MICROCLONAL PROPAGATION OF *Vaccinium* SP. AND *Rubus* SP. AND DETECTION OF GENETIC VARIABILITY IN CULTURE *IN VITRO*

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ABSTRACT

Five high-bush blueberry, two lingonberry, and six raspberry cultivars were evaluated in terms of their regeneration capacity when propagated *in vitro* by axillary and adventitious organogenesis. In the high-bush blueberry and the lingonberry, shoots were regenerated from isolated meristems and dormant buds and cultivated on modified Anderson's rhododendron (AN) medium. Shoot formation was induced on medium containing 0.5 mg l^{-1} zeatin. In the high-bush blueberry, the cultivar with the highest shoot proliferation intensity was 'Brigitta', with 14.2 shoots per primary explant. The cultivars with the highest adventitious shoot multiplication were 'Brigitta', with 39.1 shoots/explant, and 'Berkeley', with 18.0 shoots/explant. In the lingonberry, the cultivar with the highest shoot proliferation intensity was 'Red Pearl', with 5.2 shoots per primary explant. The cultivar with the highest adventitious shoot multiplication was 'Red Pearl' with about 44 shoots/explant. In the raspberry, shoots were regenerated from isolated meristems and dormant buds and cultivated on modified MS medium containing 1.0 mg l^{-1} BAP and 0.1 mg l^{-1} IBA. The cultivar with the best meristem formation and shoot proliferation was 'Bulharský Rubín', with 56.6% of the explants producing shoots. Adventitious shoot regeneration was also highest in 'Bulharský Rubín', with 25.75 of leaf explants producing adventitious shoots on MS medium containing 0.5 mg l^{-1} TDZ and 0.2 mg l^{-1} 2,4-D.

RAPD proved to be a simple and efficient technique for identifying high-bush blueberry and lingonberry clones, which could easily be distinguished by their

characteristic polymorphic banding patterns. However, there were no differences in the DNA profiles of the mother cultivars and any of the clones derived from them. Therefore, either no somaclonal variation occurred during the micropropagation process, or more sensitive techniques are needed to detect it. Flow-cytometry did not detect any changes in ploidy level, which confirms that no relative changes in DNA content took place during the micro-propagation process.

Key words: *Vaccinium* sp., *Rubus* sp., *in vitro* regeneration, RAPD, flow-cytometry

Abbreviations: AN – Anderson rhododendron medium; TDZ – thidiazuron; BAP – 6-benzylaminopurine; 2-iP – dimethylallylaminopurine; IBA – indole-3-butyric acid; 2,4-D – 2,4-dichlorophenoxyacetic acid; DNA – deoxinucleic acid; RAPD – Random amplified polymorphic DNA analysis.

INTRODUCTION

Berry fruits are an economically important crop in many countries. Interest in berry fruits has recently increased because they are excellent sources of health-promoting vitamins, anti-oxidants and other valuable nutrients (Song and Sing, 2004). In Slovakia, however, berry production sharply decreased during the 1990s in spite of the fact that soil and climate conditions in Slovakia are excellent for the intensive cultivation of berry fruits. There are also many unique berry cultivars which lend themselves to intensive cultivation. Recently, interest in growing various berry fruits had been increasing not only among home gardeners, but also among small farmers. Berry culture is increasing in sub-mountainous areas of the country, where soil and climate conditions are more favorable for intensive berry culture than in the lowland areas.

Some berry cultivars recently introduced into Slovakia are potentially profitable alternative and non-conventional fruit crops. Among them are cultivars of the high-bush blueberry (*Vaccinium corymbosum* L.), the lingonberry (*Vaccinium vitis-idaea* L.), and the raspberry (*Rubus idaeus* L.). In Slovakia, lingonberry production has predominantly relied on the collection of the berries from stands of native varieties growing wild in the mountains. Habitat destruction has lead to a reduction in the supply of native lingonberries. Although these native wild varieties are not as suitable for intensive culture as are the new cultivars, they are still very valuable because of their high dietary value and breeding potential. They can be a versatile raw material for the food processing and pharmaceutical industries.

High-bush blueberry and lingonberry plantations have already been established at the Research Station of the Grassland and Mountain Agricultural Research Institute in Krivá na Orave in northern Slovakia. Various berry cultivars are propagated by cuttings at this station. The genus *Rubus* includes many valuable cultivated species and varieties. *Rubus* varieties are propagated vegetatively. However, traditional techniques are not

very efficient. The number of propagules which can be produced is limited by seasonal growth rate. *Rubus* species are susceptible to a wide variety of viral and fungal diseases. For this reason, plantations should be planted at higher altitudes. Intensive *Rubus* breeding and propagation programs are underway at the Research Institute of Fruit and Ornamental Plants in Bojnice in western Slovakia.

