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In vitro propagation of *Vaccinium* species

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Abstract

The aim of the work was direct regeneration of shoots from meristems, as well as adventitious shoot production from leaves for regeneration and effective production of highbush blueberry *Vaccinium corymbosum* L. and lingonberry *Vaccinium vitis-idaea* L. The results showed that the shoot proliferation intensity of individual cultivars differed. Therefore, optimisation of cytokinin type and concentration for each cultivar was necessary. For meristem regeneration, improved multiplication was achieved on medium with zeatin in comparison with N^6-Δ^2-isopentenyl adenine. Thidiazuron was effective in adventitious shoot regeneration from leaf tissue of highbush blueberry, and both thidiazuron and zeatin for lingonberry leaf tissue. Microshoot rooting was achieved on Anderson culture medium supplemented with indole-3-butyric acid or directly in peat after dipping of shoots into indole-3-butyric acid solution under *ex vitro* conditions.

Key words: adventitious organogenesis, meristem culture, regeneration *in vitro*, *Vaccinium corymbosum* L., *Vaccinium vitis-idea* L.

Introduction

Highbush blueberry (*Vaccinium corymbosum* L.) and lingonberry (*Vaccinium vitis-idaea* L.) are important and biologically valuable small fruit species. Both species have been succesfully grown in USA and in many European countries for a long period (Jaakola et al. 2001). Some introduced cultivars of these species are suitable also for cultivation in the conditions prevailing in Slovakia. Effective mass production of plants is needed for commercial plantation establishment (Ostrolucká et al. 2002). Vegetative propagation is not very successful and is considerably limited by seasonal growth. Generative propagation is not only time demanding, but also does not give the opportunity to obtain homogeneous progeny. Tissue culture propagation techniques can be used as a system for effective plant production, production of virus-free plants which are genetically identical with maternal plant, and also allow for induction of genetic variability (George 1993; Marcotrigiano et al. 1996). This paper presents our results obtained during experimental micropropagation of different cultivars of highbush blueberry *V. corymbosum* and lingonberry *V. vitis-idaea*.

Materials and methods

The regeneration ability of some cultivars in species *Vaccinium corymbosum* L. and *Vaccinium vitis-idaea* L. was tested by direct organogenesis from apical and axillary buds, and isolated meristems, and by adventitious organogenesis from whole leaves derived from microshoots. The primary explants were obtained from dormant shoots collected from the plantation at the Research Station, Krivá (Research Institute in Banská Bystrica, Slovakia). Shoot segments were sterilised in 70 % ethanol (2 min) and 0.1 % $HgCl_2$ (6 min). The explants were cultivated on Anderson (1980) culture medium (AN) supplemented with 3 % sucrose and the growth regulators zeatin, N^6-Δ^2-isopentyl adenine (2iP), thidiazuron (TDZ), indole-3-butyric acid (IBA) in different concentrations. The medium pH was adjusted to 5.0 in all variants of culture media with the exception of an experiment aimed at modification of the pH values for cultivar 'Duke' cultivation.

For *V. corymbosum* cv. 'Duke', the intensity of shoot proliferation on AN culture medium with 0.5, 1.0, 2.0 mg l^{-1} zeatin (in pH 5.0) was tested. Also effect of different medium pH on shoot regeneration was investigated. The medium pH was tested in the range from 3.0 to 5.5 using AN culture medium with 2.0 mg l^{-1} zeatin.

The effect of zeatin and 2iP was compared for *V. corymbosum* cvs. 'Blueray', 'Darrow', 'Berkeley', 'Bluecrop' and 'Duke' on AN culture medium with 2.0 mg l^{-1} zeatin or 15 mg l^{-1} 2iP in combination with 0.5 mg l^{-1} IBA.

Regeneration *in vitro* for *V. vitis-idaea* cvs. 'Red Pearl' and 'Koralle' was tested on AN culture medium supplemented with zeatin and 2iP in three different concentrations (0.25, 0.50, 1.0 mg l^{-1} zeatin and 2.50, 5.0, 10.0 mg l^{-1} 2iP). As a control, AN culture medium without cytokinins was used.

Explants were incubated at 25 °C, light intensity 50 μmol m^{-2} s^{-2} and 16/8 h photoperiod. Each experiment was repeated three times and the number of primary explants was 30 per experiment. The regeneration ability of cultivars was evaluated on the basis of shoot proliferation intensity parameters: mean number of shoots per explant, mean total number of regenerants per explant (mean number of shoots per explant and one-node segments per shoot) after five weeks of cultivation of *V. corymbosum* and after five subcultures of *V. vitis-idaea* with a subculture interval of 5 to 6 weeks. Isolated microshoots were rooted *in vitro* (AN culture medium with 0.8 mg l^{-1} IBA and 8 g l^{-1} charcoal) or *ex vitro* (directly in peat substrate after dipping into 0.5 to 0.8 mg l^{-1} IBA solution).

