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RESEARCH PAPER

Effects of pH of a nutrient medium on parameters of growth and rhizogenesis of wild-type blue honeysuckle (*Lonicera edulis* (Turcz. ex Herder) Turcz. ex Freyn) in *in vitro* culture

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ABSTRACT

Under natural conditions, species of blue honeysuckles grow on soils with a wide range of pH. In this work, for the first time under artificial *in vitro* conditions, the influence of nutrient medium pH of 3.8 to 8.3 on the viability and growth and development parameters was studied in a species *Lonicera edulis* from the blue-honeysuckle subsection. It was found that nutrient medium pH does not affect the propagation coefficient of *Lonicera edulis*; however, more alkaline media caused shortening of its shoots by reducing the length of internodes. Our data suggested that pH affects the rooting of *in vitro* microshoots of the species under study. Two optima for the rhizogenesis were found: pH 3.8–4.3 (56.5–60.1 % rooting capacity) and pH 6.3–6.8 (53.8–61.1 %). Meanwhile, the height of the shoots and the number of leaves at the rooting stage did not significantly differ among pH values of the medium. Thus, these *in vitro* culture experiments confirm the tolerance of *Lonicera edulis* to a wide pH range.

Keywords: blue honeysuckle, *in vitro* propagation, pH range, tolerance

РЕЗЮМЕ

Эрст А.А., Боярских И.Г., Банаев Е.В., Томошевич М.А. Влияние pH питательной среды на параметры роста и ризогенеза дикорастущей голубой жимолости (*Lonicera edulis* (Turcz. ex Herder) Turcz. ex Freyn) в культуре *in vitro*. В естественных условиях голубые жимолости произрастают на почвах с широким диапазоном pH. В данной работе впервые в искусственных условиях *in vitro* исследовано влияния pH питательной среды от 3.8 до 8.3 на жизнеспособность и показатели роста и развития *Lonicera edulis*. Отмечено, что pH питательной среды не оказывает влияния на коэффициент размножения *Lonicera edulis*, однако более щелочные среды вызывают укорочение побегов за счет сокращения длины междоузлий. Полученные данные свидетельствуют о том, что значение pH влияет на укореняемость *in vitro* микропобегов. Выявлено два оптимума для укоренения: pH 3.8–4.3 (56.5–60.1 %) и pH 6.3–6.8 (53.8–61.1 %). При этом высота побегов и количество листьев на стадии укоренения достоверно не различались при различных значениях pH среды. Проведенные в культуре *in vitro* исследования подтверждают устойчивость *Lonicera edulis* к широкому диапазону pH.

Ключевые слова: голубые жимолости, *in vitro* размножение, диапазон pH, толерантность

Blue honeysuckles (subsection *Caeruleae* Rehd., family Caprifoliaceae Juss.) are a new promising berry crop with an extensive geographical range restricted to the Holarctic region and covering vast areas of Northern Europe, Asia and North America (Plekhanova 2000, Renata 2001). The taxonomy of the subsection *Caeruleae* remains a controversial issue. In a book on the genus honeysuckle, Rehder (1903), in asserting the subsection *Caeruleae*, expressed the opinion that it consists of only one polymorphic circumpolar species: *Lonicera caerulea* L. Skvortsov & Kuklina (2002) also propose to adhere to a broad definition of the species *L. caerulea* sensu lato and clearly defined the geographical origin of its individual specimens, singling out *L. iliensis* Pojark. also as an independent species. According to Poyarkova (1958) in 'Flora of the USSR', there were 10 species of honeysuckle growing in the territory of the former Soviet Union. This point of view is convenient in practical work with this berry crop and is widespread among plant breeders, although due to the lack of clarity in differentiation of these species, confusion in collections has arisen. Plekhanova (2006) recognises the existence of one tetraploid polymorphic species *L. caerulea* with a large geographical range, dividing it into subspecies and three endemic diploid species *L. iliensis*, *L. edulis* (Turcz. ex Herder) Turcz. ex Freyn, and *L. boczkarnikowae* Plekhanova. In recent years, the taxonomy of the subsection *Caeruleae* proposed by Plekhanova (2006) has been increasingly used in scientific research. Researchers mainly use subspecies of *L. caerulea* to create varieties, as well as for food and medicinal purposes. Data on the characteristics of the species *L. edulis* are practically absent.

