions, while the rest occur in subtropical, temperate, and boreal regions of the Northern Hemisphere (Retamal et al. 2012). Blueberries exhibit distinctive characteristics: fleshy, small-sized fruit containing abundant antioxidant compounds such as phenolic, flavonoids, tannin, and ascorbic acid, along with minerals and fruit colorants such as anthocyanin and carotenoid (Ştefănescu et al. 2020). The reputed health benefits of berries are often attributed to their potent bioactive phenolic compounds, which potentially synergize to enhance human health.

Besides their significance in commercial production, berries have medicinal properties and are utilized as ornamental landscape ground cover (Ştefănescu et al. 2020). Blueberries are extensively cultivated commercially in various countries across Asia, Australia, Europe, New Zealand, and South America (Smolarz & Pluta 2014; Titok et al. 2021). Blueberries in nurseries are usually propagated using cuttings, a simple method but time-consuming in the case of large-scale production. Micropropagation, a more efficient method, facilitates planting material and enhances production areas (Chen & Henny 2008; Chen et al. 2018). Various media are used for *in vitro* blueberry culture, including MS (Murashige & Skoog 1962), Anderson (Anderson 1980), Z-2 (Zimmerman & Broome 1980), and WPM medium (Lloyd & McCown 1981).

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Shoot proliferation on MS medium is characterized by a slow rate (Ružić et al. 2012); in contrast, WPM has been identified as a more suitable medium for micropropagation than Anderson's (Sedlak & Papstein 2009). For shoot proliferation, commonly used growth regulators are 6-(4-hydroxy-3-methylbut-2-enylamino)purine (zeatin, ZT), 6-(γ,γ -dimethylallylamino)purine (2iP), used alone or in combination with indole-3-acetic acid (IAA) (Litwińczuk & Wadas 2008). Despite the advancement and success of *in vitro* culture, rooting of *in vitro* propagated shoots remains a critical bottleneck. Rooting *in vitro* is crucial to successfully transitioning from *in vitro* to *ex vitro* conditions. The current study aimed to optimize rooting conditions under the influence of auxins and activated carbon (AC) for *in vitro* propagated shoots of blueberry to enhance rooting efficiency and uniformity for bulk production.

MATERIALS AND METHODS

Plants source

Two commercially significant blueberry cultivars, 'Lanmie#1' (easy to root) and 'Brightwell' (recalcitrant) were studied, using explants from disease-free mother plants cultivated hydroponically at Çukurova University, Türkiye. 'Lanmei #1' was derived from an interspecific hybrid line with a genetic composition of 33% *V. corymbosum* (25%), *V. darrowii* (8%), *V. ashei* (1%), *V. angustifolium* (25%), *V. elliotii* (8%), *V. constablaei*. This cultivar is known for being easy to root. 'Brightwell' is also commercially important but has a rooting problem. Young shoots approximately 10 cm long were surface sterilized and immediately transferred to *in vitro* culture.

Culture initiation

Surface sterilization is critical to ensure the purity and viability of blueberry explants. The initial phase involved removing leaves from young blueberry shoots. The explants were then subjected to sterilization steps to remove potential contaminants, such as fungi and bacteria. A preliminary step involved a gentle cleansing of explant surfaces with tap water. Subsequently, explants were sectioned into pieces, each measuring 1.5–3 cm and containing two buds.

Initially, explants were exposed to a solution containing 0.1 g·L⁻¹ Rifampicin, agitated for 5 minutes, and rinsed thrice with distilled water. Further, explants were sterilized in a solution containing 20% sodium hypochlorite (NaOCl) with a few drops of Tween 2 for 10 min. Finally, explants were rinsed 3–4 times with distilled sterile water and placed singly in test tubes containing Anderson medium, with 2 mg·L⁻¹ 2iP + 0.1 mg·L⁻¹ IBA + 0.15 mg·L⁻¹ GA₃, 15 g·L⁻¹ sucrose, 100 mg·L⁻¹ myo-inositol, and 6.5 g·L⁻¹ agar, with a pH level of 5.3. Initially, explants were kept in the dark, at 24 °C for three days, and transferred to light conditions.

Shoot multiplication

For multiplication, Anderson's medium was used, consisting of 2.0 mg·L⁻¹ 2iP, 0.25 mg·L⁻¹ zeatin, 0.1 mg·L⁻¹ IBA, 0.1 mg·L⁻¹ GA₃, 20 g·L⁻¹ sucrose, 200 mg·L⁻¹ myo-inositol, and 6.5 g·L⁻¹ agar, pH 5.3. Nine explants were cultured per magenta vessel. The multiplication medium was changed every month. The growth room was maintained at 24 ± 2 °C with 55% humidity. Fluorescent lamps were calibrated at 2500–3000 lux light intensity for 16 hours.

