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OBTAINING HIGH-QUALITY PLANTING MATERIAL OF FOREST BERRY PLANTS BY CLONAL MICROPROPAGATION FOR RESTORATION OF CUTOVER PEATLANDS

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Abstract. The results of studies on the improvement of technology for producing high-quality planting material of half-high blueberry and Arctic bramble by the method of clonal micropropagation are presented in the current paper. Creation of forest berry plantations in peat extraction areas allows reducing environmental damage and significantly increasing the efficiency of the timber industry. In recent decades, there has been an increasing interest in the creation of forest berry plantations on drained and cutover peatlands in Russia and other countries. It is necessary to use varietal planting material for the successful cultivation of forest berry plants on an industrial scale. Clonal micropropagation is the most effective of the vegetative methods for obtaining planting material, which allows receiving a huge amount of healthy planting material all year round in the conditions of a small laboratory area. Chloride-free ecosterilizer and bleaching agent based on sodium hypochlorite "Belizna" with an exposure of 15 and 20 min showed high efficiency in sterilization of explants of half-high blueberry and Arctic bramble. The highest viability of explants of the studied forest berry crops was observed when sterilized with a 0.1 % mercuric chloride solution and 15 min exposure, and its sharp decrease at 20 min exposure. At the stage of micropropagation, with an increase in the concentration of cytokinin 6-BAP from 0.5 to 1.0 mg/L on the nutrient Woody Plant Medium the number of shoots in regenerated plants of half-high blueberry (Northcountry and Northblue cultivars) and Arctic bramble (Anna and Sofia cultivars) increased. The effect of the concentration of IBA-derived auxin on the number and length of roots of regenerated plants was observed at the *in vitro* rooting stage.
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Keywords: Arctic bramble, clonal micropropagation, cultivar, cutover peatlands, growth regulator, half-high blueberry, *in vitro*.

Introduction

The negative environmental consequences of peat excavations include the violation of the unique ecological marsh systems, the deterioration of the living conditions of marsh and forest flora and fauna, and the associated reduction in the number of its populations up to complete destruction, as well as a decrease in the reserves of ground, underground and surface waters. In addition, valuable berry and medicinal plants growing in swamps are destroyed significantly, and the likelihood of peat fires increases, as a result of which a huge amount of smoke and other peat combustion products that can be transported over distances of tens and hundreds of kilometers are released into the atmosphere.

The reclamation of cutover peatlands is one of the important issues of forestry. Creation of plantations of forest berry plants in peat extraction areas allow to reduce environmental damage and significantly increase the efficiency of the forest industry. In recent decades, in Russia, as in other countries of the world, there is a growing interest in the creation of plantations of forest berry plants on drained and cutover peatlands. For these purposes, it is best to use berry crops that have traditionally grown in forest biocenoses, such as blueberry, cranberry, lingonberry and Arctic bramble. This is becoming increasingly important due to the constantly increasing anthropogenic load, as a result of which the resources of wild forest berry crops are steadily decreasing, their productivity is decreasing, and their quality is deteriorating [6, 13, 19].

It is necessary to use varietal planting material for successful plantation cultivation of forest berry plants on an industrial scale. Selection of forest berry crops is carried out by both Russian and foreign scientists. So far, Russian varieties have already been created and hybrid forms of forest berry plants have been selected. These varieties and hybrid forms are promising for cultivation in southern taiga and coniferous-deciduous forest areas of the European part of the Russian Federation.

Such forest berry crops as blueberry and Arctic bramble can be successfully used for the recultivation of cutover peatlands. The fruits of these forest berry plants are dietary products with high nutritional value. They contain vitamins and biologically active substances necessary for the normal functioning of the human body. Moreover, these plants have medicinal properties. For example, blueberry fruits are rich in vitamin P substances that regulate the functioning of endocrine glands, have anti-inflammatory and antitumor effects, and are effective in prevention and treatment of atherosclerosis, hypertension, rheumatism and other diseases [1, 3, 9, 17, 18, 20]. Arctic bramble fruits are used in folk medicine as an antizingotic, antipyretic agent, as well as for kidney stone disease, gout, gastritis, colitis, anemia, acute respiratory diseases, and bronchial asthma. Fresh leaves are used for wound healing, and tincture of leaves is used for rheumatism [2, 4, 5, 7, 15].

