ŽEMĖS ŪKIO MOKSLAI. 2007. T. 14. Nr. 2. P. 40–44


*Sodininkystė ir daržininkystė • Horticulture • Садоводство и овощеводство*

# Adaptation of American cranberry to substrate pH *in vitro* and *ex vitro*

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The aim of the present work was to investigate the properties of physiological and morphological changes during adaptation of American cranberry (*Oxycoccus macrocarpus* (Aiton) Pursh) to nonoptimal acidity of the substrate *in vitro* and *ex vitro*. The investigation was carried out with three cranberry cultivars: 'Bergman', 'Bain 10' and 'Black Weil'. Changes in the morphological parts of plants during adaptation were measured. The content of mono-carbohydrates in microshoots growing in media of different acidity was evaluated by high-pressure liquid chromatography. 'Bergman' and 'Black Weil' were found to be more adaptive than 'Bain 10' to the pH of the substrate. The content of mono-carbohydrates in microshoots depended on the acidity of the medium. The pH of the substrate influenced microshoot growth and the pigmental system of photosynthesis in plants. Cranberry plants tolerate a more alkaline pH when growing *in vitro* than *ex vitro*.

**Key words:** American cranberry, adaptation, substrate pH, *in vitro, ex vitro*

## INTRODUCTION

The adaptation of plants determines their ability to survive in unfavourable conditions. There are many factors influencing the growth of plants. One of them is soil acidity. The assimilation of nutritives depends on the acidity of the soil [4]. For many plants, there are more toxic metals (Mn, Cu, Cd et cetera) in acid soils than in alkaline ones. Acid soil hampers agricultural productivity because of P, Ca, Mg, Mo deficiency and aluminum toxicity [13]. Profusion of toxic Al compounds in acid soils makes plants accumulate Fe, Mn and other heavy metals [1, 11]. Several plant species have evolved the mechanism that enables them to grow on acid soils with a toxic concentration of $Al^{3+}$. In some Al-tolerant wheat varieties, Al tolerance depends on Al-inducible release of malate; root exudation of oxalate in buckwheat also correlates with Al tolerance [10]. Al-tolerant cultivars of wheat have a single dominant locus designated as *Alt1*. The *Alt1* gene was cloned [12]. High pH also results in low phosphate availability and in reduced micronutrient (Zn, Fe, Mn) availability. The pH below 4 $H^+$ ions prevails in the composition of the soil solution when competing with other cations for root absorption sites, interfering with ion transport and uptake by roots [7].

American cranberry (*Oxycoccus macrocarpus* (Aiton) Pursh) is an important commercial crop which has adapted to grow in acid soils. The best pH of soil for cranberry is between 2.9–5.5. High concentrations of heavy metals are not dangerous for cranberry. It has adapted to accumulate Fe, Mn and other heavy metals and is widely valuated for a high content of microelements (Na, K, Ca, Mg, P, I, Fe, Mn, Cu, Cl).

It is still unknown what determines the adaptation abilities of acidophilic plants and what possibilities there are to regulate the adaptation. Various biotechnological methods may be used for solving these problems. It was noticed that blueberry (*Vaccinium corimbosum)* tolerates higher pH growing *in vitro* [3]. *In vitro* media give possibility to grow plants in optimal conditions with all necessary nutritives well available for plants. Besides, an *in vitro* system allows to analyse precisely the effect of any factor on growing plants. It has been supposed that cultivating cranberry *in vitro* may widen its tolerance of substrate pH *in vitro* and *ex vitro*.

The aim of the present work was to investigate the physiological and morphological changes during adaptation of cranberries to a nonoptimal acidity of the substrate *in vitro* and *ex vitro*.