Shoot rooting

½ WPM medium with pH 5.3 was supplemented with different concentrations of IBA, NAA, and IAA 0, 0.5, 1, 2, and 3 mg·L⁻¹. AC at concentrations of 0, 0.5, 1, 2, and 3 g·L⁻¹ was independently applied, without adding auxins. From the fourth subculture, single shoot explants 2–2.5 cm long were excised and transferred to test jars containing one of the prepared media. Explants were incubated in darkness at 24 °C for three days to promote root initiation. Following this, test jars were transferred to the culture room. Rooting percentage, root number, and root length per explant were recorded after one month of culture. Nine shoots were used per replication, and the experiment was replicated three times.

Statistical analysis

In all experiments, a randomized complete block design was used, and data were statistically analyzed using SPSS software (version 16), followed by Duncan's test with $p > 0.05$.

RESULTS AND DISCUSSION

Our results demonstrated a comparative assessment of three different auxins (IBA, IAA, and NAA) at four different concentrations (0, 0.5, 1, 2, and 3 mg·L⁻¹) and AC at five concentrations (0, 0.5, 1.0, 2.0, 3.0 and 4.0 mg·L⁻¹) on growth shoots and roots of two blueberry cultivars. ‘Lanmei #1’ cultivar’s parameters were higher at each treatment than ‘Brightwell’ cultivar.

Effect of IBA on growth parameters

The effect of IBA on shoot number in ‘Lanmei #1’, was insignificant across different IBA concentrations (1.7–1.9 shoots per explant). In ‘Brightwell’, a slight increase in shoot number (up to 1.5 shoots per explant) was observed at 1 mg·L⁻¹ IBA. The shoot length in ‘Lanmei #1’ increased with increasing IBA concentration (4.2–7.8 cm), but these values were statistically insignificant. ‘Brightwell’ shoot length slightly increased at 1 mg·L⁻¹ (2.7 cm) compared with another concentration. Root number in ‘Lanmei #1’ increased with IBA concentrations (1.4–3.7 roots per explants), with no statistical difference between 1, 2, and 3 mg·L⁻¹ IBA. The ‘Brightwell’ root number was generally low, with an increase at 2 mg·L⁻¹ (1.6 roots per explant) followed by a decrease at high concentrations (0.4 roots per explant). Root length in ‘Lanmei #1’ increased at 1 mg·L⁻¹ IBA (3.6 cm), with no significant difference between 3 mg·L⁻¹ and 2 mg·L⁻¹. In ‘Brightwell’, the root number was higher at 1 mg·L⁻¹ (1.5 per explant). Callus growth was observed only at 2 and 3 mg·L⁻¹ in both cultivars. In ‘Lanmei #1’, the root percentage remains consistently (90–96%) high across all IBA concentrations, while in ‘Brightwell’, the rooting percentage was significantly lower (Table 1).

Effect of NAA on growth parameters

Shoot number in ‘Lanmei #1’ increased only at 0.5 mg·L⁻¹ NAA (1.8 shoots per explant) compared with the other concentrations. In ‘Brightwell’, the shoot number remained stable across NAA concentrations. Shoot length in ‘Lanmei #1’ did not change, except that NAA at 2 mg·L⁻¹ significantly decreased it, whereas in ‘Brightwell’, shoot length was not changed. The root number in ‘Lanmei #1’ was the highest at 0.5 mg·L⁻¹ and 3 mg·L⁻¹ NAA (4.9–4.4 roots per explant) compared to other concentrations, while in ‘Brightwell’, the number of roots was highest at 2 and 3 mg·L⁻¹ NAA. Root length in ‘Lanmei #1’ was lowest at control and highest at

0.5 mg·L⁻¹ NAA. ‘Brightwell’ root length was not changed depending on auxin concentration. In both cultivars, callus development increased with increasing NAA concentrations. In ‘Lanmei #1’ the root percentage was similar to that in response to IBA, while ‘Brightwell’ responded well to NAA but still showed a lower percentage than ‘Lanmei #1’ (Table 2).