Blueberry and Arctic bramble can be propagated both by seeds and by stem and root cuttings (vegetative), as well as by using *in vitro* tissue cultivation. It should be taken into account that that plants produced by seed propagation do not retain the features of the original mother variety, therefore this method is not suitable for varietal plants. Clonal micropropagation is the most effective of vegetative methods of obtaining planting material. This method allows receiving in conditions of small laboratory area a huge amount of healthy (virus-free) planting material all year round [10, 11].

Materials and methods

Our studies are devoted to improving the technology for producing high-quality planting material of high-half blueberry and Arctic bramble using the clonal micropropagation. The research was carried out in the Laboratory of Clonal Micropropagation of Plants on the basis of the Central European Forest Experimental

Station of the All-Russian Research Institute for Silviculture and Mechanization of Forestry in 2018–2019. As research objects We used two cultivars of high-half blueberry (Northblue and Northcountry), obtained by crossing *V. corymbosum* and *V. angustifolium* [8, 12, 14], and two cultivars of Arctic bramble (*Rubus arcticus* L., Anna and Sofia) [16].

At the stage of introduction to *in vitro* culture, we studied the effect of various sterilizers with different exposures on the viability of explants of high-half blueberry and Arctic bramble. We used mercuric chloride (0.1 % solution), chloride-free ecosterilizer and bleaching agent based on sodium hypochlorite “Belizna” as the main sterilizers, with exposures of 10, 15 and 20 min. After 14 days, the viability of explants was determined by the ratio of live explants to the total number introduced into the culture. In each variant, 100 explants were introduced. Also we carried out a series of experiments to study the effect of cytokinin 6-BAP at the concentrations of 0.5 and 1.0 mg/L on the biometric parameters of shoots and IBA-derived auxin at the concentrations of 0.5 and 1.0 mg/L on *in vitro* rhizogenesis. Plants were cultivated on the Woody Plant Medium (WPM) with pH in the range of 4.5–4.8. The number and length of shoots and roots were recorded. 10-fold repeat.

Results and discussion

At the stage of *in vitro* culture introduction, we found that the ecosterilizer and bleaching agent were the most effective at an exposure of 15 and 20 min, where the explant viability was 75–91 %. The explants viability of both berry crops is quite high (80–90 %) when treated with corrosive sublimate for 15 min, but it sharply decreased to 25–28 % with an increase in exposure to 20 min. This is apparently due to the phytotoxicity of mercuric chloride. The percentage of viable explants when treated with the studied sterilizing agents (except chloride-free ecosterilizer) was very low (20–24 %) at an exposure time of 10 min (table 1). The rest explants died from infection.

Table 1

The explants viability of high-half blueberry and Arctic bramble depending on sterilizing agents and exposure

Sterilization time, min	Explants viability, %					
	High-half blueberry			Arctic bramble		
	Mercuric chloride	Chloride-free ecosterilizer	Bleaching agent	Mercuric chloride	Chloride-free ecosterilizer	Bleaching agent
10	20	50	15	24	56	22
15	90	90	75	80	91	80
20	25	95	95	28	93	91

At the stage of micropropagation itself, increasing the concentration of cytokinin 6-BAP from 0.5 to 1.0 mg/L on the WPM medium increased the number of shoots in regenerated plants of high-half blueberry cultivars Northcountry and Northblue and Arctic bramble cultivars Anna and Sofia. At the concentration of 6-BAP 0.5 mg/L the number of shoots of high-half blueberry and Arctic bramble was

2.1–2.4 and 2.5–2.6 pcs, respectively. At the concentration of 1.0 mg/L it was 3.2–3.5 and 4.6–4.7 pcs, respectively. No significant differences were found in the studied cultures depending on the variety (table 2).

Table 2

The number of shoots of high-half blueberry and Arctic bramble depending on the cultivar and 6-BAP concentration, pcs

Species	Cultivar	6-BAP concentration, mg/L		LSD_{05}
		0.5	1.0	
High-half blueberry	Northblue	2.1	3.5	1.41
	Northcountry	2.4	3.2	1.93
Arctic bramble	Anna	2.6	4.6	0.13
	Sofia	2.5	4.7	0.22

LSD_{05} – least significant difference at 5 % significance level.

The average shoot length of high-half blueberry cultivars Northblue and Northcountry with an increase in cytokinin 6-BAP in the nutrient medium slightly decreased from 3.9–4.1 cm at 0.5 mg/L to 3.0–3.3 cm at 1.0 mg/L. A similar pattern was observed for Arctic bramble: the average length was 1.5–1.7 cm at the concentration of 6-BAP 0.5 mg/L, and 1.1–1.2 cm at 1.0 mg/L (table 3).