When establishing commercial berry plantations, it is essential to plant certified pathogen-free new cultivars which produce well at higher elevations. Microbial pathogens can be transferred during vegetative propagation of plants. Traditional propagation methods are also not very efficient in terms of the number of propagules generated. These limitations can be overcome by the use of *in vitro* techniques, which ensure a reliable supply of healthy, pathogen-free planting material (Tsao et al., 2000; O'Herlihy et al., 2003).

The aim of this study was to develop and test efficient *in vitro* regeneration and propagation systems which can be used with high-bush blueberry, lingonberry and raspberry cultivars. Another goal was to evaluate RAPD as an inexpensive technique to identify *Vaccinium* cultivars and to genetically characterize clones derived from in vitro culture. Flow-cytometry was used to evaluate clonal fidelity and variability in vegetatively propagated plant material which had been cultured *in vitro* for several months.

MATERIAL AND METHODS

Five high-bush blueberry, two lingonberry, and six raspberry cultivars were evaluated in terms of their regeneration capacity when propagated *in vitro*.

The high-bush blueberry cultivars tested were: 'Berkeley', 'Bluecrop', 'Blueray', 'Duke' and 'Brigitta'. The lingonberry cultivars tested were: 'Red Pearl', 'Koralle'. The raspberry cultivars tested were: 'Bojana', 'Afrodita', 'Granát', 'Ada', 'Medea' and 'Bulharský Rubín'.

Shoots were regenerated directly from isolated meristems, dormant apical buds, and dormant axillary buds. Stem cutting with dormant buds were collected in January and February from mature plants growing in the field. Nodal segments bearing one bud apiece were washed under running water, sterilized for two minutes in 70% ethanol, soaked for six minutes in a solution containing $HgCl_2$ and Tween, and finally washed three times for fifteen minutes in sterile distilled water.

In vitro culture

All cultures were maintained in a growth chamber at $23 \pm 2^{o}C$ using a 16 h light/8 h dark photoperiod. Light was supplied using white fluorescent lamps at an intensity of 50 $\mu mol\ m^{-2}\ s^{-1}$

High-bush blueberries and lingonberries

Shoots were regenerated from isolated meristems and dormant buds and cultivated on Anderson's rhododendron medium supplemented with 30 mg l^{-1} sucrose, 8 mg l^{-1} phyto agar, and 0.5 mg l^{-1} zeatin, pH 4.8 to 5.0 (Anderson, 1980). Leaves with cut margins were placed horizontally on the medium abaxial surface down.

In the high-bush blueberry, adventitious buds were induced on AN medium containing 0.5 mg l^{-1} zeatin.

In the lingonberry, adventitious buds were induced by first culturing the explants on AN medium containing either 2.19 mg l-1 or 4.38 mg l^{-1} zeatin. After six weeks, the explants were transferred to AN medium containing 0.5 mg l^{-1} zeatin.

After three subculture cycles each lasting five or six weeks, the high-bush blueberry and lingonberry cultivars were evaluated in terms of regeneration ability based on shoot proliferation intensity. Shoot proliferation was enhanced by dividing regenerated elongated micro-shoots into single-node segments. Shoot proliferation intensity is defined as the average number of shoots produced by the end of the third subculture cycle by the primary explant and the micro-shoot cuttings derived from it. The percentage of leaves which had produced callus and regenerated shoots after six weeks of culture was also recorded.

Raspberries

Shoots were regenerated from isolated meristems and dormant buds and cultivated on modified MS medium containing 1.0 mg l^{-1} BAP and 0.1 mg l^{-1} IBA (Gajdošová et al., 2000). The same medium was used for shoot multiplication.

Adventitious buds were induced by cultivating leaves and stem cuttings of *in vitro* plants on modified MS medium and AN medium containing the following growth regulators:

TDZ: 0.1, 0.5 or 1.0 mg l^{-1}, alone or in combination with 0.2 mg l^{-1} 2,4-D; and

BAP: 0.5 or 1.0 mg l^{-1} in combination with 0.5 mg l^{-1} IBA.

Shoots were rooted on modified MS medium containing 1mg l^{-1} IBA.