Effect of IAA on growth parameters

In both cultivars, IAA did not affect shoot number or callus weight. The shoots of ‘Lanmei #1’ were significantly higher at 2 mg·L⁻¹ IAA (7.4 cm), similar to the root number per explant (2.8). ‘Brightwell’ showed no significant difference in shoot length, root number, and root length across IAA concentrations. The root number in ‘Lanmei #1’ was significantly higher at 2 and 3 mg·L⁻¹ IAA than at the other concentration (2.7 and 1.8 roots per explant). ‘Brightwell’ showed variability across different IAA dosages with no clear trend. Root length in ‘Lanmei #1’ was highest at 2 mg·L⁻¹ (5.0 cm) compared with other concentrations (0.8–1.9 cm). In ‘Lanmei #1’ the rooting percentage ranged from 46% to 100%, while in ‘Brightwell’ the rooting percentage was less effective, similar to the IBA results (Table 3).

Effect of AC on growth parameters

AC did not affect growth parameters. The two exceptions were found only for 3 and 4 mg·L⁻¹ as they increased root number in ‘Lanmei #1’. However, AC does not improve the rooting percentage in either cultivars (Table 4).

The effects of auxins on plant growth and development *in vitro* have been studied intensively (De Klerk 2002; Kumar et al. 2019; Pasternak & Steinmacher 2024). *Vaccinium* spp., as a plant propagated mainly *in vitro*, has often been the subject of studies on the role of auxins (Debnath 2007; Litwińczuk & Wadas 2008; Ružić et al. 2012; Guo et al. 2019). *In vitro* rooting of *Vaccinium* depends on many factors, including growth regulators, mineral medium constitutions, and technology applied but to a great extent on the plant genotype (Debnath & McRae 2005; Ružić et al. 2012; Guo et al. 2019; Georgieva & Kondakova 2021; Wang et al. 2023; Zhou et al. 2023). Both the kind and concentration are crucial for effectivity. Generally, IBA is considered the most effective for woody plants (Hartmann et al. 2010), although Khadr et al. (2020) pointed to IAA for *Vaccinium* rooting.

The most obvious factor in our experiment was the genetic ability to root. Cultivar ‘Lanmei #1’ could root without auxin, but auxins increase root number, length, and shoot length. NAA showed the most instantiable influence, mainly on root number and callus growth, probably due to its durability in the medium.

Cultivar ‘Brightwell’ was a recalcitrant for rooting. It did not root without auxin or root sporadically, forming only 0.2 roots per shoot. Adding 1 mg·L⁻¹ IBA increased root number to 1.5 roots per shoot, whereas IAA at 3 mg·L⁻¹ increased root number to 0.7. The most effective was NAA; the number of roots grew with concentration up to 2.1 at 3 mg·L⁻¹.

Georgieva and Kondakova (2021) and Fan et al. (2017) also detected a great variability in blue-

berry cultivars both in shoot formation and for rooting. These findings underscore the critical need for cultivar-specific protocols in optimizing regeneration and rooting process for *Vaccinium* spp., reinforcing the importance and potential impact of our research.

Our findings highlight the unique genetic makeup of ‘Lanmei #1’ that leads to a specific response to each auxin and concentration. This unique responsiveness, whether due to more efficient auxin receptors or signaling pathways or more efficient use of self or obtained auxin, highlights the need for further studies, such as transcriptomic and proteomic analyses, to provide deeper insight into these differential responses.

Table 1. Effect of IBA on shoot number and length, root number and length, and callus formation in two blueberry cultivars

Parameter	Cultivar	IBA concentration (mg·L ⁻¹)				
		0	0.5	1	2	3
Shoot number	‘Lanmei #1’	1.7 ± 0.5a	1.7 ± 0.3a	1.7 ± 0.2a	1.5 ± 0.2ab	1.8 ± 0.3a
	‘Brightwell’	1.1 ± 0.3bc	1.0 ± 0.0c	1.4 ± 0.2ab	1.0 ± 0.0c	1.0 ± 0.0c
Shoot length (cm)	‘Lanmei #1’	4.1 ± 0.6a	5.9 ± 2.0a	7.7 ± 2.3a	4.6 ± 1.2ab	5.4 ± 0.7a
	‘Brightwell’	2.1 ± 0.7bc	1.5 ± 0.2c	2.7 ± 0.9ab	1.4 ± 0.3c	1.6 ± 0.4c
Root number	‘Lanmei #1’	1.4 ± 0.7cd	1.8 ± 0.5bc	2.8 ± 0.6ab	3.6 ± 1.0a	3.7 ± 0.8a
	‘Brightwell’	0.0 ± 0.0e	0.0 ± 0.0e	1.5 ± 1.40c	0.4 ± 0.3de	0.3 ± 0.4de
Root length (cm)	‘Lanmei #1’	0.7 ± 0.4c	3.6 ± 1.1a	3.4 ± 1.4ab	2.3 ± 0.9b	2.4 ± 0.9ab
	‘Brightwell’	0.0 ± 0.0c	0.0 ± 0.0c	1.0 ± 0.9c	0.324 ± 0.3c	0.1 ± 0.1c
Callus weight (g)	‘Lanmei #1’	0.0 ± 0.0d	0.0 ± 0.0cd	0.0 ± 0.0d	0.3 ± 0.1a	0.1 ± 0.2abc
	‘Brightwell’	0.0 ± 0.0d	0.0 ± 0.0bcd	0.0 ± 0.3bcd	0.0 ± 0.0bcd	0.1 ± 0.0ab
Root percentage	‘Lanmei #1’	91.1 ± 14.4a	92 ± 10.9a	96 ± 8.9a	90 ± 1a	96 ± 5.4a
	‘Brightwell’	20 ± 19.8c	0 ± 0c	50 ± 37.4b	20 ± 15.8c	14 ± 15.1c