For induction of adventitious shoot regeneration from leaf tissue of cv. 'Duke' AN medium supplemented with TDZ (2.2 mg l^{-1}) was used. For the cvs. 'Red Pearl' and 'Koralle', AN medium with 2.2 mg l^{-1} TDZ or 2.19 mg l^{-1} zeatin was tested. Leaves were placed horizontally with the abaxial surface on the medium and cultivated under light.

The data were statistically evaluated by using Statgraphics one-way analysis of variance and multiple range analysis.

Results and discussion

Our observations showed that multiplication (shoot proliferation intensity) depends not only on the concentration of cytokinins in culture medium but also on the response of individual species and cultivars, previously shown by Popowich and Filipenya (1997).

Our experiments confirmed a positive and significant influence of the cytokinin

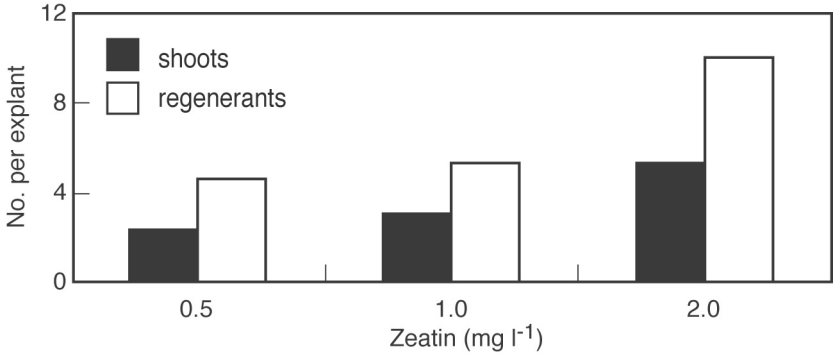

Fig. 1. Effect of different concentrations of zeatin on regeneration of *Vaccinium corymbosum* cv. 'Duke' on Anderson culture medium after 5 weeks of cultivation.

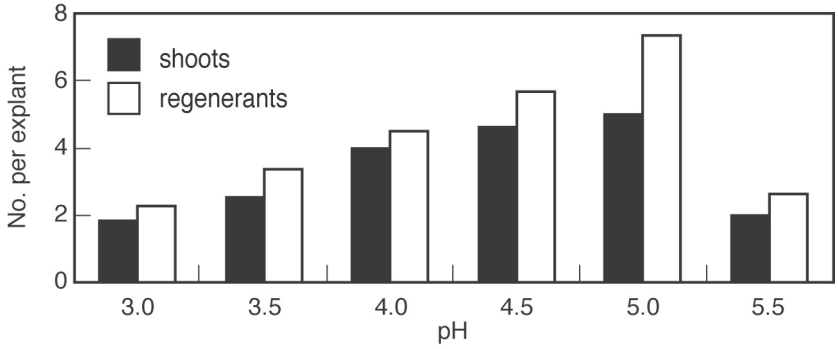

Fig. 2. Effect of medium pH on regeneration of *Vaccinium corymbosum* cv. 'Duke' on Anderson culture medium with 2 mg l^{-1} zeatin after 5 weeks of cultivation.

zeatin on shoot differentiation in cultivar 'Duke'. A higher concentration of zeatin was more effective. The highest mean number of shoots and total number of regenerants per explant was achieved on medium with 2 mg l^{-1} zeatin (Fig. 1). Shoot proliferation was absent on medium without zeatin. Our results are in agreement with work of other authors (Chandler, Draper 1986; Reed, Abdelnour-Esquivel 1991), who showed that zeatin is suitable for stimulation of shoot multiplication in *V. corymbosum*.

Several reports in the literature show that pH of the medium can influence *in vitro* shoot and root formation in some plants and that pH changes during culture (Finn et al. 1991; George 1993). It is known that some plants can tolerate a broader pH range, while in others pH tolerance is limited. Therefore, it is necessary to determine optimal pH levels. The *Vaccinium* sp. are acidophilic plants. *In vitro* screening system allows to investigate the response of plants to different pH levels. Our preliminary experiment with cultivar 'Duke' confirmed differences in shoot proliferation intensity depending on pH of the medium (pH 4.0 to 5.5). A higher multiplication effect was obtained at pH 5.0 and the lowest in pH 3.0 (Fig. 2).

