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⁻¹ 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. Therefore 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
**Medium pH and shoot proliferation in Vaccinium vitis-idaea**

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 × 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 g l⁻¹ sucrose, 8 g l⁻¹ Phyto agar and 0.75 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°C and 118 kPa. AN medium was supplemented with 30 g l⁻¹ sucrose and 0.5 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 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°C with a 16 h photoperiod and light intensity 50 μmol m⁻² 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 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 sub-cultured on the same medium. The cultures were used as the source of single-node explants for testing 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; Anderson 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 were significant at lower pH 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.
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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. Stanile 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-
different 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 (apoplas 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).

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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REFERENCES

ANDERSON WC. 1980. Tissue culture propagation of Red and Black Raspberries, Rubus idaeus and Rubus occidentalis. Acta Horticulturae 112: 30–31.

ANDERSONE U, and IEVINIS G. 2008. Medium pH affects regeneration capacity and oxidative enzyme activity of Pinus sylvestris in tissue culture. Acta Universitatis Latviensis 745: 25–35.

BHATIA P, and ASHWATH N. 2005. Effect of medium pH on shoot regeneration from the cotyledonary explants of tomato. Biotechnology 4: 7–10.

BORKOWSKA B. 1996. Wymagania roślin borówki wysokiej pochodzacych z in vitro. Ogrodnictwo 2: 17–18.
Brissette L, Tremblay L, and Lord D. 1990. Micropropagation of lowbush blueberry from mature field-grown plants. HortScience 25: 349–351.

Debnath SC. 2003. Micropropagation of small fruits. In: Jain SM, and Ishii K [eds.], Micropropagation of Woody Trees and Fruits, 465–506. Kluwer Academic Publishers, Dordrecht.

Debnath SC. 2004. In vitro culture of lowbush blueberry (Vaccinium angustifolium Ait.). Small Fruits Review 3: 393–408.

Debnath SC. 2009. Propagation and cultivation of Vaccinium species and less known small fruits. Latvian Journal of Agronomy 12: 22–29.

De Wulf O, and de Wulf PH. 1993. Activated charcoal catalyses sucrose hydrolysis during autoclaving. Plant Cell, Tissue and Organ Culture 32: 97–99.

De Klerk GJ, Hanečková J, and Jasik J. 2008. Effect of media type on blueberry germplasm for higher pH tolerance. Russian Journal of Plant Physiology 55: 399–405.

Debnath SC. 2005. Effects of carbon source and concentration on development of lingonberry (Vaccinium vitis-idea L.) shoots cultivated in in vitro from nodal explants. In vitro Cellular and Developmental Biology – Plant 41: 145–150.

Druart PH, and de Wulf O. 1993. Activated charcoal catalyses sucrose hydrolysis during autoclaving. Plant Cell, Tissue and Organ Culture 32: 97–99.

Drury HR, Wengero D, and Miller AR. 1991. Culture medium pH is influenced by basal medium, carbohydrate source, gelling agent, activated charcoal, and medium storage method. Plant Cell Reports 10: 583–586.

Easton EA, and Filipenya VL. 1997. Effect of exogenous cytokinin on viability of Vaccinium corymbosum L. explants in vitro. Russian Journal of Plant Physiology 44: 104–107.

Easton EA, and Filipenya VL. 1997. Effect of exogenous cytokinin on viability of Vaccinium corymbosum L. explants in vitro. Russian Journal of Plant Physiology 44: 104–107.

Finn CE, Luby JJ, Rosen CJ, and Ascher PD. 1991. Evaluation in vitro of blueberry germplasm for higher pH tolerance. Journal of the American Society for Horticultural Science 116: 312–316.

Georgiou DI, and Druart PH. 1987. In vitro micropropagation of Vaccinium vitis-idea L. species. In: Jain SM, and Häggman H [eds.], Micropropagation of Woody Trees and Fruits, 465–506. Kluwer Academic Publishers, Dordrecht.

George EF. 1993. Plant Propagation by Tissue Culture, part 1. The Technology. Exgetics Ltd.

