



## EFFECT OF MEDIUM pH ON AXILLARY SHOOT PROLIFERATION OF SELECTED *VACCINIUM VITIS-IDAEA* L. CULTIVARS

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The influence of initial medium pH on long-term axillary shoot proliferation was tested in Anderson's media supplemented with 0.5 mg.l<sup>-1</sup> zeatin with initial pH adjusted to 4.0, 4.5, 5.0, 5.5 and 6.0 before autoclaving, with the aim of increasing shoot proliferation in *Vaccinium vitis-idaea* L. cv. Koralle and Red Pearl. Shoot proliferation using in-vitro-derived single-node segments was markedly influenced by the cultivar and medium pH. Shoot proliferation was significantly higher in cv. Koralle (6.92 shoots/explant) than in cv. Red Pearl (5.61 shoots/explant) at different culture medium pH. The study confirmed the importance of properly adjusted initial culture medium pH for effective shoot proliferation. For cv. Koralle, medium pH 5.5 was the most favorable for shoot proliferation. For cv. Red Pearl, the number of shoots formed was highest at pH 4.

**Key words:** *Vaccinium vitis-idaea* L., cv. Koralle, cv. Red Pearl, in vitro axillary shoot proliferation, culture medium pH.

### INTRODUCTION

Lingonberry (*Vaccinium vitis-idaea* L.) is a commercially important and biologically valuable plant species suitable for cultivation as a fruit crop, medicinal plant and ornamental ground cover. To satisfy the increasing demand for lingonberry plants and fruits, in vitro production of large amounts of good quality planting material is needed for commercial plantations. It is known that different cultivars of the same species differ in their requirements for growth conditions in vitro. Thus the search for optimal culture conditions, to achieve satisfactory micropropagation rates.

Besides the composition of basal culture medium and growth regulators, another factor limiting successful plant micropropagation is the hydrogen ion concentration of the medium, expressed by the pH value. It influences the utilization of medium components such as macro- and microelements and growth regulators. In general, pH values in the range of 5.5–5.8 are recommended for in vitro culture of the majority of plant species (George, 1993). However, the most effective pH value is specific to the

individual plant species and even cultivars, and has to be determined experimentally. For acidophilous plants, including lingonberry, effective values of soil pH in natural conditions range from 3.5 to 4.8 (Brissette et al., 1990; Hričovský et al., 2002), and they need acidic culture media as well. Certain changes in medium pH occur during medium autoclaving and culture, but correctly adjusted initial medium pH can ensure regulated uptake of single medium components and increased growth (Selby et al., 1989; Rossi-Hassani and Zryd, 1995).

We observed shoot proliferation from in vitro-derived single-node explants of *Vaccinium vitis-idaea* cv. Red Pearl and Koralle on Anderson's (1980) medium (AN) at different initial medium pH, with the aim of optimizing culture conditions for maximal axillary shoot proliferation.

### MATERIALS AND METHODS

#### CULTURE ESTABLISHMENT

The initial plant material, stems with dormant buds, was collected from mature plants of *Vaccinium*

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*vitis-idaea* cv. Koralle and Red Pearl during February and March 2009. Stems were cut into single-node segments carrying an apical or axillary bud. The segments were washed under running water for 1 h and sterilized 2 min in 70% ethanol, 6 min in 0.1% solution of mercuric chloride with 3 drops of Tween, followed by washing  $3 \times 15$  min in sterile distilled water. After sterilization the upper scales were removed from the buds. For shoot initiation the explants were cultivated horizontally on AN medium supplemented with  $30 \text{ g l}^{-1}$  sucrose,  $8 \text{ g l}^{-1}$  Phyto agar and  $0.75 \text{ mg l}^{-1}$  zeatin at pH adjusted to 4.8–5.0 (Gajdošová et al., 2007).