Table 3

The average shoot length of high-half blueberry and Arctic bramble depending on the cultivar and 6-BAP concentration, cm

Species	Cultivar	6-BAP concentration, mg/L		LSD_{05}
		0.5	1.0	
High-half blueberry	Northblue	4.1	3.0	0.29
	Northcountry	3.9	3.3	0.47
Arctic bramble	Anna	1.5	1.2	0.05
	Sofia	1.7	1.1	0.08

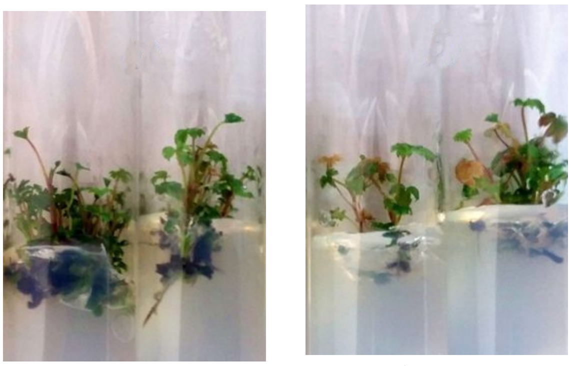
The total shoot length in both studied berry crops was greater at the concentration of 6-BAP 1.0 mg/L and reached 10.5–10.6 cm for half-high blueberry and 5.2–5.5 cm for Arctic bramble. It was 8.6–9.4 cm and 3.9–4.2 cm, respectively, at the concentration of 6-BAP 0.5 mg/L (fig.).

There were no significant differences in both berry crops depending on the cultivar (table 4).

Table 4

The total shoot length of high-half blueberry and Arctic bramble depending on the cultivar and 6-BAP concentration, cm

Species	Cultivar	6-BAP concentration, mg/L		LSD_{05}
		0.5	1.0	
High-half blueberry	Northblue	8.6	10.5	0.60
	Northcountry	9.4	10.6	0.34
Arctic bramble	Anna	3.9	5.5	0.19
	Sofia	4.2	5.2	0.33



a *b*

Arctic bramble plants *in vitro* at the stage of micropropagation itself with the addition of 6-BAP to the nutrient medium in the following concentrations, mg/L: *a* – 1.0; *b* – 0.5

At the *in vitro* rooting stage, we studied the effect of IBA-derived auxin concentrations on the number and length of roots of regenerated plants of high-half blueberry and Arctic bramble. The number of roots increased from 1.9–2.1 up to 3.9–4.1 pcs for half-high blueberry with an increase in the concentration of IBA-derived auxin in the nutrient medium from 0.5 to 1.0 mg/L, and from 3.7–3.8 to 4.5–4.6 pcs for Arctic bramble. Significant differences in the cultivars of the studied berry crops by the number of roots were not detected (table 5).

Table 5

The number of roots of high-half blueberry and Arctic bramble depending on the cultivar and IBA-derived auxin concentration, pcs.

Species	Cultivar	IBA-derived auxin concentration, mg/L		LSD_{05}
		0.5	1.0	
High-half blueberry	Northblue	1.9	4.1	0.34
	Northcountry	2.1	3.9	0.41
Arctic bramble	Anna	3.8	4.5	0.69
	Sofia	3.7	4.6	0.51

The average root length decreased slightly with increasing concentration of IBA-derived auxin. It was 1.5–1.6 cm at 0.5 mg/L, and 1.3 cm at 1.0 mg/L for half-high blueberry, and 1.1–1.2 and 0.8–0.9 cm, respectively, for Arctic bramble. There were practically no differences in the average root length of both berry crops depending on the cultivar (table 6).

Table 6

The average root length of high-half blueberry and Arctic bramble depending on the cultivar and IBA-derived auxin concentration, cm

Species	Cultivar	IBA-derived auxin concentration, mg/L		LSD_{05}
		0.5	1.0	
High-half blueberry	Northblue	1.6	1.3	0.45
	Northcountry	1.5	1.3	0.52
Arctic bramble	Anna	1.1	0.9	0.13
	Sofia	1.2	0.8	0.18

The total root length of half-high blueberry was increased from 3.0–3.1 cm to 5.1–5.3 cm with an increase in the concentration of IBA-derived auxin from 0.5 to 1.0 mg/L. It decreased from 4.2–4.4 cm to 4.1–4.2 cm for Arctic bramble. Cultivar differences in the total root length were not significant (table 7).

