Peculiarities of propagation *in vitro* of *Vaccinium vitis-idaea* L. and *V. praestans* Lamb.

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As a crop, commonly a few *Vaccinium* genus species are spread. In search for new, unique, physiologically important for human food and health and active compounds, other *Vaccinium* species became of interest as well. Micropropagation conditions for *V. praestans* Lamb. and *V. vitis-idaea* L were investigated. Differences between species were established regarding shoot growth, rhizogenesis under *in vitro* conditions, adaptation and growth in non-sterile environment. The potential to propagate quickly a big amount of *V. praestans* plants by utilizing the *in vitro* propagation method and differently from lingonberry to produce planting material fast is shown.

Key words: *Vaccinium vitis-idaea* L., *V. praestans* Lamb., micropropagation

INTRODUCTION

The importance of the genus *Vaccinium* plants in the world has been increasing [1]. From natural habitats they are transferred to plantations, cultured varieties are being developed and plant cultivation technologies prepared. So far as a crop only a few *Vaccinium* genus species have been commonly spread. In search for new, unique, physiologically important for human food and health and active compounds, other *Vaccinium* species became of interest as well. [2, 3]. *Vaccinium praestans* Lamb. is a creeper bush distributed in Far East [4]. Big-size berries are 1.5 cm in diameter, red, of tender consistency, sweet acid with a peculiar strong aroma [5]. Berries of *V. praestans* differ from lingonberry, European blueberry and bog blueberry in chemical composition by higher amounts of vitamin C, cellular tissues, zinc and chromium. In them have been detected 17 amino acids, of which 7 are irreplaceable [2]. Wider dissemination of the plant is limited by the lack of planting material. Germination of newly stratified seeds reaches 70%. In a year the seeds loose germination power. In the wild, seedlings in the first year grow very slowly and only in the 4th–5th year start side shooting. Propagation by rhizome partition is workable only since the 40th–50th year of plant growth [6].

The objective of the work was to study the conditions of *V. praestans* Lamb. and *V. vitis-idaea* L. micropropagation and to establish development peculiarities *in vivo* of propagated *in vitro* plants.

METHODS

The study included the lingonberry varieties 'Sanna' and 'Koralle' and *Vaccinium praestans* Lamb. seedlings No 115; No 119 and No 120. For induction of *in vitro* culture, apices of plants grown in greenhouse were employed. Explants were sterilized for 10 min in 9% calcium hypochlorite solution, 3 times washed with sterile water. From shoots rescued leaves and shoot segments with 3–5 internodes were planted on WPM [7] nutrient medium supplemented with 5 mg/l 2iP before autoclaving.

The nutrient medium for microshoot rhizogenesis was prepared based on high peat. High peat extraction at the ratio 1:1 (according to volume) was poured with distilled water. The mixture was shaken up and set aside for 24 h. Hereafter the extraction was filtered, pH adjusted to 5.4, agar agar introduced and the medium autoclaved.

Explants were cultured in a phytochamber at a temperature of 25 ± 2 °C, lighting 3000 lx, photoperiod 16 h. Subcultivation lasted 4–8 weeks. After 4 weeks the number of explants growing under *in vitro* conditions, the number and length of shoots regenerated in adapted culture, and the reproduction coefficient were computed. After 8 weeks of growth on rhizogenesis medium, shoot rooting and tillering in

non-sterile environment were assessed. Data were processed by the dispersion analysis method for alternative characters [8].

RESULTS AND DISCUSSION

After a 4-week cultivation on the medium, part of explants started growing: from their axillary buds new shoots regenerated. No essential differences among the *Vaccinium* species studied were revealed. The rate of explants inducing new shoots depended on the ability to dispose of fungal and bacterial infection. The latter rate depended on cultivation place and conditions of mother plants. When explants were rescued from outdoor plants, 33.3% of explants regenerated shoots, while isolating shoot apices from greenhouse plants 56% of explants regenerated shoots. In both cases, if the apical meristem was absent in an explant, apparently due to elimination of apical domination a bigger part of axillary buds regenerated shoots. If an explant contained the apical meristem, elongated growth manifested. New shoots did not form.

Growth parameters in adapted culture are presented in Table 1. The table illustrates that shoot growth parameters of various genotypes of both species in *in vitro* culture are rather similar. Lingonberry shoots are nearly twice longer than *V. praestans* microshoots. The internode number of lingonberry shoots, though reliably, is slightly higher than the internode number of *V. praestans*. All this brings closer the regeneration coefficients of various genotypes of these species. The internodes of lingonberry microshoots are longer. This mainly predetermines the differences in microshoot length and the lower output of lingonberry shoots from explant. *V. praestans* explants of similar length with shorter internodes concomitantly contain more axillary buds. Therefore, their shoot number per explant is usually higher.

