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MICROPROPAGATION OF HIGHBUSH BLUEBERRY CULTIVARS
AUGSTKRŪMU MELLEŅU ŠĶIRŅU MIKROPAVAIROŠANA

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Abstract
The aim of this study was to determine an efficient micropropagation system for the highbush blueberry cultivars 'Spartan', 'Bluecrop' and 'Berkeley'. The shoot tips of the selected three genotypes were successfully established *in vitro* using mercuric chloride in a concentration of 0.15 % as a sterilization solution. Anderson's rhododendron medium (AN), half-strength Murashige and Skoog medium (half-MS) and McCown woody plant medium (WPM) containing cytokinin zeatin in concentrations 0.5, 1 or 2 mg l^{-1} were tested. Multiplication rates varied depending on the cultivar, medium and concentration of the zeatin. The highest multiplication 4.8 ± 0.2 was noted for 'Berkeley' on WPM medium with zeatin (2 mg l^{-1}). Out of three media tested, the WPM medium was found to be more effective than the AN medium and half-MS medium for shoot multiplication. The *in vitro* rooting on the WPM medium is also reported.

Kopsavilkums
Pētījuma mērķis bija noteikt augstkrūmu melleņu šķirņu 'Spartan', 'Bluecrop' un 'Berkeley' efektīvāko mikropavairošanas sistēmu. Izvēlēto trīs genotipu dzinumu gali tika veiksmīgi ievietoti *in vitro* izmantojot 0.15% koncentrācijas dzīvsudraba hlorīdu kā sterilizācijas šķīdumu. Tika pārbaudīta Andersona rododendra barotne (AN), pus-stiprā Murashige un Skoog barotne (pus-MS) un McCown koksnaino augu barotne (WPM), kas satur citokinīna zeatīnu 0.5, 1 vai 2 mg l^{-1} koncentrācijā. Pavairošanas ātrums bija atkarīgs no šķirnes, barotnes un zeatīna koncentrācijas. Augstākais pavairošanas koeficients 4.8 ± 0.2 bija vērojams šķirnei 'Berkeley' WPM barotnē ar zeatīnu (2 mg l^{-1}). No trim pārbaudītājām barotnēm dzinumu pavairošanai WPM barotne bija efektīvāka par AN barotni un pus-MS barotni. WPM barotrnē tika novērota arī dzinumu *in vitro* apsakņošanās.

Key words: explant, *in vitro*, zeatin, multiplication, rooting

Introduction
The highbush blueberry (*Vaccinium corymbosum* L.), native to North America, is a commercially important fruit crop (Zmarlicki, 2006). Although plants of the *Vaccinium* genus have not been cultivated on a large scale in the Czech Republic, there is potential for commercial highbush blueberry production in some mountain regions. A number of research programs continue to find suitable high yielding highbush blueberry cultivars with superior berry qualities for commercial growing in the rural countryside of the Czech Republic (Paprstein *et al.*, 2006; Paprstein and Ludvikova, 2006). In recent years, the blueberry has received considerable attention for its nutritional quality and health benefits (Howell, 2009).
If suitable cultivars are to receive wide distribution rapid propagation techniques will be essential. Highbush blueberry can be propagated vegetatively by multiple-node softwood or hardwood cuttings. This method, although generally successful, is slow and labor intensive. Success with