## METHODS AND CONDITIONS

The investigation was carried out with three cranberry cultivars: 'Bain 10', 'Bergman' and 'Black Weil'. Explants were taken from plantlets cultivated *in vitro* in a medium pH 5.6. Twenty 15–27 mm long rootless, uncontaminated explants of all three cultivars were planted in all five nutrient media of different acidity: pH 4, pH 5, pH 6, pH 7, and pH 8. Woody Plant Medium (WPM)

[9] was used. Additionally, 30 g/l sucrose, 7 g/l agar, 2iP were added into the nutrient medium. The acidity of nutrient medium was fixed by using 1 mol/l caustic soda and hydrochloric acid solutions. Isolated explants were grown in a chamber at 50 μmol/$m^2$s photosynthetic photon flex density (PPFD) (photoperiod 16 h and temperature 20/25 °C, day/night). Subcultivation was done every two months. Every fifteen days the length of microshoots, the number and the length of roots were fixed. In addition, the amount of mono-carbohydrates in microshoots was measured using high-pressure liquid chromatography [2]. Plants 1.5 g in mass were ground, infused with 4 ml ~70 °C distilled water, extracted for 24 h and filtered through 0.45 μm filter. For high-pressure liquid chromatography, the Adsorbil $NH_3$ column 5 μm (150 mm × 4.6 mm) was used. Mobile phase: acetonitrile / $H_2O$ 75 / 25, flow rate 1 ml/min, refractive index detector (Shimadzu LC–10A) [5]. After 12 months of growing in a nutrient medium of various acidity *in vitro*, cranberry microplants were planted *ex vitro* in peat of three different acidities (pH 4.2, pH 5.5 and 6.45). The acidity of peat was fixed by using caustic soda solution. Cranberries grew *ex vitro* for two months; the number of limbs and the length of plants were measured.

The means and their standard errors were calculated using Microsoft Excel and analysis of variance (ANOVA).

## RESULTS AND DISCUSSION

For fourteen months cranberry microshoots grew *in vitro* in a medium of different acidity. Microshoots of several cultivars differently reacted to the pH (Fig. 1). Within the first two months (first subcultivation) of the experiment, a moderately alkaline medium (pH 8) provided to be most adverse to microshoots of all three cultivars. 'Bain 10' microshoots growing in an extremely acid (pH 4) nutrient medium were even smaller than the ones growing in a moderately alkaline medium (pH 8). Within the first two months the growth of cranberry microshoots was slower in media where pH differed more from pH in a previously used medium.

After six months (third subcultivation) since the beginning of the experiment, the growth trends of 'Bergman' and 'Black Weil' microshoots were changed (Fig. 2). The average length of microshoots increased respectively 3.3 and 2.5 times in the extremely acid (pH 4), 2.7 and 1.9 times in strongly acid (pH 5) and only 1.3 and 1.5 times in slightly acid (pH 6) nutrient media. On the average, microshoots growing in a moderately alkaline (pH 8) medium were 7.0 and 5.5 times longer in the first two months of experiment.

The longest microshoots of 'Bain 10' grew in a slightly acid (pH 6) medium. Their length increased 1.75 times more than during the first two months of the experiment. The increase of microshoots in the extremely acid (pH 4) medium was even 8.8 times larger (average increasement during the first and second months of experiment was only 11.2 mm). The average length of microshoots in the extremely acid (pH 4) medium became the same as in less acid (pH 5) and almost the same as in slightly acid (pH 6) media. In the extremely alkaline medium (pH8) the length of microshoots increased even 2.7 times. A longer period of time (6–7 months) was required for cranberry microshoots to adapt to grow successfully in a medium with the acidity level customary to cranberries.