RAPD analysis

RAPD analysis was performed on clones of selected high-bush blueberry and lingonberry cultivars after several months of *in vitro* culture. Leaves of the field grown mother plants were used as the controls.

DNA was isolated using the King Fisher™ Genomic DNA Purification Kit in accordance with the procedure recommended by the manufacturer (Thermo Labsystems, Helsinki, Finland). The extraction protocol had to be modified to prevent problems caused by the presence of polyphenols. 1% PVP was added to the lysis buffer, and later also to the PCR reaction mixture.

DNA polymorphism was analysed using the Operon decamer primer Kit (Operon Technologies, Inc.). Twenty OPU primers of arbitrary sequence were screened.. PCR was repeated at least twice for each primer. The products were then separated and the obtained DNA patterns were analyzed.

Flow cytometry

Flow cytometry was performed using the PA II ploidy analyzer with a mercury arc lamp using UV excitation. Samples were prepared in a two-step procedure using the Partec CyStain UV precise P reagent kit containing DAPI. Analysis was carried out on young high-bush blueberry and lingonberry leaves from *in vitro* plants. The high-bush blueberry cultivar tested was 'Berkeley', and the lingonberry cultivar tested was 'Red Pearl'. Four clones of each cultivar were tested: three derived from regenerated adventitious shoot, and one derived from meristem.

RESULTS

High-bush blueberries and lingonberries

Our results confirm that high-bush blueberries and lingonberries can be efficiently propagated by direct organogenesis.

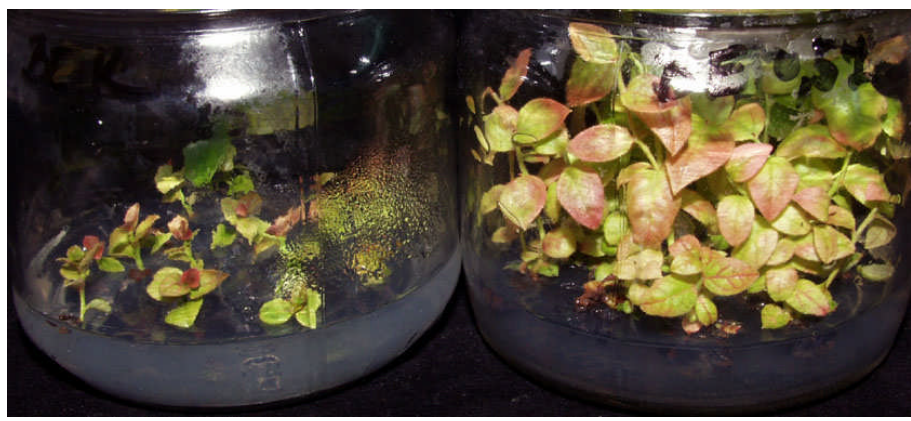

Figure 1. Shoot proliferation from dormant buds in the high-bush blueberry cultivar 'Berkeley' on both AN medium without zeatin and AN medium containing 0.5 mg l^{-1} zeatin after ten weeks of culture

In the high-bush blueberry, multi-shoot cultures were obtained by cultivating apical and axillary buds on AN culture medium containing 0.5 mg l^{-1} zeatin. Shoot proliferation intensity varied widely depending on cultivar. The cultivar with the highest shoot proliferation intensity was 'Brigitta', with 14.2 shoots per primary explant. The cultivars with the lowest shoot proliferation intensities were 'Duke', with 2.3 shoots/explant, and 'Blueray', with 2.9 shoots/explant (Fig. 1). Previous experiments on high-

bush blueberries showed that shoot proliferation intensity depends on the concentration of zeatin in the culture medium (Ostrolucká et al., 2002). In our study, 'Duke' formed 2.3 shoots/explant on medium containing 0.5 mg l^{-1} zeatin, and 5.3 shoots/explant on medium containing 2.0 mg l^{-1} zeatin.

In the lingonberry, multi-shoot cultures were obtained by initially cultivating apical and axillary buds on AN culture medium containing either 2.19 mg l-1 or 4.38 mg l^{-1} zeatin, and then transferring then to AN medium containing 0.5 mg l^{-1} zeatin after six weeks. Shoot proliferation intensity depended on cultivar. 'Red Pearl' formed 5.2 shoots/explant, and 'Koralle' formed 2.8 shoots/explant. The number of shoots produced per primary explant could be increased by dividing micro-shoots into single-node segments. Segmentation was particularly effective with the blueberry cultivar 'Duke' and the lingonberry cultivar 'Koralle'. With segmentation, shoot proliferation intensity increased to 4.6 in 'Duke', and to 4.4 in 'Koralle' (Fig. 2).