cuttings also varies markedly with the individual genotype, age of the stock plant and the vegetation period (Kosina and Sedlak, 2006).
The micropropagation can potentially multiply selected cultivars more rapidly than traditional nursery methods utilizing softwood or hardwood cuttings. Over the last three decades, *in vitro* culture propagation methods on various basal media using axillary bud proliferation and adventitious shoot regeneration has been achieved with varying success (Reed and Abdelnour, 1991; Noe *et al.*, 1998; Gajdosova *et al.*, 2006; Li *et al.*, 2006). Unfortunately, these results are not broadly applicable, because the effectiveness of the medium and morphogenesis of *Vaccinium in vitro* plants seems to be highly dependent on plant growth regulators and the media used for the culture, and this dependence is genotype specific (Ostrolucka *et al.*, 2004; Debnath, 2007). Moreover, the response of individual genotypes can vary during the whole cycle of micropropagation (Mehri-Kamoun *et al.*, 2004). The suitability and genetic stability of blueberry plants micropropagated in tissue culture have also been discussed for a long time (El-Shiekh *et al.*, 1996; Smolarz and Chlebowska, 1997).
As a part of the project to introduce highbush blueberry culture to the Czech Republic, studies were conducted to investigate new genetic resources and their possible multiplication by *in vitro* culture (Paprstein *et al.*, 2005). The objective of this study was to compare various basal media with different zeatin concentrations for shoot proliferation and to determine the best one for micropropagation of three highbush blueberry cultivars.

Materials and Methods
For the *in vitro* culture establishment, twenty actively growing shoot tips (5 to 15 mm in length) were cut from shoots of three blueberry cultivars ('Spartan', 'Bluecrop' and 'Berkeley') sprouting in laboratory conditions. The donor shoots were removed from mature shrubs growing in field germplasm collection of RBIP Holovousy in March. After removal of most of the leaves, the initial explants were dipped in a 0.15 % solution of $HgCl_2$ with a wetting agent added (0.05 % Tween-20) for 1 min. This was carried out under sterile conditions under a laminar flow hood. Following sterilization, the tips were rinsed in sterile distilled water and cultured in 200 ml glass culture flasks (seven shoots per flask), each with 35 ml of WPM (woody plant medium) according to Lloyd and McCown (1981). The initial WPM medium was with 1 mg l^{-1} zeatin. Culture vessels were glass bottles capped with clear permeable polypropylene caps. The contamination rate, the survival and development of shoots from excised shoot tips were analyzed after sterilization. Uncontaminated shoots established on WPM medium were transferred after one month to a fresh proliferation medium. All shoot cultures were serially subcultured for at least 4 months on a WPM medium supplemented with 2.0 mg l^{-1} zeatin. This provided a stock collection of shoots for proliferation studies.
All initiation and multiplication media contained 7.0 g l^{-1} Difco agar. The pH of the media was adjusted to 5.2 before autoclaving at 120 °C at 100 kPa for 15 minutes. Cultures were grown in rooms under cool-white fluorescent tubular lamps at 60 $\mu mol.m^{-2}.s^{-1}$ (16-hour photoperiod) at 22 ± 1 °C.
For the multiplication phase, three basal nutrient media WPM, AN (Anderson's rhododendron medium) according to Anderson (1980) and modified MS (Murashige and Skoog, 1962) medium containing half macro and micronutrients (half-MS) were tested. The shoot tip cultures were multiplied by removing several elongating shoots from the basal mass and subculturing the shoots on a fresh medium. To induce new shoots, basal nutrient media included three concentrations 0.5, 1 or 2 mg l^{-1} of the cytokinin zeatin. The zeatin was filter sterilized (25 mm, Acrodisc Syringe Filter 0.2 μm, Pall Gelman, USA) and added to multiplication media after autoclaving. Uniform single shoot tips (5 to 10 mm in length) excised from apical parts of established proliferating cultures were used in all multiplication experiments. The morphological appearance of the shoots (primarily callus formation, hyperhydricity etc.) was also noted.
The multiplication rate was defined as the number of newly formed shoots (>10 mm) per initial shoot tip after four weeks of culture. The shoot formation was recorded between the fifth and fifteenth subculture. In all experiments 25 shoot tips were used. Each experiment was repeated four times. Data from four independent experiments were pooled and expressed as the mean. To evaluate the accuracy of the estimate of the mean of population, treatment means were compared