#### TESTING EFFECT OF MEDIUM pH ON SHOOT PROLIFERATION

The shoots derived in vitro were cut into single-node segments which were further cultivated on AN media in which the initial medium pH was adjusted to 4.0, 4.5, 5.0, 5.5 and 6.0 before autoclaving for 20 min at  $120^\circ\text{C}$  and 118 kPa. AN medium was supplemented with  $30 \text{ g l}^{-1}$  sucrose and  $0.5 \text{ mg l}^{-1}$  zeatin, which we used successfully for long-term axillary shoot proliferation in previous experiments (Gajdošová et al., 2007). Zeatin always was added to the culture medium after autoclaving. We used Phyto agar ( $8 \text{ g l}^{-1}$ ) as gelling agent in the media; it is characterized by high gel strength, for satisfactory gelling at low pH. Changes in basal medium pH after autoclaving and after addition of zeatin were recorded as averages of three measurements.

Shoots were cultured at  $25^\circ\text{C}$  with a 16 h photoperiod and light intensity  $50 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . We used 18 glass jars with 30 ml medium and 5 explants per jar, in two replicates of 9 jars for each treatment. The influence of initial medium pH in the presence of zeatin on shoot proliferation was followed at 5–6-week intervals during three subcultures and expressed as mean number of shoots per explant. The results were evaluated statistically using Statgraphics Plus 5 for Windows including Scheffe's test, at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### CULTURE ESTABLISHMENT

The cultures were established by shoot initiation from dormant apical and axillary buds of both cultivars of *Vaccinium vitis-idaea* on AN medium with  $0.75 \text{ mg l}^{-1}$  zeatin. After 5 weeks of culture the mean number of shoots per primary explant was 5.0 for cv. Koralle and 6.8 for cv. Red Pearl. The shoots were vigorous with good elongation growth. Shoots 30–40 mm long were separated and subcultured on the same medium. The cultures were used as the source of single-node explants for test-

ing the influence of initial medium pH on the efficiency of long-term axillary shoot proliferation of the lingonberry cultivars.

### EFFECT OF MEDIUM pH ON SHOOT PROLIFERATION

Many authors have examined the effect of culture medium pH on the morphogenic reaction and tissue growth in vitro in different plant species (George, 1993; Leifert et al., 1992, 1995; Williams, 1995; Ruzić, 2004; Bhatia and Ashwath, 2005; Andersone and Levinsh, 2008; de Klerk et al., 2008). In our experiments we tested axillary shoot proliferation in lingonberry on culture media having different acidity levels without any added buffer in order to determine the most suitable initial medium pH for effective routine micropropagation. We recorded changes in the pH of basal medium without zeatin after autoclaving and after adding zeatin (Tab. 1). The measurements of basal medium pH after autoclaving showed a shift in medium pH to higher acidity; the extent of the change depended on the initial pH value. At initial medium pH 4 it decreased after autoclaving by 0.24 units, while at pH 6 the decline was sharper (1.63 units). Adding zeatin to the autoclaved media raised the pH: the final pH of the media that had been at pH 4.0, 4.5 and 5.0 before autoclaving reached virtually the same values as the initial ones, but for media whose pH was 5.5 and 6.0 before autoclaving the final pH differed markedly. These changes were most probably due to the chemistry of zeatin added to the culture medium, as well as the NaOH used in preparing the zeatin storage solution.

According to Thorpe et al. (2008) the drop in pH after autoclaving may vary according to the pH to which the medium was initially adjusted. Selby et al. (1989) also confirmed changes in medium pH after autoclaving dependent on initial medium pH: at low initial pH the medium acidified by 0.1–0.2 units, and at higher initial pH the value decreased by 0.8–0.9 units. Heat sterilization can significantly alter medium pH through denaturing of proteins, hydrolysis of carbohydrates and dissolution of salts. Several authors have called attention to changes in medium pH caused by changes in medium components (Owen et al., 1991; Druart and Wulf, 1993). The changes of medium pH after we added zeatin confirm the important influence of components added to the medium after autoclaving.

Table 2 shows the results of multiple range analysis of shoot number per explant at different culture medium pH. Two repeated experiments confirmed the significant effect of initial medium pH on shoot proliferation in both cultivars (Fig. 1). For cv. Koralle, shoot proliferation was highest on medium with zeatin at pH 5.5 (6.92 shoots/explant), and lowest (5.54 shoots/explant) at pH 4.0 (Fig. 2a,b). For

TABLE 1. Changes in initial medium pH after autoclaving

Initial medium pH before autoclaving without zeatin	Medium pH after autoclaving	
	without zeatin	after zeatin addition
4.0	3.76	3.88
4.5	4.14	4.50
5.0	4.69	5.09
5.5	4.70	4.93
6.0	4.37	4.64

Note: pH values after autoclaving are means of three measurements.