Table 7

The total root length of high-half blueberry and Arctic bramble depending on the cultivar and IBA-derived auxin concentration, cm

Species	Cultivar	IBA-derived auxin concentration, mg/L		LSD_{05}
		0.5	1.0	
High-half blueberry	Northblue	3.0	5.3	0.74
	Northcountry	3.1	5.1	0.93
Arctic bramble	Anna	4.2	4.1	0.69
	Sofia	4.4	3.7	0.97

Thus, a change in the concentration of IBA-derived auxin influenced the number of roots, the average root length and the total root length of high-half blueberry and Arctic bramble plants.

Conclusion

The following conclusions can be drawn according to the research results. Chloride-free ecosterilizer and bleaching agent with an exposure of 15 and 20 min showed quite high efficiency in surface sterilization of explants of high-half blueberry and Arctic bramble. The explants viability of the studied berry plants was high during sterilization with a 0.1 % mercuric chloride solution and an exposure of 15 min, and it sharply decreased with an exposure of 20 min.

An increase in the concentration of cytokinin 6-BAP from 0.5 to 1.0 mg/L on the WPM medium contributed to an increase in the number and total length of shoots of high-half blueberry and Arctic bramble, while the average shoot length slightly decreased. Therefore, it's advisable to add cytokinin 6-BAP at the concentration of 1.0 mg/L to the nutrient medium at the stage of micropropagation itself.

The number of roots of the studied berry plants increased, and its average root length decreased slightly with an increase in the concentration of IBA-derived auxin from 0.5 to 1.0 mg/L. The total root length of high-half blueberry increased and decreased for Arctic bramble. Therefore, it is rational to add 1.0 mg/L of IBA-derived auxin for half-high blueberry, and 0.5 mg/L of IBA-derived auxin for Arctic bramble when cloning at the *in vitro* rooting stage.

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ПОЛУЧЕНИЕ ВЫСОКОКАЧЕСТВЕННОГО ПОСАДОЧНОГО МАТЕРИАЛА ЛЕСНЫХ ЯГОДНЫХ РАСТЕНИЙ МЕТОДОМ КЛОНАЛЬНОГО МИКРОРАЗМНОЖЕНИЯ ДЛЯ РЕКУЛЬТИВАЦИИ ВЫРАБОТАННЫХ ТОРФЯНИКОВ

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Аннотация. Приведены результаты исследований по совершенствованию технологии получения высококачественного посадочного материала голубики полувысокой и княженики арктической методом клонального микроразмножения. Создание плантации лесных ягодных растений в районах добычи торфа позволяет снизить ущерб, наносимый окружающей среде, и значительно повысить эффективность лесной промышленности. В последние десятилетия в России и других странах мира растет интерес к созданию на осушенных и освоенных торфяниках насаждений лесных ягодных растений, для успешного выращивания которых в промышленных масштабах необходимо использовать сортовой посадочный материал. Наиболее эффективный метод (из вегетативных) его разведения – клональное микроразмножение, позволяющее в условиях небольшой лабораторной площади круглый год получать огромное количество оздоровленного посадочного материала. Экостерилизатор бесхлорный и отбеливающее средство на основе гипохлорита натрия «Белизна» показали высокую эффективность при стерилизации эксплантатов голубики полувысокой и княженики арктической с выдержкой 15 и 20 мин. Наиболее высокая жизнеспособность эксплантов исследуемых лесных ягодных культур отмечена при стерилизации 0,1 % раствором хлорида ртути и

выдержке 15 мин, низкая – при выдержке 20 мин. На стадии собственно микроразмножения при повышении концентрации цитокинина 6-БАП с 0,5 до 1,0 мг/л на питательной среде WPM увеличивалось количество побегов у регенерированных растений голубики полувысокой (сортов Northcountry и Northblue) и княженики арктической (сортов Anna и Sofia). На стадии укоренения *in vitro* отмечено влияние концентрации ауксина ИМК на количество и длину корней регенерированных растений.
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Ключевые слова: княженика арктическая, клональное микроразмножение, сорт, выработанные торфяники, регулятор роста, голубика полувысокая, *in vitro*.

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