with the standard error (SE) of the mean as a measure of variance. For three genotypes ('Spartan', 'Bluecrop', 'Berkeley'), shoots (10 to 20 mm in length) derived from the best proliferation medium, were excised and rooted on WPM medium with 1 mg l^{-1} IBA. Although several media were evaluated for the induction of roots, only results from medium that showed maximal root induction are presented in this report. Culture conditions during root initiation and root growth were the same as during shoot culture. A hundred microcuttings were used for this treatment. The number of rooted *in vitro* plants was recorded five weeks after transfer to rooting medium. The treatment means were compared with the standard error (SE) of the mean. Shoots with roots were rinsed in water to remove remnants of the medium and then transferred to Jiffy 7 peat pellets (AS Jiffy Products, Norway) soaked with water. The shoots were misted with water to prevent wilting during transplanting. The Jiffy 7 pellets with rooted plants were placed on a greenhouse bench equipped with transparent plastic covers (100 % air humidity) under the standard greenhouse condition. The plants were gradually acclimated by opening the covers over fourteen days.

Results and Discussion

The numbers of uncontaminated explants that survived and developed into shoots are shown in Table 1. Selected three genotypes were successfully established *in vitro* using mercuric chloride in a concentration of 0.15 % as a sterilization solution. Bacterial and fungal contamination was infrequent. Of the 60 shoot tips taken only one explant of 'Spartan', one explant of 'Berkeley' and two explants of 'Bluecrop' were visibly contaminated with micro-organisms. These explants were later discarded. The use of mercuric chloride had a direct beneficial effect and overcame the contamination from the microflora of the field germplasm collections of blueberry. On the other hand, the toxicity to tissues caused by mercuric chloride was high. In the case of the cultivars 'Spartan' and 'Bluecrop', about 50 % of initial uncontaminated explants did not develop shoots and turned brown. However, the remaining uncontaminated initial explants of these two cultivars had a greenish color and produced shoots. Debnath and McRae (2001) reported that although regeneration from primary explants is a first necessary step in any micropropagation of *Vaccinium* genus, the regeneration frequency has no effect on the further success of the micropropagation program. Many shoots could be obtained from a few clean shoots regenerated from the primary explant.

Table 1. Surface sterilization of highbush blueberry cultivars by 0.15 % mercuric chloride

Cultivars	Explants contaminated		Explants died without contamination		Established explants which developed shoots	
	Number	(%)	Number	(%)	Number	(%)
Spartan	1	5	11	55	8	40
Bluecrop	2	10	10	50	8	40
Berkeley	1	5	5	25	14	70

After 5 months in the culture, all surviving explants showed active and uniform shoot growth and multiplication. Dividing and subculturing the basal shoot mass did not cause tissue breakdown or exudation. The results of the multiplication of highbush blueberry cultivars are shown in Tables 2 - 4. The number of newly formed shoots varied with the cultivar, the medium tested and the concentration of zeatin. Across all experiments, the highest multiplication rate (4.8) was obtained for 'Berkeley' on WPM medium with the highest concentration 2 mg l^{-1} of zeatin. On three tested media, 'Berkeley' was the cultivar with the highest ability to produce vigorous multiple shoot cultures. On the contrary, for the cultivar 'Spartan', neither of the three tested media containing different concentrations of zeatin promoted markedly *in vitro* shoot formation and the number of newly formed shoots was thus very low (from 1.3 to 1.8). Within the same range of zeatin concentration, the three genotypes gave higher multiplication rates on the WPM medium. The woody plant medium (WPM) was found to be more effective than an AN and a half-MS medium for the initiation of new shoots in our study. The lowest multiplication rates were noted for 'Bluecrop' and 'Berkeley' on a half-MS medium. The lowest multiplication rates for 'Spartan' were noted on the AN medium. Short shoots (shorter than 10 mm) were frequently observed in the case of the cultivar 'Bluecrop' on half-MS medium with all concentrations of zeatin tested. The

shoots shorter than 10 mm were not counted for our multiplication studies. These small shoots did not elongate and were difficult to use directly in further procedures. The half-MS medium proved to be less suitable for the multiplication of three highbush blueberry cultivars.