Gustavsson BA. 1997. Breeding strategies in lingonberry culture (Vaccinium vitis-idea). Acta Horticulturae 446: 29–137.

Hrabovský I, Cigáňová I, Horčík V, and Šmáda D. 2002. Small fruits and less known fruit species. Priroda, Bratislava. (In Slovak).

Jaakola L, Tolvaren A, Laine K, and Hohtola A. 2001. Effect of N6-isopentenyladenine concentration on growth initiation in vitro and rooting of bilberry and lingonberry microshoots. Plant Cell Tissue and Organ Culture 66: 73–77.

Leifert C, Pryce S, Lumsden PJ, and Waites WM. 1992. Effect of medium acidity on growth and rooting of different plant species growing in vitro. Plant Cell, Tissue and Organ Culture 30: 171–179.

Leifert C, Murphy KP, and Lumsden PJ. 1995. Mineral and carbohydrate nutrition of plant cell and tissue cultures. Critical Reviews in Plant Sciences 14: 83–109.

Lloyd G, and McCown B. 1980. Commercially feasible micropropagation of mountain laurel (Kalmia latifolia), by use of shoot tip culture. Proceedings of the International Plant Propagators' Society, vol. 30, 421–427.

Moog PR, and Bruggemann W. 1994. Iron reductase systems on the plant plasma membrane. A review. Plant and Soil 165: 241–260.

Ostrolucká MG, Libárová G, Ondrušková E, and Gajdošová A. 2004. In vitro propagation of Vaccinium species. Acta Universitatis Latviaeensis, Biology 676: 207–212.

Owen HR, Wengero D, and Miller AR. 1991. Culture medium pH is influenced by basal medium, carbohydrate source, gelling agent, activated charcoal, and medium storage method. Plant Cell Reports 10: 583–586.

Popowich EA, and Filipenya VL. 1997. Effect of exogenous cytokinin on viability of Vaccinium corymbosum L. explants in vitro. Russian Journal of Plant Physiology 44: 104–107.

Poocnacht U, and Darnell R. 2004. Effect of ammonium and nitrate on ferric chelate reductase and nitrate reductase in Vaccinium species. Annals of Botany 93: 399–405.

Rossi-Hassani BD, and Zryd JP. 1995. In vitro culture and plant regeneration of large flowered purslane. Plant Cell Tissue and Organ Culture 41: 281–283.

Ruzic D. 2004. Specifičnost Mineralne Ishrane u Kulturi In Vitro. Izd. Zadužbina Andrejević, Beograd.

Sedlak J, and Papestein F. 1997. Micropropagation of high-bush blueberry cultivars. Latvian Journal of Agronomy 12: 108–113.

Selby C, Lee R, and Harvey BMR. 1989. The effects of culture medium rigidity on adventitious bud production and tissue vitrification in needle cultures of Sitka spruce (Picea sitchensis (Boog.) Carr.). New Physiologist 113: 203–210.

Shirvin RM, Chu MC, Mann ML, Young H, Sullivan J, and Fermainian T. 1986. Stability of tissue culture medium pH as a function of autoclaving, time, and cultured plant material. Plant Cell Reports 5: 292–294.

Stanene G, and Stanic R. 2007. Adaptation of American cranberry to substrate pH in vitro and ex vitro. Žemes ukio moksliai 14: 40–44.

Thorpe T, Stasolla C, Yeung EC, de Klerk G-J, Roberts A, and George EF. 2008. The components of plant tissue culture media II: Organic additions, osmotic and pH effects and support systems. In: George EF et al. [eds.], Plant Propagation by Tissue Culture. 3rd edition, vol. 1. The Background. 115–175. Springer, Dordrecht.

Williams RR. 1995. The chemical microenvironment. In: Aitken-Christie J, Kozai T, and Smith LM [eds.], Automation and Environmental Control in Plant Tissue Culture, 405–439. Kluwer Academic Publishers, Dordrecht.

Wolfe D, Chin CK, and Eck P. 1986. Relationship of the pH of medium to growth of 'Bluecrop' highbush blueberry in vitro. HortScience 21: 296–298.