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OPTIMIZATION OF MICROPROPAGATION, EX VITRO ROOTING, AND ADAPTATION OF BLACKBERRY (*RUBUS* L.) USING GROWTH STIMULANTS

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Abstract

The article presents the results of optimizing micropropagation and ex vitro adaptation conditions for blackberry plantlets of the *Rubus* L. cultivars *Natchez* and *Heaven Can Wait*. During the micropropagation stage, the most effective shoot multiplication was achieved on MS medium supplemented with 0.5 mg/L BAP and 0.1 mg/L GA_3, resulting in the formation of 5.3–5.8 shoots per explant with an average height of 5.27–5.43 cm. For ex vitro rooting and adaptation, the best results were obtained using a peat and perlite substrate (3:1) moistened with a 0.01% solution of succinic acid. After 8 weeks, the *Natchez* cultivar showed a shoot length of 8.47 cm, and *Heaven Can Wait* reached 7.10 cm. Both cultivars developed more than 11.42 roots on average, with root lengths ranging from 10.35 to 11.52 cm. The use of growth stimulants (humic acid, nanosilicon, and succinic acid) significantly improved rooting efficiency, with the succinic acid treatment achieving the highest results – rooting and adaptation rates of 87% to 97%. These findings confirm the effectiveness of growth stimulators in enhancing the propagation and adaptation of blackberry plantlets under ex vitro conditions.

Key words: blackberry, in vitro culture, cultivar, ex vitro regenerant plants, growth stimulants

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Introduction. Blackberry is gaining popularity as a valuable berry crop due to its high bioflavonoid and pectin content and adaptability to various climates [1]. However, modern cultivars with extended fruiting periods face low propagation rates. Traditional methods (root suckers, cuttings, tip layering) do not yield enough disease-free planting material. Clonal micropropagation can accelerate reproduction and improve plant health, but the adaptation of roots to ex vitro conditions remains a major challenge [2].

The transition from in vitro to ex vitro is the most critical stage, with up to 95% plant loss, emphasizing the need for optimal conditions for rooting and adaptation [3,4]. Rooting microcuttings directly in substrate under ex vitro conditions is a promising solution, combining rooting and adaptation in one stage [5,6].

Growth stimulators such as humic acids, nanosilicon, and succinic acid can enhance rooting and stress resistance [7–9]. Studies confirm the effectiveness of ex vitro rooting in blackberry and raspberry cultivars, particularly with stimulators like IBA and succinic acid [9–12].

Despite progress, adaptation depends on both external and internal factors, particularly substrate composition, climate, and growth stimulators.

This study aims to optimize the micropropagation stage and evaluate the effects of growth stimulants (humic acid, succinic acid, and nanosilicon) on rhizogenesis and ex vitro adaptation of blackberry plantlets. The use of stimulators can enhance rooting and adaptation efficiency, accelerating propagation of promising cultivars and improving the quality of planting material.

Material and methods. The research was conducted at the Plant Bioengineering Laboratory of the M. A. Aitkhozhin Institute of Molecular Biology and Biochemistry in 2023–2025. The objects of the study were blackberry plants of two cultivars: *Rubus* L. *Natchez* and *Heaven Can Wait*.

The *Natchez* cultivar is an American dessert variety known for its high adaptability to different climatic zones and its low soil requirements. The yield is consistently high – 13–20 kg per bush or 2.3–3.2 kg of blackberries per plant.

The *Heaven Can Wait* cultivar is an American early-season variety. The berries have an excellent taste, are sweet, and are characterized by high levels of anthocyanins and antioxidants, which is the hallmark of this variety. The yield is high – 15–25 kg per bush.

The explants used were axillary buds taken from one-year-old blackberry shoots. The sterilization of the plant material was carried out according to the following scheme: washing with running water for 30 min; treatment with 70% ethanol for 1 min; treatment with a 1% sodium hypochlorite solution for 15 min; washing with sterile water three times for 5 min each.

For culturing the explants during the initiation and micropropagation stages, Murashige and Skoog's [13] nutrient medium (MS) was used with the addition of phytohormones. During the initiation stage, 0.1 mg/L of 6-benzylaminopurine (BAP) was applied. For clonal micropropagation, blackberry microshoots regen-

erated under in vitro conditions were divided into segments 5 to 10 mm in length and transferred to fresh MS medium supplemented with various concentrations of phytohormones. Specifically, BAP was added at concentrations of 0.5 and 1.0 mg/L, and gibberellic acid (GA_3) at 0.1, 0.3, and 0.5 mg/L. Sucrose was used as a carbon source at a concentration of 30 g/L. The semi-solid medium was prepared using 6–8 g/L agar-agar, and the pH was adjusted to 5.70. Cultures were maintained at 24–26 °C under a light intensity of 3000 lux, provided by fluorescent lamps, with a photoperiod of 18/6. The subculturing duration was 4 weeks.