Table 2. Multiplication rates for highbush blueberry cultivars on WPM medium with zeatin

Zeatin (mg l^{-1})	Cultivar		
	Spartan	Bluecrop	Berkeley
0,5	1.4 ± 0.1	1.3 ± 0.1	3.0 ± 0.1
1	1.8 ± 0.1	1.5 ± 0.1	4.0 ± 0.2
2	1.7 ± 0.1	2.0 ± 0.1	4.8 ± 0.2

Table 3. Multiplication rates for highbush blueberry cultivars on AN medium with zeatin

Zeatin (mg l^{-1})	Cultivar		
	Spartan	Bluecrop	Berkeley
0.5	1.4 ± 0.1	1.2 ± 0.0	1.4 ± 0.0
1	1.3 ± 0.1	1.2 ± 0.1	2.2 ± 0.1
2	1.3 ± 0.1	1.9 ± 0.1	2.3 ± 0.1

Table 4. Multiplication rates for highbush blueberry cultivars on half MS medium with zeatin

Zeatin (mg l^{-1})	Cultivar		
	Spartan	Bluecrop	Berkeley
0.5	1.5 ± 0.1	1.2 ± 0.0	1.2 ± 0.1
1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
2	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1

In the case of the cultivars ‘Bluecrop’ and ‘Berkeley’, the increasing zeatin concentration in the tested media also increased the shoot multiplication without excessive callus formation. Zeatin proved its ability to stimulate adventitious shoot development in *Vaccinium in vitro* culture. The highest multiplication rates were always noted on media with the highest concentration of zeatin (2 mg l^{-1}). The zeatin level 2 mg l^{-1} can be recommended for the multiplication of the cultivars ‘Bluecrop’ and ‘Berkeley’. Earlier reports indicated that zeatin was an important plant hormone for efficient multiplication and growth in *Vaccinium* micropropagation (Reed and Abdelnour, 1991; Debnath and McRae, 2001; Ostrolucka *et al.*, 2004; Jiang *et al.*, 2009). According to Reed and Abdelnour (1991), the cultivation medium with relatively high levels of zeatin (4 mg l^{-1}) promoted a significantly higher initiation of axillary shoots in eight of twelve *Vaccinium corymbosum* genotypes than on the control medium. On the contrary, Gajdosova *et al.* (2006) pointed out the effectiveness of zeatin in low concentration (0.5 mg l^{-1}) for inducing multiple shoot development in meristem cultures of *Vaccinium* sp. Zeatin concentrations of 2 mg l^{-1} and higher promoted callus formation and suppressed shoot regeneration in Gajdosova’s experiments, which is contradictory to our findings. In our experiments on all media, any physiological disorders or morphological abnormalities such as excessive callus formation or the production of abnormally narrow leaves were not observed during the *in vitro* shoot proliferation stage. For the cultivar ‘Spartan’ the highest multiplication rate 1.8 was noted on media with the zeatin concentration of 1 mg l^{-1}. However this multiplication rate (1.8) achieved on a WPM medium with 1 mg l^{-1} of zeatin can be sufficient only for *in vitro* culture establishment and maintenance, but is not satisfactory for larger scale *in vitro* shoot production. Future research and testing of other media and plant growth regulators is needed in the case of ‘Spartan’.

The results of rooting are summarized in Table 5. There was considerable variation in the rooting percentage of used blueberry cultivars. WPM medium with a high concentration of IBA (1 mg l^{-1}) was effective for root induction in the case of cultivars ‘Berkeley’ and ‘Bluecrop’. Root initiation started within two weeks. The percent of rooting was 70 % for the cultivar ‘Berkeley’ and 61% for ‘Bluecrop’. However, the same treatment yielded considerably fewer rooted plants (9 %) in the case of ‘Spartan’. On an average, IBA promoted development of two to six good quality roots per shoot without callusing at the basal portion of shoots. Roots originated directly from the base of the main shoot. High survival (more than 80 %) was obtained after acclimatization of rooted plants in *ex vitro* conditions. These plants showed normal growth and developmental characteristics,

compared to conventionally grown plants. Ostrolucka *et al.* (2007) reported 80 % rooting and 80 – 90 % survival after transfer to *ex vitro* conditions for *V. corymbosum* genotypes with both *in vitro* and *ex vitro* rooting using 0.8 mg l^{-1} IBA for root induction.