During the thirteenth–fourteenth months (after the seventh subcultivation) of the experiment, the growth trends of cranberry microshoots did not change much as compared to the sixth–seventh months, but it was different from the growth trends during the first and second months. It means that the cranberry culture *in vitro* had become adapted (Fig. 3). The average length of microshoots was shorter than in the sixth–seventh months. 'Bergman' microshoots grew statistically equally in all acid and neutral media. The growth tendencies of 'Black Weil'

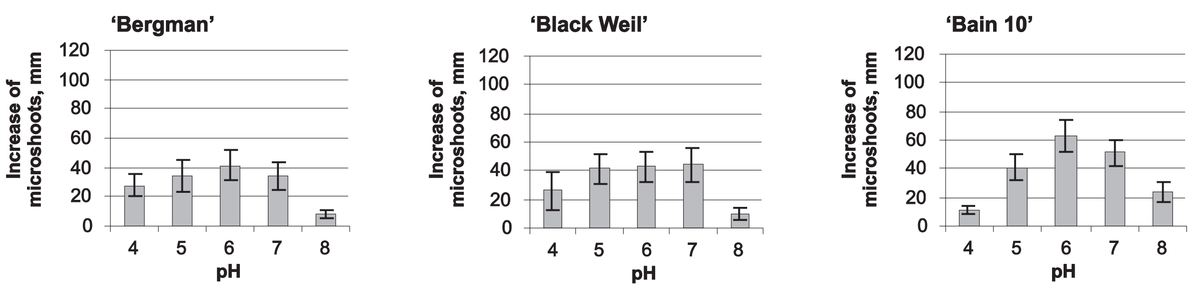


**Fig. 1.** Increase of microshoot length of American cranberry cultivars 'Bergman', 'Black Weil' and 'Bain 10' in nutrient medium of different acidity *in vitro* during the first–second months of experiment

**1 pav.** Stambiauogės spanguolės veislių 'Bergman', 'Black Weil' ir 'Bain 10' mikroūglių prieaugis skirtingo rūgštingumo terpėse per pirmus du kultivavimo *in vitro* mėnesius

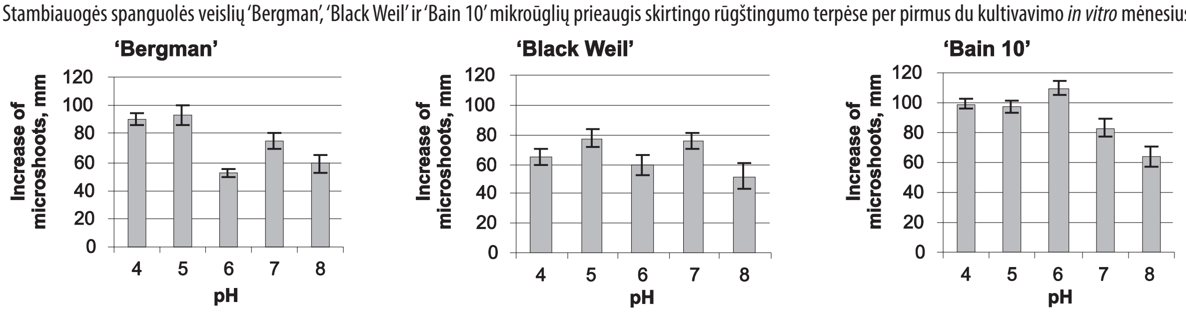


**Fig. 2.** Increase of microshoot length of American cranberry cultivars 'Bergman', 'Black Weil' and 'Bain 10' in nutrient medium of different acidity *in vitro* during the sixth–seventh months of experiment

**2 pav.** Stambiauogės spanguolės veislių 'Bergman', 'Black Weil' ir 'Bain 10' mikroūglių prieaugis skirtingo rūgštingumo terpėse per šeštą–septintą kultivavimo *in vitro* mėnesius microshoots remained the same as during the sixth–seventh months of experiment. The growth tendencies of 'Bain 10' microshoots became almost the same as those of 'Black Weil'. 'Bain 10' microshoots needed more time for adaptation to varied pH of the medium. Thus, cranberry cultivars were conditionally divided into two groups: adaptive ('Bergman' and 'Black Weil') and non-adaptive ('Bain 10') according to the response of microshoots to the different acidity of the nutrient medium and the duration of adaptation.