The value of blue honeysuckles is primarily a high concentration (in plant organs) of vitamin C, of biologically active phenolic compounds (Sharma & Lee 2021), and of biologically significant macro- and microelements (Golba et al. 2020), which have antioxidant, immunomodulatory, antibacterial, antiviral, antifungal, antiallergic, and other types of effects (Minaeva 1991, Jurikova et al. 2012, Golba et al. 2020). Because of the ultra-early ripening of blue honeysuckle fruits,

they have attracted attention as a source of biologically active substances in early summer. The popularity of this crop is growing every year in various countries, thereby increasing the need for original data on its propagation and cultivation as a crop.

Natural habitats of blue honeysuckle are located in the valleys of rivers and streams, along the margins of bogs, under the canopy of forests and in the subalpine belt of mountains of northern latitudes. According to Kuklina & Vozna (1988), in nature the pH of soil environment in the habitats of blue honeysuckle varies from strongly acidic (pH 3.3) to slightly basic (pH 7.7). Judging from our studies conducted in natural growing conditions of blue honeysuckle (Russia: Gorny Altai and southern taiga of the West Siberian Plain), such soils are heterogeneous in pH of the soil medium (salt pH is 3.7–7.3) (Boyarskikh et al. 2018, Boyarskikh & Siromlya 2022a). Even in individual small habitats, soil acidity can vary from 3.3 to 6.9 (Boyarskikh & Siromlya 2022b)

Currently, soil pH and fertiliser requirements for optimum growth of blue honeysuckle are based on the optimum conditions for other small-fruited crops such as raspberries and tall blueberries. Therefore, it has been proposed to establish plantations of this crop on slightly acidic soils and to apply significant amounts of nitrogen (N), phosphorus (P) and potassium (K) annually (Gagnon 2015). Experimental studies of the effect of soil acidity on vegetative growth of blue honeysuckle in Canada show different optimal soil pH ranges, from slightly acidic (salt pH 5.5–6.0 or water pH 5.9–6.5) (Tremblay et al. 2011) to varying from slightly acidic to basic (pH 5.5–8.0) (Dawson 2017). Proper soil care and preparation prior to plantation establishment is critical for maximising rhizogenesis, vegetative growth (both above and below ground) and plant health (Tremblay et al. 2019).

When developing clonal micropropagation technology for blue honeysuckle, nutrient media with pH 5.7–5.8 are commonly used (Dziedzic 2008, Fira et al. 2014, Marcelina & Ireneusz 2014, Kolobanova 2023). Plant cells and tissues require optimal hydrogen ion concentration (pH) for crop growth and development. pH affects nutrient uptake as well as enzymatic and hormonal activities of plants (Bhatia & Ashwath 2005). The pH value of the nutrient medium has previously been shown to affect shoot regeneration, shoot height, root length, bacterial contamination and antibiotic activity added to plant tissue culture medium to suppress contaminants, as well as the level of secondary metabolite biosynthesis and antiradical activity (Leifert et al. 1992, Poonam & Ashwath 2005, Naik et al. 2010, Kovacevic et al. 2013, Cuce et al. 2016, Figiel-Kroczyńska et al. 2022, Banaev et al. 2024). The pH also affects the state of the solidifying agent in the medium, with pH above 6 producing a very hard medium and pH below 5 producing an insufficiently hard medium (Bhatia & Ashwath 2005). Investigation of the effect of different pH levels in the nutrient medium on growth and developmental parameters has not been done before on blue honeysuckle species, which are very tolerant to this factor in nature. In contrast to field screening, *in vitro* screening allows faster determination of resistance/susceptibility to stress under control conditions. Over the past few decades, *in vitro* selection based on tissue culture has become a feasible and cost-effective tool for developing stress tolerant plants (Rai et al. 2011, Erst & Karakulov 2023, Sahu et al. 2023).

The aim of this work was to study the effect of pH of the nutrient medium on the growth and development of a natural specimen of *L. edulis* (from Buryatia, Russia) in *in vitro* culture.

MATERIAL AND METHODS

Plant material

The sampling location: Russian Federation, Buryatia, Pribaikalskii District, the Turka River mouth, a shore of Baikal Lake; 52°58'14"N 108°14'43.9"E; date: 23 July 2021 [collector(s): E.V. Banaev & M.A. Tomoshevich. Det.: I.G. Boyarskikh. Barcode: NSK3001597, $2n = 18$] (Marhold et al. 2022).