Table 5. Rooting of highbush blueberry on WPM medium with 1 mg l^{-1} IBA

Cultivar	Rooting shoots (%)	Root number per shoot ± SE
Spartan	9	6.0 ± 0.6
Bluecrop	61	2.3 ± 0.1
Berkeley	70	2.6 ± 0.1

Conclusions

Micropropagation techniques described in this paper increased multiplication mainly in the highbush blueberry cultivar 'Berkeley' on WPM medium. A rapid *in vitro* shoot multiplication procedure could have a crucial impact on our ability to rapidly proliferate and maintain desirable highbush blueberry cultivars, while at the same time allowing for initial plant material availability throughout the year. By using a zeatin supplemented WPM medium for shoot initiation and proliferation, thousands of plants a year could be produced from a single initial shoot. In comparison, conventional nursery techniques using multiple-node softwood or hardwood cuttings produce only a few plants annually. However, some cultivars of highbush blueberry would still require further research to optimize the proliferation media.

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INFLUENCE OF DRYING TECHNOLOGY ON THE QUALITY OF DRIED CANDIED *CHAENOMELES JAPONICA* DURING STORAGE
KALTĒŠANAS TEHNOLOĢIJU IETEKME UZ *CHAENOMELES JAPONICA* SUKĀŽU KVALITĀTI UZGLABĀŠANAS LAIKĀ

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Abstract
In the Baltic region, the development of Japanese quince as a fruit crop started in Latvia in 1951. The fruits of *Chaenomeles japonica* are very firm, acidic, with too low sugar content to be consumed fresh, but they are useful for processing and valuable because of the high content of organic acids, vitamin C, phenolic compounds and fresh aroma. The interest for growing Japanese quince in Latvia reached a peak in 1993, but the processing was not solved. Only juice and puree were produced. Therefore a new Japanese quince processing technology was worked out and patented (RL patent Nr. LV 12779 B) at Dobele Horticultural Plant Breeding Experimental Station (presently Latvia State Institute of Fruit-Growing) and Latvia University of Agriculture in 2002.
In order to enlarge the product spectrum made from Japanese quince, investigations were conducted at the Latvia State Institute of Fruit-Growing during the 2007. The aim of the present work was to characterize the influence of the drying technology on the quality of dried candied Japanese quince during storage for six months. Two different drying technologies were used: with forced air circulation and vacuum-microwave. The sweet dried Japanese quince products were tested for content of vitamin C, phenolic compounds and changes of colour by using the CIE L*a*b* colour system.
The obtained data showed that the content of vitamin C decreased on average by 40 %, but the content of phenolic compounds by 17 % in the product. The over colour of the product changed after two months of storage.

Kopsavilkums
Baltijas reģionā krūmcidoniju selekcija uzsākta Latvijā kopš 1951. gada. *Chaenomeles japonica* augļi ir cieti, skābi, ar pārāk mazu cukura daudzumu, lai tos varētu patērēt svaigā veidā, bet, pateicoties augstajam organisko skābju, C vitamīna, fenolu savienojumu saturam un patīkamā aromāta dēļ, tie ir noderīgi pārstrādei. Interese par Japānas krūmcidoniju audzēšanu Latvijā virsotni sasniedza 1993. gadā, bet netika atrisināta augļu pārstrāde. Galvenokārt tika ražota sula un biezenis. Tādēļ Dobeles Dārzkopības selekcijas un izmēģinājumu stacijā (šobrīd Latvijas Valsts augļkopības institūtā) un Latvijas Lauksaimniecības universitātē 2002. gadā tika izstrādāta un