Fifteen days from the beginning of subcultivaton, microshoots began to take roots. In the adapted culture (thirteenth–fourteenth months, the seventh subcultivation) the growth of roots showed the same trends as the increase of microshoots (Fig. 4). Microplants of 'Bain 10' raised few and short roots in all pH variants. Even 26.3% of this cultivar microshoots in pH 6 and pH 8 had no roots at all. Meanwhile, more than half of 'Black Weil' microplants gained many and long (more than 20 mm) roots. The adaptive cultivars 'Bergman' and 'Black Weil' grew significantly longer roots in a medium of all pH than did the non-adaptive cultivar 'Bain 10'. Substrate pH influences the absorption of microelements (P, N, Fe, etc.) by roots and also influences root growth, branching, root hair length. The effect of medium pH depended on the genotype (Fig. 4). In Arabidopsis, the low-P-induced root growth is directed toward increasing the P-uptake capacity of the plant root system by modulating root architecture and by inducing the expression of genes that are involved in P uptake [8]. In Arabidopsis, increasing N availability reduces primary root elongation, whereas an increase in P supply has the opposite effect. We may suppose that the differences among cranberry cultivar rooting intensity are determined by the nature of genes responsible for rooting and the uptake of microelements.

After fourteen months of cultivation *in vitro* in media of different acidity the content of glucose, fructose and sucrose in microplants was measured (Fig. 5). No consistent pattern of sucrose change according to the acidity of nutrient medium was noted. Glucose and fructose proportion did not depend on the acidity of a medium. 'Bergman' and 'Bain 10' had more glucose; 'Black Weil' had equal levels of both mono-carbohydrates. The

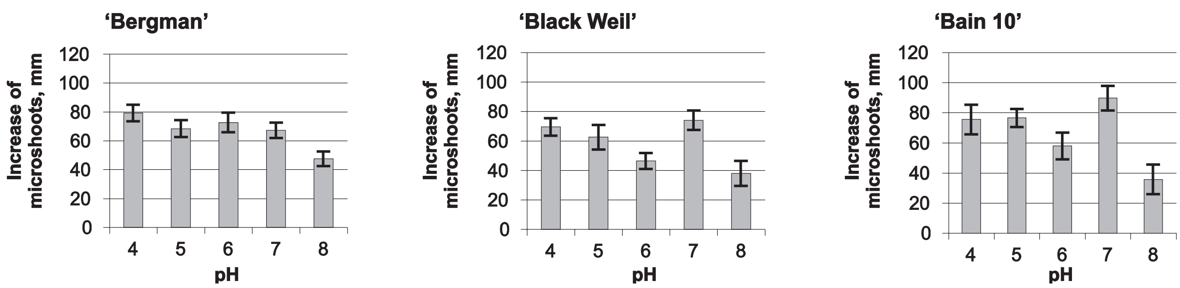


**Fig. 3.** Increase of microshoot length of American cranberry cultivars 'Bergman', 'Black Weil' and 'Bain 10' in nutrient medium of different acidity *in vitro* during the thirteenth–fourteenth months of experiment

**3 pav.** Stambiauogės spanguolės veislių 'Bergman', 'Black Weil' ir 'Bain 10' mikroūglių prieaugis skirtingo rūgštingumo terpėse per tryliktą–keturioliktą kultivavimo *in vitro* mėnesius