Values are expressed as mean ± standard deviation (n = 3); Values with the same letter in the same line and column are not statistically different (p < 0.05)

Table 2. Effect of NAA on shoot number and length, root number and length, and callus formation in two blueberry cultivars

Parameter	Cultivar	NAA concentration (mg·L ⁻¹)				
		0	0.5	1	2	3
Shoot number	‘Lanmei #1’	1.3 ± 0.3bc	1.7 ± 0.3a	1.3 ± 0.1bc	1.4 ± 0.0b	1.2 ± 0.1bcd
	‘Brightwell’	1.1 ± 0.1cd	1.1 ± 0.1cd	1.0 ± 0.0d	1.0 ± 0.0d	1.0 ± 0.0d
Shoot length (cm)	‘Lanmei #1’	4.7 ± 1.7a	5.4 ± 0.7a	4.7 ± 1.1a	3.3 ± 0.3b	4.5 ± 0.6a
	‘Brightwell’	2.3 ± 0.5bcd	2.1 ± 0.4cd	2.3 ± 0.1bcd	1.34 ± 0.2d	2.85 ± 0.6bc
Root number	‘Lanmei #1’	0.8 ± 0.4de	4.9 ± 0.9a	4.1 ± 2.1b	3.5 ± 0.8b	4.4 ± 0.7ab
	‘Brightwell’	0.2 ± 0.2e	0.4 ± 0.3e	0.6 ± 0.6de	1.6 ± 1.1cd	2.1 ± 0.7c
Root length (cm)	‘Lanmei #1’	0.9 ± 1.0cd	2.9 ± 0.5a	1.8 ± 0.7b	1.2 ± 0.1bc	1.6 ± 0.4b
	‘Brightwell’	0.1 ± 0.2e	0.2 ± 0.2e	0.2 ± 0.2e	0.3 ± 0.1de	0.5 ± 0.1cde
Callus weight (g)	‘Lanmei #1’	0.2 ± 0.0cd	0.3 ± 0.1b	0.5 ± 0.2a	0.6 ± 0.0a	0.5 ± 0.1a
	‘Brightwell’	0.0 ± 0.0d	0.1 ± 0.0cd	0.1 ± 0.0bc	0.3 ± 0.1b	0.0 ± 0.0d
Root percentage	‘Lanmei #1’	91.1 ± 14.4a	100 ± 0a	94 ± 8.9a	88 ± 8.3a	98 ± 4.4a
	‘Brightwell’	20 ± 19.8c	24 ± 13.4c	34 ± 26.0c	58 ± 17.8b	64 ± 15.1b

Note: see Table 1

Table 3. Effect of IAA on shoot number and length, root number and length, and callus formation in two cultivars of blueberry