In vitro propagation

Seeds of *L. edulis* were used to introduce this plant into *in vitro* culture. Seeds were surface sterilised using 20 % Domestos solution (Unilever, UK) (20 min) followed by three times rinsing with sterile distilled water. Seeds were germinated on 0.6 % aqueous agar (Difco, USA) at 24±1°C under 16 h/8 h light/dark photoperiod conditions at 2–3 klx illumination. After one month of cultivation, roots were cut off from the seedlings and transplanted to Murashige and Skoog (MS) medium (Murashige & Skoog 1962) supplemented with 0.5 μM 6-benzylaminopurine (BAP) (Sigma-Aldrich, St. Louis, MO, USA). The shoots were then cut into single-node segments and transplanted onto medium of the same composition for micropropagation. For rooting of the resulting microshoots, ½ MS medium supplemented with 1 μM indole-3-butyric acid (IBA; Sigma-Aldrich, Shanghai, China) was used. All media tested for *in vitro* propagation contained 3 % sucrose and 0.6 % Bacto® agar (PanReac®, Barcelona, Spain). The culture vessels were 100 ml conical flasks containing 20 ml of culture medium. The culture medium was autoclaved at 121°C for 20 min and the pH of the medium was adjusted to 5.8. Cultures were incubated at 24±1°C on a cycle of 16 hours light/8 hours dark at a light intensity of 2–3 klx from cold fluorescent lamps.

The impact of pH of the nutrient medium

Since genotypes of the same species may react differently to changes in nutrient medium, one random genotype of *L. edulis* was selected for the purity of the experiment.

The evaluation of the effect of nutrient medium pH on the growth and development of *L. edulis* microshoots was based on culturing single-node segments (microcuttings) for 30 days on MS medium supplemented with 0.5 μM BAP, with different pH levels and agar concentrations (pH/agar: 3.8/1 %; 4.3/0.8 %; 4.8/0.7 %; 5.3/0.7 %; 5.8/0.6 %; 6.3/0.6 %; 6.8/0.5 %; 7.3/0.5 %; 7.8/0.4 %; 8.3/0.3 %).

The evaluation of the effect of nutrient medium pH on rhizogenesis of *L. edulis* microshoots was based on culturing single-node segments (microshoots) for 30 days on ½ MS medium supplemented with 1 μM IBA and having pH levels and agar concentration as above.

Adaptation to ex vitro conditions

The *in vitro* rooted plants were planted into a soil mixture (peat with 20 % of perlite, pH 5.5–6.0) and covered with

film for 14 days to create high humidity. The cultures were incubated at 24±1°C under a photoperiod of 16-h light and 8-h dark with a light intensity of 2–3 klx from cool fluorescent lights.

Statistical analyses

The following parameters were evaluated: viability (%), propagation rate (the number of shoots per explant), plant height (mm), rooting capacity (% of shoots that grow roots), the number of roots per shoot, and root length (mm).

Experiments were conducted twice with three replicates each. Data were subjected to one-way analysis of variance (ANOVA with Duncan's multiple-range test, $p \leq 0.05$) using STATISTICA 10.0 software (TIBCO Software, USA). Data are presented as the mean and standard error (mean ± SE).

RESULTS AND DISCUSSION

The surface sterilization method used allowed us to obtain 100 % sterile plant material. Seed germination on 0.6 % water agar was 100 %. On MS medium supplemented with 0.5 μM BAP, 1–3 shoots 18–24 mm tall with six leaves developed.

The viability of *L. edulis* shoots at pH of nutrient medium from 3.8 to 8.3 was 100 %. The multiplication rate did not differ significantly on different nutrient media. The shoot height was greater on acidic media compared to alkaline media: 24.18 mm at pH 3.8 and 13.78 mm at pH 8.3. The number of leaves in shoots of different heights did not differ significantly (Fig. 1A, B, Table 1).