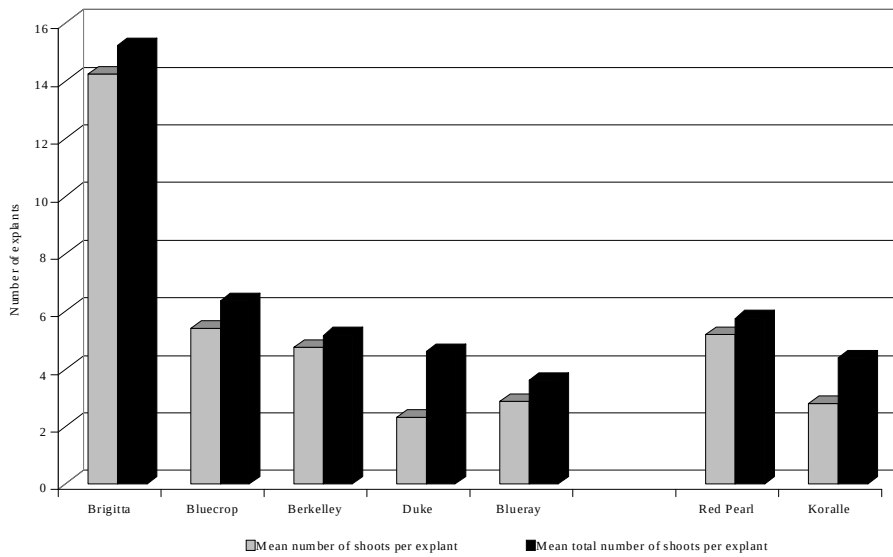

Figure 2. Influence of zeatin (0.5 mg l^{-1}) on regeneration in of high-bush blueberry and lingonberry cultivars

Adventitious organogenesis can be used to scale up clonal production in selected *Vaccinium* genotypes. We have developed a very efficient procedure for regenerating adventitious shoots from leaf tissue in high-bush blueberries and lingonberries.

In the high-bush blueberry, leaf explants produced multiple shoots after six weeks of culture on AN medium containing 0.5 mg l^{-1} zeatin without going through an intermediary callus phase. Regenerative capacity varied

widely depending on cultivar. Two cultivars had particularly high shoot multiplication rates: 'Brigitta', with 39.1 shoots/explant; and 'Berkeley', with 18.0 shoots/explant. Both of these cultivars exhibited a high regenerative capacity, as expected for apical and axillary bud cultures (Tab. 1).

Table 1. Adventitious shoot regeneration from leaf tissue in the high-bush blueberry (*Vaccinium corymbosum* L.)

Cultivar	Number of leaf explants	Percentage of explants regenerating shoots	Mean shoots per explant	Multiplication of adventitious shoots	
				first subculture	second subculture
Berkeley	20	35	18.0	110.0	237.1
Bluecrop	20	10	5.8	13.3	50.3
Brigitta	20	40	39.1	129.4	257.5

In the lingonberry, leaf explants produced adventitious shoots only after going through an intermediary callus phase. After six weeks on AN medium containing either 2.19 or 4.38 mg l^{-1} zeatin, leaf and stem explants formed callus tissue, but did not regenerate shoots. A higher percentage of callus formation was observed in stem explants. At this stage, the calluses were transferred to AN medium containing 0.5 mg l^{-1} zeatin. In 'Red Pearl', adventitious shoots formed on calluses derived from both leaf and stem explants (Tab. 2). More buds formed on callus derived from leaf explants (Fig. 3). The shoots produced were directly rooted in a peat substrate after being dipped in a solution containing 1.0 mg l^{-1} IBA. In 'Koralle', although the explants formed callus tissue, they did not regenerate shoots even after being transferred to AN medium containing 0.5 mg l^{-1} zeatin.

Table 2. Indirect regeneration of adventitious shoots in the lingonberry (*Vaccinium vitis-idaea* L.)