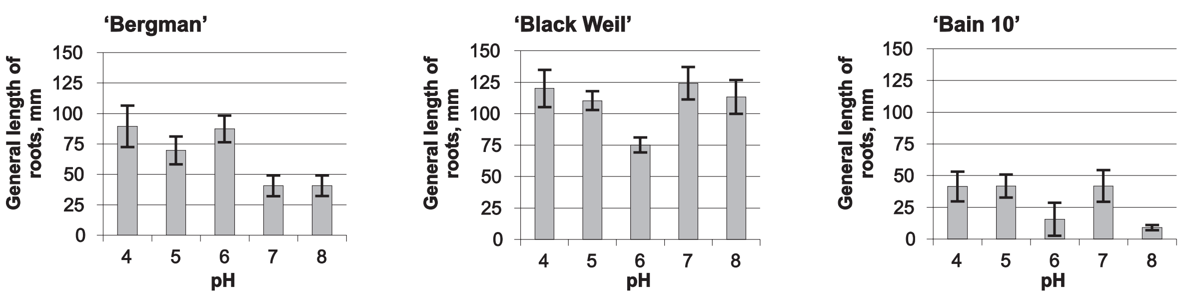


**Fig. 4.** General length of microshoot roots of different cultivars growing in media of different acidity during the thirteenth–fourteenth months of experiment

**4 pav.** Skirtingų veislių mikroaugalų šaknų ilgis skirtingo rūgštingumo terpėse per tryliktą–keturioliktą kultivavimo *in vitro* mėnesius

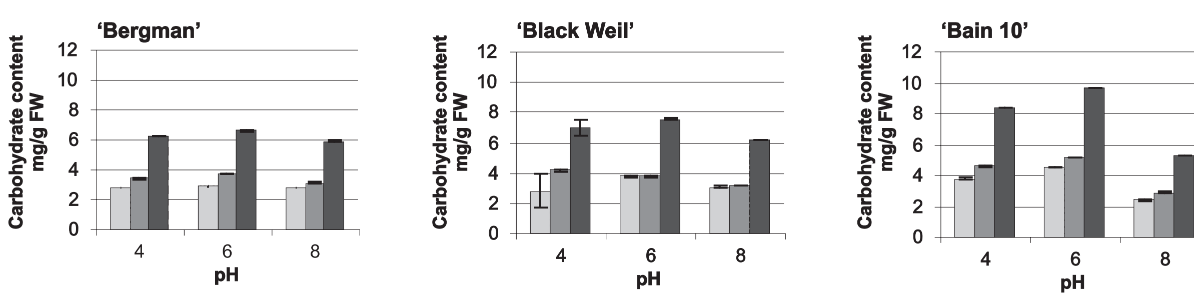


**Fig. 5.** Carbohydrate content in microshoots growing on media of different acidity. ▢ glucose, ▢ fructose, ■ general content of glucose and fructose

**5 pav.** Angliavandenių kiekis mikroūgliuose, užaugintuose skirtingo rūgštingumo terpėse. ▢ gliukozė, ▢ fruktozė, ■ bendras gliukozės ir fruktozės kiekis