It has been previously reported that various factors influence the growth and development parameters of *L. edulis in vitro*: mineral composition of the medium, type and concentration of growth regulators, plant genotype, and others (Sedlák & Paprtein 2007, Marcelina & Ireneusz 2014, Orlova et al. 2022). In our study on one genotype of *L. edulis*, we found that the pH of the nutrient medium did not affect the propagation rate, but a more alkaline medium caused shoot shortening by reducing internode length. For five genotypes of white poplar (*Populus alba* L.), it was also shown that the longest shoots and roots are formed on nutrient medium with low pH (3.0) (Kovacevic et al. 2013). For three species of the genus *Vaccinium*, it has been reported that increasing pH to 6.0 results in shoot elongation and an increase in the number of nodes per shoot (Cuce et al. 2016), while for *V. corymbosum* 'Liberty' it has been shown that pH has no significant effect on shoot height (Figiel-Kroczyńska et al. 2022). In *Solanum lycopersicum* 'Red Coat', both high (6.5–7.5) and low (4.5–5.0) pH values have been shown to reduce shoot length compared to the control (Poonam & Ashwath 2005). Optimal pH is known to regulate cytoplasmic activity affecting cell division and shoot growth and does not impair cell membrane function or buffered cytoplasmic pH (Brown et al. 1979). The pH of the medium can also facilitate or inhibit nutrient uptake into the medium, for example, *in vitro* ammonium uptake is facilitated at a stable pH of 5.5 (George et al. 2008).

The rooting rate of the selected *L. edulis* genotype on the tested nutrient media varied from 23.8 to 61.1 %, while in the control it was 33.3 % (Fig. 1C, Table 2). We identified two rooting optima: pH 3.8–4.3 and pH 6.3–6.8. At the rooting stage, shoots 38.5–48.4 mm tall with eight, less frequently 10, leaves and 1–2 roots 28.5–46.6 mm long developed. We found no significant differences in the analysed parameters between pH levels, except for root length (the lowest value was observed at

Table 1 Effects of nutrient medium pH on the growth and development of *Lonicera edulis* microshoots cultured on the MS medium supplemented with 0.5 μM BAP, after 30 days of the cultivation.

pH	Agar, %	Propagation coefficient, shoots/ explant	Shoot height, mm	Number of leaves
3.8	1.0	1.33±0.24a	24.18±2.21a	6.00±0.47abc
4.3	0.8	1.18±0.12a	21.54±3.49ab	5.83±0.30abc
4.8	0.7	1.44±0.24a	19.85±3.78ab	5.54±0.40bc
5.3	0.7	1.00±0.00a	19.36±1.54ab	6.73±0.30ab
5.8 (control)	0.6	1.42±0.19a	20.00±1.66ab	6.24±0.34abc
6.3	0.6	1.43±0.29a	15.47±1.66b	5.65±0.31abc
6.8	0.5	1.43±0.20a	15.70±3.41b	5.60±0.50abc
7.3	0.5	1.20±0.13a	14.00±2.40b	5.00±0.39c
7.8	0.4	1.30±0.21a	14.54±1.84b	6.92±0.58a
8.3	0.3	1.29±0.18a	13.78±1.39b	6.22±0.40abc

Note: Values followed by the same letter(s) within a row are not significantly different at $p \leq 0.05$ according to Duncan's multiple-range test.