Cultivar	Primary explants	Number of explants	Shoot regeneration by callogenesis				
			Anderson culture medium				
			2.19 mg l^{-1} zeatin (A)	4.38 mg l^{-1} zeatin (B)	0.5 mg l^{-1} zeatin A	B	mean shoots per explant
			callus [%]	callus [%]	number of shoots from callus		
Red Pearl	leaf	36	13.3	23.3	864	728	44.22
	stem	6	83.3	83.3	207	59	44.33
Koralle	leaf	30	13.3	16.6	0	0	0
	stem	6	16.6	16.6	0	0	0

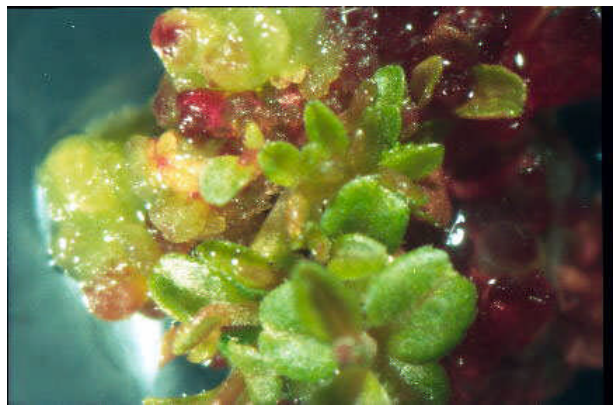

Figure 3. Induction of adventitious buds in callus derived from leaf tissue in the lingonberry cultivar 'Read Pearl' after eight weeks of culture on AN medium containing 0.5 mg l^{-1} zeatin

Raspberries

Figure 4. Shoot regeneration from dormant buds in the raspberry cultivar 'Bulharský Rubín' cultured on modified MS medium containing 1.0 mg l^{-1} BAP and 0.1 mg l^{-1} IBA

Shoot regeneration in the raspberry cultivars tested depended on genotype, culture medium and growth regulators. Apical and axillary buds produced adventitious shoots on modified MS medium containing 1 mg l^{-1} BAP and 0.1mg l^{-1} IBA. Regenerative capacity varied widely depending on cultivar. The percentage of buds producing shoots was 56.6% in 'Bulharský Rubín', 53.8% in 'Afrodita', 52.6% in 'Granát', 47.1% in 'Ada', and 44.8% in 'Bojana' (Fig. 4). The mean number of shoots per explant followed the same trend, ranging from a high of 13.9 in 'Bulharský Rubín' to a low of 2.3 in 'Medea'. Shoot multiplication was also efficient on modified MS medium containing 1mg l^{-1} BAP and 0.1mg l^{-1} IBA.

The cultivars also differed in their capacities to form adventitious buds. Adventitious bud induction was successful in three cultivars: 'Miral', 'Afrodita' and 'Bulharský Rubín'. In 'Miral' and 'Afrodita', adventitious buds formed on hypocotyl explants cultured on medium containing 1.0 mg l^{-1} BAP and 0.5 mg l^{-1} IBA.

Figure 5. Adventitious shoot regeneration in the raspberry cultivar 'Bulharský Rubín' cultured on modified MS medium containing 0.5 mg l^{-1} TDZ and 0.2 mg l^{-1} 2,4-D

The best results were obtained with leaf explants of 'Bulharský Rubin' cultured on MS medium containing 0.5 mg l^{-1} TDZ and 0.2 mg l^{-1} 2,4-D (Fig. 5). 27.5% of these explants regenerated adventitious shoots. On medium containing only 0.1 mg l^{-1} TDZ, shoot regeneration was sporadic. On the other hand, on medium containing 1.0 mg l^{-1} TDZ, explant necrosis occurred.

RAPD analysis

RAPD analysis using King Fisher™ equipment proved to be a simple and efficient technique for identifying high-bush blueberry and lingonberry cultivars. The high-bush blueberry cultivars 'Bluecrop', 'Blueray' and 'Duke' could easily be distinguished by their characteristic banding patterns (Fig. 6).