The critical phase of *in vitro* propagation of lignified or half-lignified plants is rhizogenesis and the following adaptation of microshoots in non-sterile environment. Plants growing under *in vitro* conditions are supplied with an exogenic source of carbohydrates. Due to this and to insufficiently intensive lighting in cultivation dishes their ability to accomplish photosynthesis is limited. Leaves of microshoots are hardly covered with a wax layer and their physiological homeostasis is more susceptible. In Table 2 there are presented rhizogenesis results of the plant species studied. They show that rhizogenesis primarily depended on plant genotype. Lingonberry rhizogenesis of both investigated plant genotypes studied was inhibited by cytokinin 2iP in WPM nutrient medium. No rhizogenesis was found after three months of cultivation when cytokinin was decomposed or its concentration decreased. All genotypes of *V. praestans* on WPM medium supplemented with 2iP after three months of cultivation when cytokinin was decomposed or its concentration decreased regenerated roots at different frequency depending on plant genotype. Microshoot rhizogenesis of the genus *Vaccinium* depended on the physiological state of a shoot and its maturity level [9]. It has been established that lingonberry shoots regenerated roots best when they were not shorter than 15 mm [10]. In rhizogenesis experiments 20–35 mm long lingonberry shoots were employed. We observed that the shoot length 15–35 mm is an important but not a sufficient indicator of its maturity level. Physiological maturity is characterized by microshoot length, its color and leaf firmness. Other parameters might be as well related to shoot ability to regenerate roots. All this proves that in the last phase of propagation it is indispensable to attain the required physiological maturity of microshoots, as is the case in propagation by soft cuttings. Propagation of *V. praestans in vitro* has not been investigated. Microshoots of this species throughout the same period grow up shorter (Table 1). Thus, in shoot regeneration experiments were employed shoots 11–20 mm long. The findings show that *V. praestans* microshoots 11 mm long are competent for rhizogenesis.

Table 1. **Comparison of *Vaccinium vitis-idaea* and *V. praestans* growth in *in vitro* adapted culture**

Plant genotype	Shoot number/explant	Shoot length (mm)*	Internode quantity*	Regeneration coefficient
Koralle	1.2	30.3 ± 1.57	7.9 ± 0.44	3.5
Sanna	1.3	28.2 ± 1.31	8.0 ± 0.45	3.7
Nr. 115	1.4	15.9 ± 1.80	6.0 ± 0.45	3.0
Nr. 119	1.6	16.9 ± 1.63	5.6 ± 0.31	3.3
Nr. 120	1.6	5.5 ± 0.52	4.6 ± 0.31	2.9

*Mean ± SD

Table 2. **Comparison of *Vaccinium vitis-idaea* and *V. praestans* rhizogenesis in *in vitro* culture**

Plant genotype	WPM medium		Nutrient medium based on peat extraction		Survived under non-sterile conditions (%)
	Planted shoots (ps)	Rhizogenesis (%) *	Planted shoots (ps)	Rhizogenesis (%) *	
Koralle	70	0 d	30	86.7 ab	69.2 c
Sanna	70	0 d	35	54.3 c	80.3 bc
No. 115	20	61.5 a	20	90.0 ab	33.3 d
No. 119	24	45.8 b	26	75.0 b	91.7 ab
No. 120	30	10.0 c	11	100.0 a	100.0 a

* Means are significantly different at $p < 0.01$ applying Duncan's multiple range test.

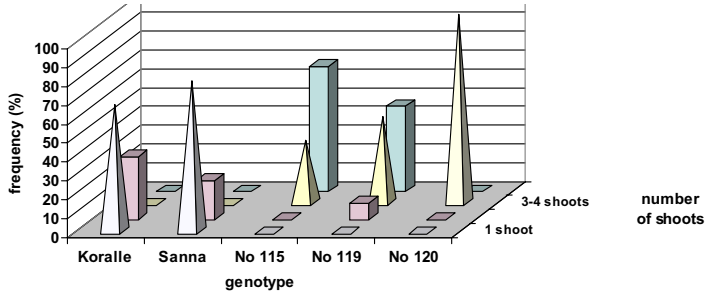

Figure. Growth of *Vaccinium vitis-idaea* and *V. praestans* plants propagated *in vitro*

Microshoot adaptation under non-sterile conditions is another critical stage. After transferring plants from cultivation dishes to high peat under non-sterile conditions adapted 69–80% lingonberry and 33–100% of *V. praestans* plants (Table 2). In five months 66–79% of established lingonberry plants failed to tiller and raised only one shoot (Figure). Only 20–33% of plants produced a second shoot. A long stretch of time (1–1.5 year) is needed for direct production of standard planting material. Over the same period all plants of *V. praestans* tillered. Most of plants (91–100% depending on genotype) had three or more shoots. Naturally *V. praestans* propagates by seeds and vegetatively. Vegetative propagation prevails. A single shoot is typical of juvenile plants. Later 1–2 dormant buds at cotyledon axils awaken and the tillering starts. Side shoots pick up intensive growth in the third year [6]. *In vitro* propagated plants, though in the juvenile state, start intensive tillering already in the fifth month. Thus, by applying the *in vitro* propagation method it is possible to propagate quickly great numbers of *V. praestans* plants and, offered differently from lingonberry, to produce planting material fast.

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VACCINIUM VITIS-IDAEA L. IR _V. PRAESTANS_ LAMB. DAUGINIMO _IN VITRO_ YPATYBĖS

S a n t r a u k a

Kultūroje plačiau paplitusios kelios *Vaccinium* genties rūšys. Ieškant naujų, unikalių, žmogaus mitybai ir sveikatai svarbių, fiziologiškai aktyvių medžiagų, pradėta domėtis ir kitomis *Vaccinium* genties rūšimis. Bandymų metu ištirtos *V. praestans* Lamb. ir *V. vitis-idaea* L. mikrodauginimo sąlygos. Nustatyti ūglių augimo, rizogenezės *in vitro* sąlygomis, adaptavimosi ir augimo nesterilioje aplinkoje skirtumai tarp šių rūšių. Parodyta galimybė taikant *in vitro* dauginimo metodą greitai padauginti didelį kiekį *V. praestans* augalų ir, skirtingai nei bruknėms, greitai išauginti sodinamąją medžiagą.