TABLE 2. Multiple range analysis of shoot number per explant of cv. Koralle and Red Pearl at different pH of culture medium with zeatin

Medium pH	Count (n)	Average number of shoots/explant	
		Koralle	Red Pearl
		Mean ± SE	Mean ± SE
4.0	90	5.54 ± 0.26 a	5.61 ± 0.30 b
4.5	90	5.75 ± 0.35 ab	4.06 ± 0.24 a
5.0	90	6.57 ± 0.30 bc	4.05 ± 0.25 a
5.5	90	6.92 ± 0.34 c	4.51 ± 0.18 a
6.0	90	6.00 ± 0.22 ab	4.38 ± 0.24 a

n – number of observations; data are means ±SE of two replicates of 45 explants each; values denoted by the same letter within column do not differ significantly by Scheffe's test at  $p < 0.05$ .

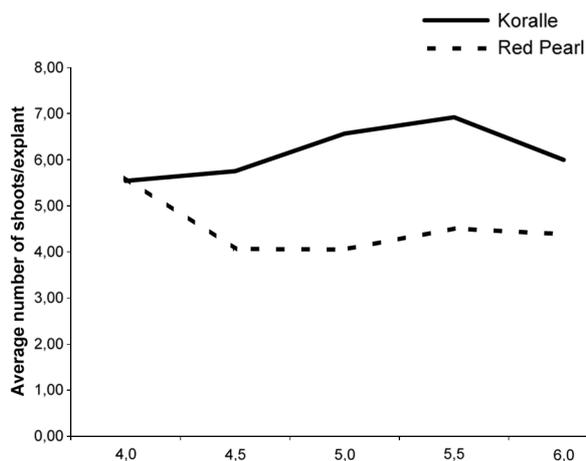


Fig. 1. Shoot number per explant of cv. Koralle and Red Pearl at different culture medium pH.

cv. Red Pearl, shoot proliferation was significantly higher (5.61 shoots/explant) at highest medium acidity (pH 4.0) than at the other tested pH levels (Fig. 3a,b).

The Koralle and Red Pearl cultivars differed significantly in shoot proliferation ability. It was significantly higher in Koralle than in Red Pearl. Those differences confirmed the dependence of shoot proliferation on cultivar genotype. Other authors have reported similar correlations (Popowich and Filipenya, 1997; Debnath, 2009; Sedlak and Paprstein, 2009).

Information on the effect of culture medium pH on shoot proliferation in *Vaccinium* spp. is sparse, especially for *V. vitis-idaea*. Testing the effect of medium pH on shoot proliferation in *Vaccinium corymbosum* cv. Duke, Ostrolucká et al. (2004) found differences in shoot proliferation depending on culture medium pH, with shoot proliferation highest at pH 5. Wolfe et al. (1986) studied the correlation between medium pH and shoot growth in *Vaccinium corymbosum* cv. Bluecrop. They found