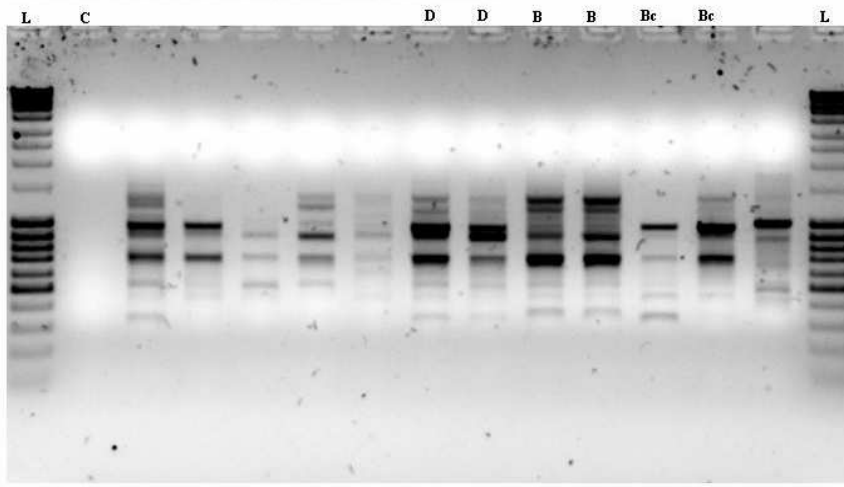

Figure 6. DNA profiles of the high-bush blueberry cultivars 'Duke' (D), 'Blueray' (B) and 'Bluecrop' (BC)

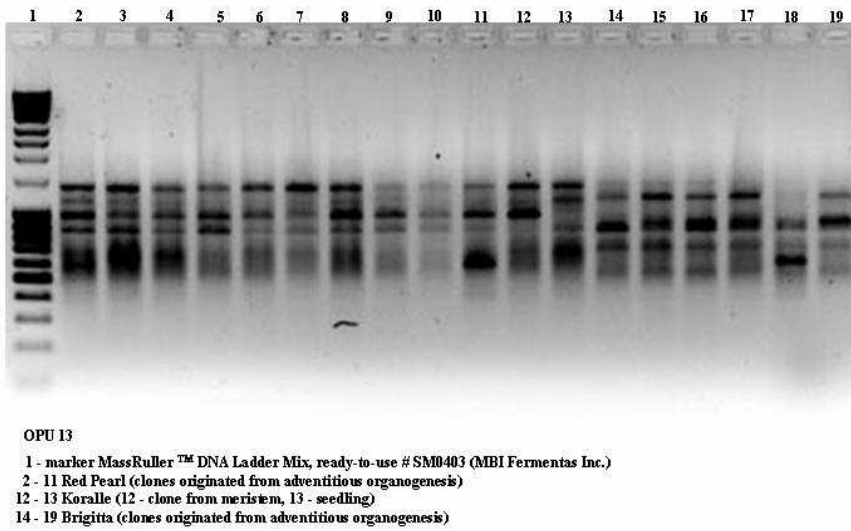

Figure 7. DNA profiles of *in vitro* derived clones of the lingonberry cultivar 'Red Pearl' and the high-bush blueberry cultivar 'Brigitta'

The primers tested differed widely in their capacity to amplify the DNA of different cultivars and clones. Of the twenty primers screened, the ones which produced the most polymorphic bands were OPU 3, OPU 13, OPU 14 and OPU 15. However, there were no differences in the DNA profiles of the mother plants and any of the clones derived from them (Fig. 7). Therefore, either no Somaclonal variation occurred during the micro-propagation process, or more sensitive techniques are needed to detect it.

Flow-cytometry analysis

Flow-cytometry did not detect any changes in the ploidy level of the clones derived from *in vitro* culture. This confirms that no relative changes in DNA content took place during the micro-propagation process (Fig. 8).

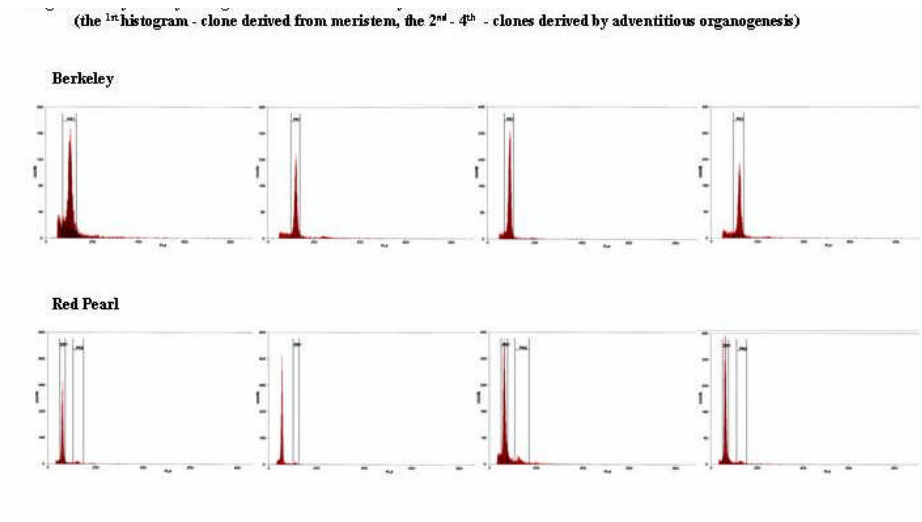

Figure 8. Flow-cytometry histograms of the high-bush blueberry cultivar 'Berkeley' and the lingonberry cultivar 'Red Pearl