Rooting and adaptation were carried out ex vitro in custom agroboxes with a peat–perlite (3:1) substrate. Microcuttings (35 per variant, in triplicate) were pretreated with 0.1 mg/L IBA. Three stimulant solutions were tested: 0.01% humic acid, nanosilicon, and succinic acid. A water-only variant served as control.

Agroboxes were kept under 18-hour light, 70% humidity, 25/22 °C (day/night). Root formation was monitored weekly; ventilation holes were added gradually for acclimatization. After 28 days, lids were removed; after 8 weeks, plants were transplanted to pots (70% peat, 30% perlite).

Rooting efficiency was evaluated based on shoot length, number of internodes, number and length of roots. Experiments were replicated three times, and statistical significance was assessed using Student's t-test ($P < 0.05$).

Results and discussion. At the multiplication stage, the goal was to achieve the highest in vitro propagation rate, influenced by genotype, medium composition, and cultivation conditions. In our study, effective shoot formation was observed four weeks after bud explant introduction, with 2–3 shoots per explant reaching 30–40 mm in length. Extending cultivation beyond this period increased the risk of mechanical damage during shoot removal.

Microshoots with 1–2 axillary buds were segmented and cultured on MS medium with varying concentrations of BAP and GA_3. The most effective combination for both *Natchez* and *Heaven Can Wait* cultivars was 0.5 mg/L BAP and 0.1 mg/L GA_3, yielding 5.3–5.8 shoots per explant with average heights of 5.27–5.43 cm.

Higher concentrations of BAP or GA_3 (up to 0.5 mg/L) did not improve outcomes and instead produced weaker, shorter shoots unsuitable for further propagation.

Thus, the optimal hormone combination for in vitro multiplication was 0.5 mg/L BAP with 0.1 mg/L GA_3.

The research demonstrated high rooting success in blackberry plantlets when combining rooting and adaptation under ex vitro conditions. To reduce stress from the transition to natural temperature, light, and humidity, unrooted microcuttings from in vitro culture were planted directly into agroboxes with an autoclaved peat-perlite substrate (3:1).

Substrates commonly used in clonal micropropagation include various mixtures of peat, perlite, sand, sphagnum moss, and vermiculite [14,15]. As shown by

Fig. 1. Experimental agroboxes with blackberry plants in the growth room

Lepse and Laugale [16], peat-based substrates significantly increase the yield of rooted blackberry plants compared to in vitro rooting. Based on this, the peat-perlite mixture (3:1) was selected as optimal in our study (Fig. 1).

The proportion of adapted plants was higher in all variants with the addition of stimulants compared to the control. In the variant with the addition of succinic acid, the highest rooting success was observed, with 97% for the *Natchez* variety and 95% for the *Heaven Can Wait* variety. The variant with humic acids showed slightly lower results: 93% for *Natchez* and 93% for *Heaven Can Wait*. In the variant with nanosilicon, the rooting success was 91% for *Natchez* and 87% for *Heaven Can Wait*. The lowest results were found in the control group: 65% for *Natchez* and 70% for *Heaven Can Wait* (Fig. 2).

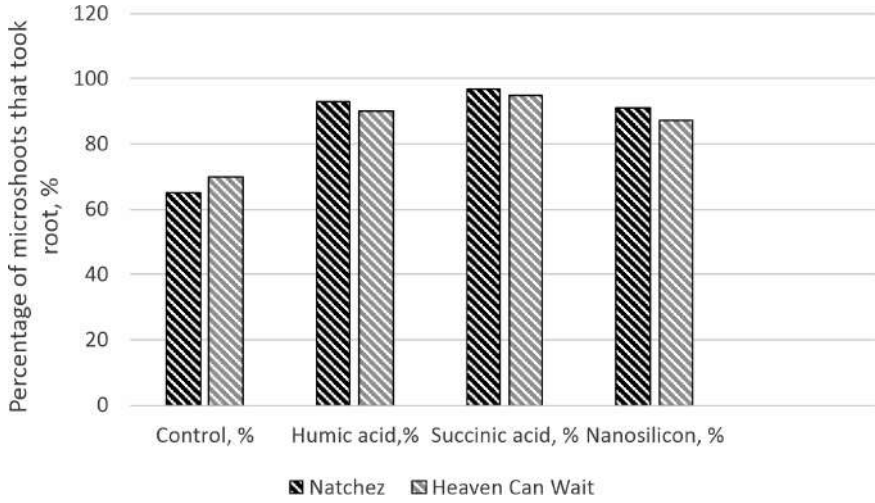

Fig. 2. Proportion of rooted microshoots of blackberry varieties under ex vitro conditions depending on different stimulants

Fig. 3. Effect of growth stimulants on the morphometric parameters of blackberry regenerant plants in agroboxes

Despite the high number of rooted cuttings in all variants with the addition of stimulants, differences were observed in the morphological development of the plants, such as shoot length, number of internodes, number of roots, and root length (Fig. 3).