Parameter	Cultivar	IAA concentration (mg·L ⁻¹)				
		0	0.5	1	2	3
Shoot number	‘Lanmei #1’	1.4 ± 0.3ab	1.7 ± 0.6a	1.2 ± 0.1bc	1.6 ± 0.3a	1.3 ± 0.3abc
	‘Brightwell’	1.0 ± 0.0c	1.0 ± 0.0c	1.0 ± 0.0c	1.0 ± 0.0c	1.2 ± 0.2bc
Shoot length (cm)	‘Lanmei #1’	4.5 ± 0.8bc	5.0 ± 2.1b	3.4 ± 1.1cd	7.4 ± 1.3a	4.1 ± 0.6bc
	‘Brightwell’	2.5 ± 1.0def	2.1 ± 0.5ef	1.7 ± 0.6f	3.2 ± 0.8def	3.2 ± 0.5cde
Root number	‘Lanmei #1’	0.7 ± 0.5c	1.7 ± 1.0b	0.7 ± 0.4c	2.7 ± 0.7a	1.8 ± 0.4b
	‘Brightwell’	0.2 ± 0.2c	0.6 ± 0.7c	0.0 ± 0.1c	0.2 ± 0.2c	0.7 ± 0.5c
Root length (cm)	‘Lanmei #1’	0.8 ± 0.5bc	1.9 ± 1.3b	1.38 ± 1.5bc	4.9 ± 2.6a	1.3 ± 0.6bc
	‘Brightwell’	0.2 ± 0.4c	0.2 ± 0.3c	0.1 ± 0.3c	0.53 ± 0.509bc	0.9 ± 0.7bc
Callus weight (g)	‘Lanmei #1’	0.0 ± 0.1a	0.0 ± 0.0ab	0.0 ± 0.2ab	0.0 ± 0.0b	0.0 ± 0.0ab
	‘Brightwell’	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.2ab	0.0 ± 0.5ab	0.0 ± 0.0b
Root percentage	‘Lanmei #1’	91.1 ± 14.4a	76 ± 42.7a	46 ± 26.0b	100 ± 0a	82 ± 8.3a
	‘Brightwell’	20 ± 19.8bc	24 ± 23.0bc	6 ± 13.4c	20 ± 18.7bc	46 ± 27.0b

Note: see Table 1

Table 4. Effect of AC on shoot number, shoot length, root number, root length and callus formation in two blueberry cultivars

Parameter	Cultivar	AC concentration (mg·L ⁻¹)					
		0	0.5	1	2	3	4
Shoot number	‘Lanmei #1’	2.2 ± 0.4abc	2.4 ± 0.9ab	2.4 ± 0.8ab	1.9 ± 1.0abcd	2.8 ± 1.5a	2.8 ± 1.5a
	‘Brightwell’	1.2 ± 0.2cd	1.5 ± 0.3cd	1.4 ± 0.4cd	1.4 ± 0.6cd	1.2 ± 0.4cd	1.0 ± 0.1d
Shoot length (cm)	‘Lanmei #1’	5.9 ± 1.0ab	6.5 ± 2.6a	6.3 ± 2.6a	6.2 ± 3.9a	6.5 ± 2.9a	5.2 ± 2.4abc
	‘Brightwell’	3.2 ± 0.8cd	4.3 ± 1.6abcd	3.5 ± 1.8cd	3.9 ± 2.1cd	3.2 ± 0.8cd	2.3 ± 0.5d
Root number	‘Lanmei #1’	1.6 ± 0.4b	1.6 ± 0.94b	1.6 ± 0.9b	1.6 ± 1.4b	2.6 ± 1.4a	2.8 ± 1.7a
	‘Brightwell’	0.4 ± 0.4c	0.9 ± 0.5bc	0.5 ± 0.6c	0.7 ± 0.8c	0.9 ± 0.9bc	0.4 ± 0.6c
Root length (cm)	‘Lanmei #1’	4.8 ± 2.4ab	7.9 ± 5.0a	5.9 ± 4.3a	4.5 ± 4.7abc	5.5 ± 3.2a	6.3 ± 4.1a
	‘Brightwell’	1.0 ± 1.1d	1.5 ± 1.2bcd	1.3 ± 1.4cd	1.8 ± 2.1bcd	0.7 ± 0.7d	0.3 ± 0.5d
Root percentage	‘Lanmei #1’	91.1 ± 14.4a	77.7 ± 43.7ab	73.3 ± 43.4abc	60 ± 54.7abcd	71.1 ± 40.5abc	80 ± 44.7ab
	‘Brightwell’	20 ± 19.8de	31.11 ± 21.3bcde	24.44 ± 27.67cde	31.11 ± 30.8bcde	31.11 ± 25.3bcde	8.89 ± 12.1e

Note: see Table 1

CONCLUSION

The current study showed that auxin type and concentration could significantly affect shoot and root development in blueberry cultivars ‘Lanmei #1’ and ‘Brightwell’. ‘Lanmei #1’, which is easier to root than ‘Brightwell’, showed a more pronounced response to auxin treatments. NAA positively influenced shoot number in ‘Lanmei #1’ beginning from 0.5 mg·L⁻¹, while in ‘Brightwell’ at 2 and 3 mg·L⁻¹. IAA promoted shoot and root length at 2 mg·L⁻¹ in ‘Lanmei #1’. AC stimulated only root number in ‘Lanmei #1’ at 3 mg·L⁻¹. Due to genetic differences,

optimizing the blueberries’ rooting for individual cultivars may be necessary.

Conflict of interest

The authors declare that they have no conflict of interest.

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