DISCUSSION

In many countries, *in vitro* micropropagation has already successfully been used to accelerate the testing and release of various new *Vaccinium* cultivars, including high-bush blueberries and lingonberries (Marcotrigiano et al., 1996; Reed and Abdelnour-Esquivel, 1991; Shibli and Smith, 1996; Popowich and Filipenya, 1997; Jaakola et al., 2001; Cao et al., 2003). In Slovakia, these techniques have not yet been widely used.

Inducing adventitious bud formation in cultures derived from leaf tissue has been reported to be promising technique not only for the propagation of

new genotypes, but for genetic transformation as well (Rowland and Ogden, 1992; Cao and Hammerschlag, 2000; Debnath and McRae, 2002; Song and Sing, 2004; Petri and Burgos, 2005). These studies have focused on determining optimal conditions for efficient *in vitro* regeneration.

Our results confirm that zeatin is a good agent for inducing multiple shoot formation in bud and meristem cultures. Zeatin also promotes adventitious organogenesis. Zeatin is more effective than 2-iP in inducing shoot regeneration in *Vaccinium sp.* (Rowland and Ogden, 1992; Ondrušková et al., 2003; Ostrolucká et al., 2002). Zeatin at a concentration of 4.0 mg l^{-1} has been used to initiate shoot formation (Reed and Abdelnour-Esquivel, 1991). Medium containing either 4.38 or 6.57 mg l^{-1} zeatin has been used to induce multiple shoot formation in leaf explants (Debnath and McRae, 2002). In our experience, zeatin at a concentration of 0.5 mg l^{-1} worked best. Higher concentrations promoted callus formation, and shoot regeneration took place only after the calluses were transferred to media containing 0.5 mg l^{-1} zeatin. Zeatin concentration influences not only shoot multiplication, but morphogenesis as well. Other studies have shown that higher concentrations of zeatin promote callus formation in leaf explants (Shibli and Smith, 1996).

The cultivars tested varied widely in terms of shoot proliferation intensity even though they were all cultured on the same medium. This confirms that shoot proliferation intensity depends on genotype, which agrees with earlier reports (Popowich and Filipenya,1997).

An *in vitro* culture system has been developed for the rapid micro-propagation of new *Rubus* cultivars and hybrids (Cousineau and Donnelly, 1991; Turk et al., 1994). *In vitro* culture of meristems has been used to produce healthy, high quality planting material which can be propagated either *in vitro* or with conventional techniques (Graham et al., 1997; Hollmann et al., 2002). Other researchers have also found that *Rubus* species can be successfully regenerated with *in vitro* techniques (Mezzetti et al., 1997; Barrett et al., 1996).

In our study, *in vitro* shoot proliferation in raspberries was successfully induced by culturing dormant buds on modified MS medium containing 1 mg l^{-1} BAP and 0.1 mg l^{-1} IBA. This agrees well with earlier studies (Donnelly et al., 1980; Palonen and Buszard, 1998). Zeatin at a concentration of 1.0 mg l^{-1} was found to stimulate shoot proliferation better than BAP at a concentration of 2.0 mg l^{-1} in combination with IBA or NAA (Debnath, 2004). However, leaf number and shoot vigor were higher with BAP and IBA. In our study, the shoots induced on medium containing 1.0 mg l^{-1} BAP and 0.1mg l^{-1} IBA were robust, grew well, and multiplied at a high rate.

Regenerative capacity in raspberries varied greatly depending on genotype, which agrees well with earlier reports (Graham et al., 1997). We succeeded in inducing adventitious shoot regeneration we obtained in ‘Miral’, ‘Afrodita’ and ‘Bulharský Rubín’. In ‘Miral’ and ‘Afrodita’, adventitious

buds formed on hypocotyl explants cultured on medium containing 1.0 mg l^{-1} BAP and 0.5 mg l^{-1} IBA. The best results were obtained with leaf explants of 'Bulharský rubín' cultured on MS medium containing 0.5 mg l^{-1} TDZ and 0.2 mg l^{-1} 2,4-D. In the blackberry, TDZ has been found to stimulate adventitious shoot formation better than BAP (Hassan et al., 1993). In some raspberry cultivars, TDZ alone or in combination with IBA effectively induced adventitious bud formation (Graham et al., 1997). However, when TDZ was used at higher concentrations or in combination with 2,4-D, shoot regeneration was completely inhibited in all cultivars. In our study, TDZ at higher concentrations caused explant necrosis. Therefore, we will use another auxin in future experiments.