Analysis of the morphometric parameters showed that after 8 weeks of ex vitro rooting, the development of plants of the studied blackberry varieties was most successful on the substrate with a 0.01% succinic acid solution (Table 1). For the *Natchez* variety, the best results were obtained on this substrate: the shoot length was 8.47 cm, and the number of internodes was 7.70. For the *Heaven Can Wait* variety, the shoot length was 7.10 cm, and the number of internodes was

Table 1

Morphometric parameters of the development of blackberry regeneration plants during ex vitro rooting depending on the substrate with different growth-promoting additives (the highest results are marked in bold)

Substrate	**Variety**	**Shoot length, cm**	**Number of internodes, pcs**	**Number of roots, pcs**	**Average root length, cm**
Control H_2O	Natchez	2.37±0.09	3.66±0.45	6.57±0.64	5.40±0.08
	HCW	2.03±0.12	3.45±0.36	5.53±0.29	4.31±0.23
Humic acid	Natchez	5.43±0.26	6.73±0.34	9.30±0.84	**12.23±0.43**
	HCW	5.30±0.06	6.40±0.10	10.77±1.4	**11.43±0.19**
Succinic acid	Natchez	**8.47±0.15**	**7.70±0.26**	**12.67±0.58**	11.52±0.58
	HCW	**7.10±0.15**	**7.30±0.29**	**11.42±1.17**	10.35±0.30
Nanosilicon	Natchez	4.60±0.21	6.60±0.29	8.33±0.05	9.83±0.12
	HCW	4.20±0.18	6.27±0.22	7.35±0.24	8.07±0.12

HCW – Heaven Can Wait

7.30. Good results were also obtained for the number of roots in both blackberry varieties (at least 11.42) on the substrate with succinic acid solution. However, the average root length of ex vitro rooted microshoots on this substrate ranged from 10.35 cm to 11.52 cm. It should be noted that the substrate with humic acid solution had a positive effect on the root length of microshoots. On this solution, the root length was 12.23 cm for *Natchez* and 11.43 cm for *Heaven Can Wait*. On the substrate with the addition of nanosilicon, low results were obtained for the shoot length (4.60 cm) and the number of internodes (6.60) for *Natchez*. For *Heaven Can Wait*, the shoot length was 4.20 cm, and the number of internodes was 6.27.

The study confirmed that growth stimulators – humic acid, nanosilicon, and succinic acid – significantly improve rooting and adaptation of blackberry microshoots.

Succinic acid, a key component of the TCA (Krebs) cycle, enhances energy metabolism and stress resistance in plants. Its application at 0.01% notably improved rooting and adaptation, aligning with the findings of Nienko et al. [17], Besedina et al. [18], and Shamanskaya and Guschina [12], who also observed positive effects on various fruit crops.

Humic acids, derived from organic matter, improve nutrient uptake, enzymatic activity, and substrate conditions. Their use in peat–perlite substrate enhanced blackberry development, consistent with prior studies [7, 18, 19] highlighting their role in plant physiology and rhizogenesis.

Nanosilicon, though not essential, benefits plants by strengthening cell walls and enhancing stress tolerance. In nanoparticle form, it is more bioavailable and activates antioxidant systems. In our research, it improved microshoot condition and rooting, supporting earlier findings [8, 20].

Overall, each stimulator aids adaptation via distinct physiological and biochemical pathways. Their use during ex vitro acclimatization enhances the efficiency and survival of blackberry plantlets in clonal propagation.

Conclusions. The study demonstrated the high efficiency of optimized micropropagation, rooting, and adaptation methods for blackberry, including the use of a peat-perlite substrate and growth stimulants (humic acid, nanosilicon, and succinic acid). The most effective micropropagation medium was MS with 0.5 mg/L BAP and 0.1 mg/L GA_3, yielding 5.3–5.8 shoots per explant and shoot height of 5.27–5.43 cm.

The best ex vitro rooting and adaptation results were achieved using 0.01% succinic acid, ensuring strong growth, good internode development, and over 11 roots per plant in *Natchez* and *Heaven Can Wait* cultivars. Combined rooting and adaptation success reached 87–97%.

These findings confirm the effectiveness of the selected phytohormone combination and succinic acid treatment, offering practical value for improving blackberry propagation and scaling up biotechnological production of planting material.

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