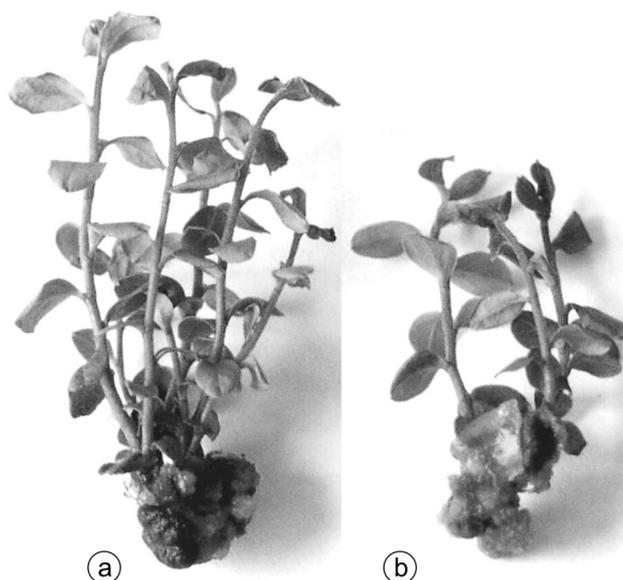
no significant differences in shoot elongation on woody plant medium (Lloyd and McCown, 1980) at pH 4.0–6.0, but shoot growth declined markedly at pH 6.5, decreasing the yield from micropropagation. Staniene and Stanyte (2007) found that substrate pH affected cranberry microshoot length both ex vitro and in vitro. In *Vaccinium* spp., Finn et al. (1991) confirmed that higher pH (6.0) had negative effects not only on seed germination in vitro but also on the vigor and dry weight of seedlings. A study testing medium pH in the 3–6 range (Borkowska, 1996) found initial pH 5.0 to be suitable for culture of *Vaccinium corymbosum*, but high medium acidity (pH 3.0) inhibited shoot growth. Debnath (2003, 2004, 2005) and Jaakola et al. (2001) determined that initial pH 4.8–5.0 was suitable for successful growth of lingonberry.

It is known that medium pH has effects not only on the uptake of medium ingredients but also on chemical reactions, especially those catalyzed by enzymes (Thorpe et al., 2008). Most *Vaccinium* species have strict soil requirements for optimal growth, requiring low pH, high iron availability, and nitrogen primarily in the form of ammonium. The limitation of acidophilous plant growth under high soil pH may be due to decreasing uptake of single elements. The activity of enzymes influencing the uptake of some elements (ferric chelate reductase, an enzyme localized to the plasma membrane, regulates the uptake and distribution of Fe through the whole plant; nitrate reductase regulates nitrogen uptake) is pH-dependent, with pH optima differing between species (Moog and Bruggemann, 1994; Poonnachit and Darnell, 2004). Lingonberry grows wild in diverse habitats ranging from lowland to upland and mountain areas, on substrate ranging from more or less acid soils to pure peat bogs (Gustavsson, 1997). For this reason a certain degree of adaptation to soil pH is characteristic of the dif-



**Fig. 2.** Shoot proliferation of cv. Koralle on Anderson's medium with 0.5 mg l<sup>-1</sup> zeatin at medium pH 5.5 (a) and pH 4.0 (b).

ferent genotypes. Skirvin et al. (1986) observed interaction between plant and medium resulting in adjustment of medium pH towards equilibrium, irrespective of the initial pH. The various compartments of cells have different pH, and this pH is maintained. Altering the pH of the external solution surrounding the tissue can alter the pH of the cells. However, essential internal controls ensure that the pH within the explant (apoplasm and symplasm pH) will be affected only slightly by medium pH. Then the effect of medium pH occurs in the medium or at the interface between explant and medium. The effect of medium pH will penetrate towards the inner tissue of the explant as the buffering capacity of the medium is increased, and thus will overcome buffering by the tissue (Thorpe et al., 2008). Anderson's (1980) medium, used in our experiments, is a weakly buffered medium, thus the plant has to expend more energy to maintain the proper physiological pH internally, and this finally affects plant growth. The period of adaptation to nonoptimal acidity of the substrate depends on the plant genotype (Staniene and Stanyte, 2007). Depending on the initial pH and buffering capacity of the medium, explants have to expend a certain amount of energy to adapt the culture medium pH to ensure optimal growth. When the initial medium pH is properly adjusted, buffering by the tissue is decreased. Adjusting the initial medium pH can enable a plant to save energy for better growth and development (Thorpe et al., 2008).



**Fig. 3.** Shoot proliferation of cv. Red Pearl on Anderson's medium with 0.5 mg l<sup>-1</sup> zeatin at medium pH 4.0 (a) and pH 6.0 (b).

The cultivars we tested differed in their response to culture medium pH. For cv. Koralle the best shoot production was achieved on media with initial pH 5.0–5.5, while for cv. Red Pearl the optimal medium pH was 4.0. In our experiments the tested pH values 4.0–6.0 never inhibited shoot proliferation completely, indicating that the tested cultivars have a certain degree of tolerance within that pH range.

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