Experiments confirmed that successful regeneration *in vitro* depends also on the reaction of specific cultivar to the cytokinin type. When the effect of zeatin and 2iP on

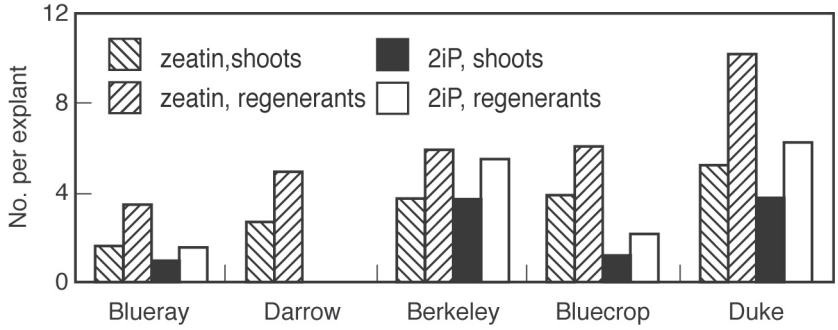

Fig. 3. Intensity of shoot proliferation in different cultivars of *Vaccinium corymbosum* on Anderson culture medium with 2 mg l^{-1} zeatin or 15 mg l^{-1} 2iP after five subcultures.

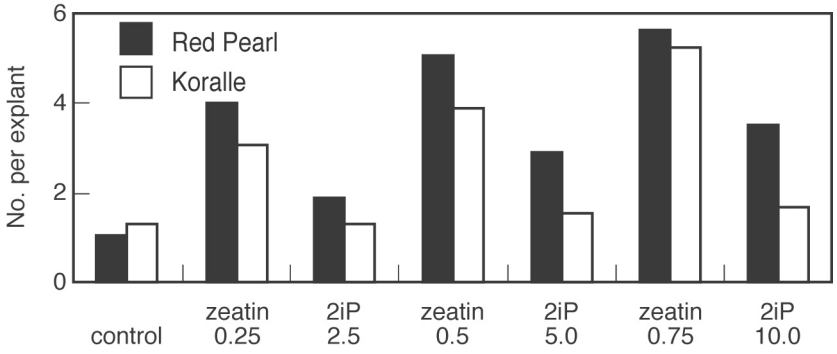

Fig. 4. Effect of different concentrations of zeatin and 2iP (mg l^{-1}) on regeneration of *Vaccinium vitis-idaea* cv. 'Red Pearl' and 'Koralle' on Anderson culture medium after five subcultures.

multiple shoot culture formation was studied in individual cultivars, differences (1.71 to 5.28 shoots per explant) among cultivars on the same medium were observed. On a medium with 2 mg l^{-1} zeatin, a higher intensity of shoot proliferation was achieved in 'Bluecrop' (3.94), 'Berkeley' (3.78) and the highest in cultivar 'Duke' (5.28). The lowest ability of regeneration was found for 'Blueray' (1.71). The differences in intensity of shoot proliferation between cultivar 'Duke' and the other cultivars were highly statistically significant. Similar results were obtained for total number of shoots per explant (3.50 to 10.06) – the lowest for 'Blueray' and the highest for 'Duke'. On medium with 2iP, lower multiplication (1.02 to 3.80 number of shoots per explant and 1.66 to 6.20 total number of regenerants per explant) were obtained in comparison with zeatin. The results confirm the importance of culture medium composition regarding shoot differentiation. The data for regeneration in cultivar 'Darrow' is absent because of contamination of cultures on the medium (Fig. 3).

There is little information about the micropropagation of *V. vitis-idaea* in literature (Hosier et al. 1985, Sidorowich et al. 1995, Jaakola et al. 2001). Our experiments on cultivation of cvs. 'Red Pearl' and 'Koralle' on medium with zeatin and 2iP showed the importance of cytokinins on regeneration (Fig. 4), demonstrated by significant differences

in the intensity of shoot proliferation between the control and AN medium with zeatin and 2iP. The exception was cultivar 'Koralle' where shoot proliferation in the control was similar to that on medium with 2iP, but zeatin had a stimulating effect on the intensity of shoot proliferation. Zeatin was more effective also in cultivar 'Red Pearl'.

Microshoot rooting was achieved on AN culture medium supplemented with IBA (0.8 mg l^{-1}) or directly in peat after dipping of shoots into IBA solution under *ex vitro* conditions (80 - 90 - 95 %), depending on the cultivar. Transfer from *in vitro* to *ex vitro* conditions was successful. Four thousand regenerants were provided to the Krivá Research Station for establishment of production plantations.

Adventitious organogenesis is an essential tool in the generation of somaclonal variants and in most methods of genetic transformation (Marcotrigiano et al. 1996). Our experiments on cultivation of leaf explants from micropropagated shoots of cvs. 'Duke', 'Red Pearl' and 'Koralle' showed an excellent alternative way of regeneration and reproduction of highbush blueberry and lingonberry. The concentrations of TDZ and zeatin used in our experiment were sufficiently effective to induce regeneration of adventitious shoots from leaf derived calli. In callus cultures, intensive production of anthocyanin pigment was observed.

Acknowledgements

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