RAPD is a simple and reliable molecular technique that had been used to identify cultivars and clones and to determine inter-relatedness in many perennial woody species, including *Vaccinium* species (Persson and Gustavsson, 2001; Arce-Johnson et al., 2002). In the bilberry (*V. myrtillus*), RAPD and AFLP (Amplified Fragment Length Polymorphism) have been tested for their usefulness in clone identification (Albert et al., 2003; 2004). Both RAPD and AFLP yielded the same results, which mean that both techniques are reliable.

Our results show that RAPD is a reliable technique for identifying blueberry cultivars. The cultivars tested produced distinct banding patterns. However, we had to screen several primers to find ones which amplified DNA in the cultivars tested. Furthermore, PCR had to be repeated several times in order to obtain readable banding patterns.

In the cranberry (*V. macrocarpon*), RAPD have been successfully used to distinguish varieties. However, RAPD has some limitations. Several reactions with several primers are required to reliably identify each clone. Also, artefacts often make accurate identification difficult (Polashock and Vorsa, 2002). In the study on the high-bush blueberry, the genetic relations among the genotypes tested determined by RAPD analysis did not agree well with known pedigree data (Levi and Rowland, 1997). In the peach, RAPD has been used to detect somaclonal variants in regenerated clones (Hashmi et al.,1997). Also in the kiwi fruit, both RAPD and SSR were used to detect somaclonal variants (Palombi and Damiano, 2002).

In our experiments, there were no differences in the DNA profiles of the mother plants and any of the clones derived from them by either axillary or adventitious organogenesis. Flow cytometry did not detect any changes in the ploidy level of the clones derived from *in vitro* culture within a single cultivar.

We can therefore conclude that more specific techniques have to be used to detect somaclonal variation in micro-propagated plants. Among the techniques which should be further investigated are SSR, EST-PCR (Rowland et al., 2003a; Rowland et al., 2003b).

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MIKROROZMNAŻANIE ROŚLIN Z RODZAJÓW *Vaccinium* I *Rubus* ORAZ WYKRYWANIE ZMIENNOŚCI GENETYCZNEJ W KULTURACH *IN VITRO*

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S T R E S Z C Z E N I E

Rośliny z rodzajów *Vaccinium* (*V. corymbosum* i *V. vitis-idaea*) i *Rubus* (*R. idaeus* L.) są znane jako źródła wielu substancji biologicznie czynnych, stąd też opracowanie metodyki ich szybkiego mnożenia w kulturach *in vitro* może być istotnym przyczynkiem do powiększenia areału uprawy tych cennych gatunków. W mikrorozmnażaniu *Vaccinium* stosowano pożywkę AN z zeatyną (0,5 mg l^{-1}). Najwyższą liczbę pędów obserwowano w odmianach Brigitta (39,12 i 14,23 pędu / eksplantat, zależnie od pochodzenia eksplantatu), Red Pearl (44 pędu / eksplantat) i Berkeley (18 pędu/eksplantat). Liczba uformowanych pędów była wyższa po przejściu przez fazę kalusa. Dla roślin z rodzaju *Rubus* inicjacja i wzrost pędów był obserwowany na zmodyfikowanej pożywce MS z 1 mg l^{-1} BAP i 0,1 mg l^{-1} IBA. Największą liczbę pędów uzyskano dla odmiany Bulharsky Rubin (56,6%), dla której stosowano z powodzeniem także pożywkę MS z 0,5 mg l^{-1} TDZ i 0,2 mg l^{-1} 2,4-D (27,5%). Zróżnicowanie genetyczne odmian i klonów uzyskanych w kulturach *in vitro* oceniano metodą RAPD. Analiza polimorfizmu DNA wykazała brak zróżnicowania między roślinami z *in vitro* i roślinami matecznymi. Ocena poziomu ploidii w analizie cytometrycznej także nie potwierdziła wystąpienia zmian związanych z procesem mikrorozmnażania testowanych roślin.

Słowa kluczowe: *Vaccinium*, *Rubus*, regeneracja, RAPD, cytometria przepływowa