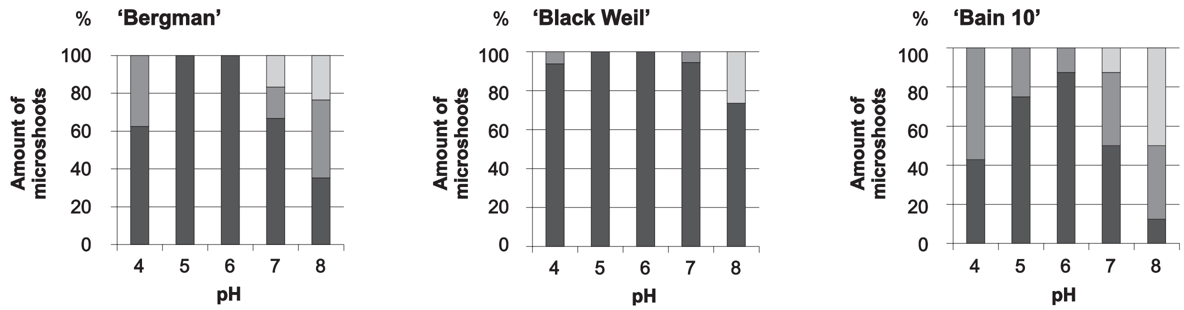


**Fig. 6.** The colour of leaves of microshoots growing in media of different acidity. ■ green, ■ light green, □ yellow

**6 pav.** Mikroaugalų. augančių kirtingo rūgštingumo terpėse, lapų spalva. ■ žalia, ■ šviesiai žalia, □ geltona

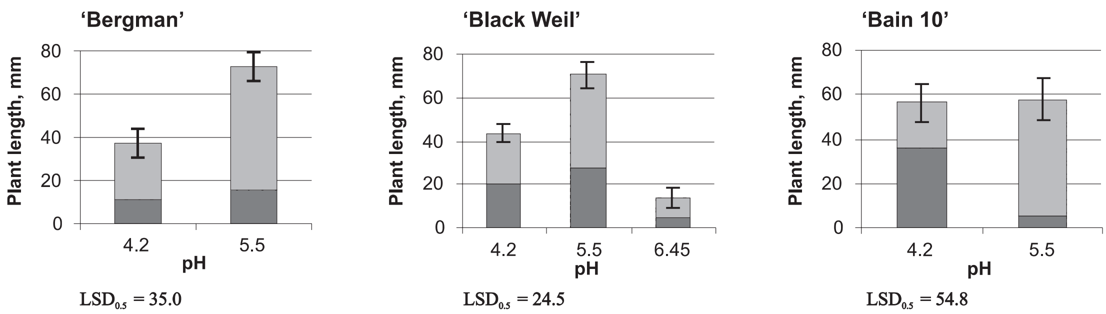


**Fig. 7.** Growth dynamics of plants in peat of different acidity *ex vitro*. Length of plants after ■ 30 days, □ 60 days

**7 pav.** Augalų augimo dinamika skirtingo rūgštingumo terpėse *ex vitro*. Augalų ilgis po ■ 30 dienų, □ 60 dienų

differences of the general amount of these two sugars according to the acidity of medium were very little in adaptive ('Bergman' and 'Black Weil') cultivars. Meanwhile, 'Bain 10' contained 1.2–1.5 times more mono-carbohydrates in acid (pH 4, pH 6) media than other cultivars, but there was 0.9 times less sugars in alkaline one. Thus, the difference between the amount of mono-carbohydrates in acid and in alkaline media was very evident in these cultivars.

The content of mono-carbohydrates depends on the intensity of photosynthesis. The intensity of photosynthesis, beside other factors, is affected also by the content of P in the plant. Acid or alkaline soils are particularly prone to P deficiency [13]. Cranberry plants are adapted to grow in acid soils, and the alkaline medium (pH 8) exerted the most negative influence on photosynthesis, especially in 'Bain 10' microshoots.

The colour of microshoot leaves after 14 months of growing *in vitro* in media of different acidity proves the premise (Fig. 6). Microshoots of all the study cultivars had yellow leaves in an alkaline medium. The main symptom of Fe deficiency in plants is chlorosis in young leaves. Iron deficiency is a problem of alkaline soils. We have found that Fe uptake rates are correlated with the requirement of the shoot rather than with iron concentration in the cells that mediate the uptake. Such behaviour is indicative of the involvement of a diffusible signal communicating the iron status between the shoot and the roots [6]. There were more microshoots of 'Bain 10' than of the other two ones, which were yellow or light green in all acid and neutral nutrient media.

After twelve months of growing *in vitro* in media of different acidity, cranberry microshoots were planted in peat of different acidity (Fig. 7). Only 'Black Weil' plants grew in the neutral (pH 6.45) substrate *ex vitro*. Plants of the rest cultivars died in the substrate of this acidity. The best substrate acidity for all cultivars was pH 5.5. The growth tendencies of 'Bergman' and 'Black Weil' were the same already after the first thirteen days. During the first month 'Bain 10' plants grew better in the very strongly acid (pH 4.2) substrate. During the second month of experiment, the length of shoots in moderately acid (pH 5.5) substrate significantly increased even 10.6 times and in the very strongly acid one decreased 1.7 times. It means that the adaptation period of 'Bain 10' plants is longer than of other cultivars.

## CONCLUSIONS

1. The length of cranberry microshoots and roots was different and depended on the time scale of adaptation, the genotype and substrate pH *in vitro* and *ex vitro*.

2. The genotype and acidity of the medium affects the process of photosynthesis. The change of the amount of mono-carbohydrates (glucose and fructose) in plants characterizes the genotype adaptation.

3. The adaptation period to a nonoptimal acidity of the substrate depends on plant genotype. It was shorter for 'Bergman' and 'Black Weil' than for 'Bain 10' plants.

4. Cranberry plants tolerate more alkaline pH growing *in vitro* than *ex vitro*. Alkaline (pH 8) nutrient medium has a negative effect on the growth of cranberries *in vitro*. Plants *ex vitro* did not survive or their growth was very weak in soil with pH 6.45.

Received 7 March 2007
Accepted 22 May 2007

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**Gražina Stanienė, Rugilė Stanytė**

## STAMBIAUOGĖS SPANGUOLĖS PRISITAIKYMO PRIE SKIRTINGO RŪGŠTINGUMO SUBSTRATO *IN VITRO* IR *EX VITRO* YPATYBĖS

*Santrauka*

Darbo tikslas – ištirti stambiauogės spanguolės (*Oxycoccus macrocarpus* (Aiton) Pursh) morfologinių ir fiziologinių parametrų kitimus prisitaikymo prie pesimalaus auginimo substrato pH *in vitro* ir *ex vitro* sąlygomis. Tirtos spanguolių veislės 'Bergman', 'Bain 10' ir 'Black Weil'. Per adaptacijos laikotarpį vertinti augalų morfologinių parametrų pakitimai. Mikroaugaluose, išaugintuose skirtingo rūgštingumo terpėse, skysčių chromatografijos metodu nustatytas monosacharidų kiekis.

Nustatyta, kad spanguolių veislių 'Bergman' ir 'Black Weil' augalai lengviau prisitaiko prie substrato pH, nei veislės 'Bain 10' augalai. Parodyta, kad substrato pH turi įtakos mikroaugalų augimui ir monosacharidų kiekiui juose. Pastebėta, kad substrato pH veikia spanguolių fotosintezės pigmentinę sistemą. Nustatyta, kad spanguolių augalai šarminį substrato pH labiau toleruoja *in vitro*, negu *ex vitro* sąlygomis.

**Raktažodžiai:** spanguolės, substratai, *in vitro*, *ex vitro*

**Гражина Станене, Ругиле Станите**

## ОСОБЕННОСТИ АДАПТАЦИИ КЛЮКВЫ КРУПНОПЛОДНОЙ К СУБСТРАТУ РАЗЛИЧНОЙ КИСЛОТНОСТИ *IN VITRO* И *EX VITRO*

*Резюме*

Целью настоящей работы явилось выявление изменения морфологических и физиологических параметров при адаптации клюквы крупноплодной (*Oxycoccus macrocarpus* (Aiton) Pursh) к субстрату различной кислотности в условиях *in vitro* и *ex vitro*. Исследованию подвергались три сорта клюквы – 'Бергман', 'Байн 10' и 'Блэк Вейл'. Морфометрические параметры микрорастений измерялись в течение всего периода адаптации. Методом жидкой хроматографии установлено количество моносахаридов в микрочеренках, выросших на питательной среде разной кислотности. Показано, что сорта 'Бергман' и 'Блэк Вейл' более адаптивны к изменениям pH питательной среды, чем сорт 'Байн 10'. Установлено влияние pH питательной среды на количество моносахаридов и процесс фотосинтеза в микрорастениях. Показано, что растения клюквы крупноплодной более терпимы к щелочному субстрату в условиях *in vitro*, чем *ex vitro*.

**Ключевые слова:** клюква крупноплодная, субстрат, *in vitro*, *ex vitro*