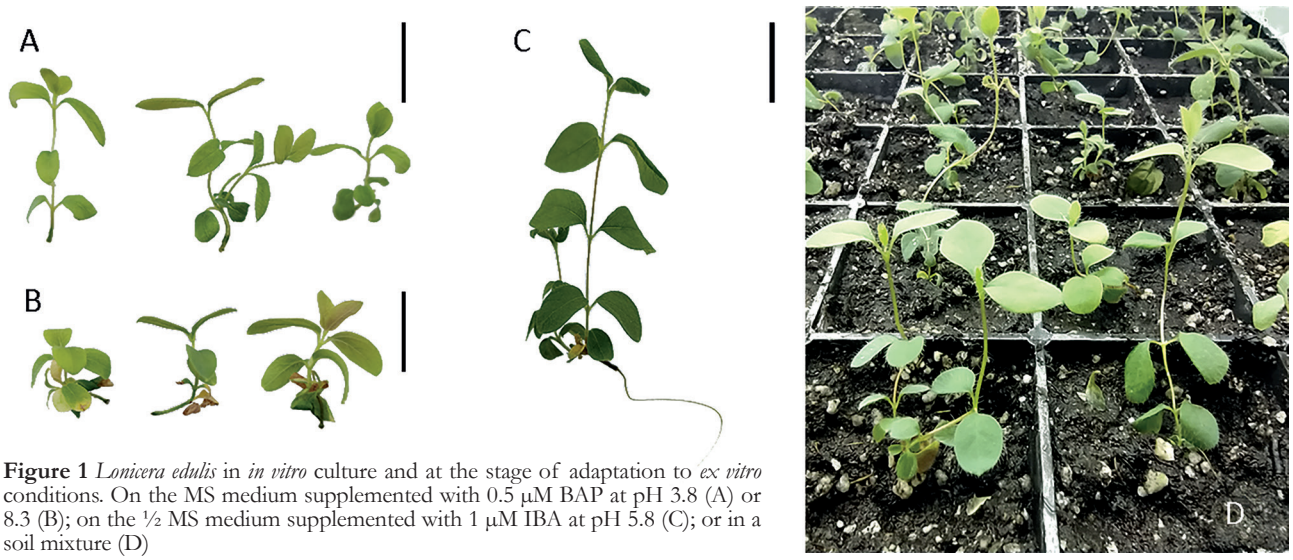

Figure 1 *Lonicera edulis* in *in vitro* culture and at the stage of adaptation to *ex vitro* conditions. On the MS medium supplemented with 0.5 μM BAP at pH 3.8 (A) or 8.3 (B); on the ½ MS medium supplemented with 1 μM IBA at pH 5.8 (C); or in a soil mixture (D)

Table 2. Effects of nutrient medium pH on rooting capacity of *Lonicera edulis* microshoots cultured on the ½ MS medium supplemented with 1 μM IBA, after 30 days of the cultivation.

pH	Agar, %	Rooting capacity, %	Number of roots	Root length, mm	Plant height, mm	Number of leaves
3.8	1.0	60.1	1–2	42.3±3.1a	38.5±3.5a	8
4.3	0.8	56.5	1–2	48.5±2.2a	47.8±4.4a	8
4.8	0.7	23.8	1–2	39.9±4.3a	40.3±3.9a	8
5.3	0.7	42.3	1–2	31.0±2.5ab	48.4±2.5a	8
5.8 (control)	0.6	33.3	1–2	46.6±3.2a	47.3±3.7a	8
6.3	0.6	53.8	1–2	28.5±3.3b	39.7±2.4a	8–10
6.8	0.5	61.1	1–2	38.2±1.2a	45.5±3.6a	8–10
7.3	0.5	40.9	1–2	40.7±2.7a	47.1±4.5a	8
7.8	0.4	26.1	1–2	39.1±1.9a	43.3±2.7a	8
8.3	0.3	33.3	1–2	43.8±1.8a	46.3±3.0a	8

Note: Values followed by the same letter(s) within a row are not significantly different at $p \leq 0.05$ according to Duncan's multiple-range test.

pH 6.3). The rooted plants successfully adapted to the culture conditions, plant viability was 72 % (Fig. 1D).

Marcelina & Ireneusz (2014) reported that microshoots rooted differently depending on treatment and genotype: the highest rooting ability (58 %) was achieved by them for Clone 44 at 2.5 mg/l IBA on MS basal nutrient medium. Sedlák & Paprtein (2007) showed that rooted shoots of blue honeysuckle on MS medium supplemented with 2.5 mg/l IBA gave 100 % rooting and good quality roots. The highest percentage of rooted microshoots (91 %) was achieved on nutrient medium supplemented with 0.3 mg/l indoleacetic acid (Orlova et al. 2022). It was previously shown that chelate forms of iron in nutrient medium affect rooting and the number of shoots and roots in *L. caerulea* var. *kamtschatica* Sevast.: 100 % rooting was obtained using Fe(III)-EDDHA (Glinushkin et al. 2023). Our results, obtained on the example of one genotype of *L. edulis*, indicate that the pH of the nutrient medium is also one of the main factors affecting rhizogenesis.

Reduced root biomass at lower soil pH has been previously reported for many cultivated and wild plants under *ex situ* conditions, as root development is impaired due to increased aluminium availability in soils with lower pH (Abdulaha-Al Baquy et al. 2017). On the other hand, alkaline soil conditions can also reduce root biomass because increased calcium activity in soil solution reduces the ability of plants to assimilate other nutrients such as phosphorus and manganese (Kerley 2000). Under *in vitro* conditions, the effects of only one or a few factors are usually modelled. For example, the MS medium used in our experiments does not contain aluminium, so this element had no toxic effect on the growth and development of *L. edulis* at low pH, in contrast to soil conditions. In future studies, we plan to use design of experiments methodology, which allows multiple factors to be tested simultaneously to understand and improve complex systems (Erst et al. 2022), as well as field trials to evaluate the effects of acid and alkaline stress on *L. edulis* clonal plant material.

CONCLUSION

In this work, the influence of nutrient medium pH on viability, as well as on growth and development parameters, was studied for the first time on the example of a natural specimen of *L. edulis in vitro*. It was found that the pH of nutrient medium does not affect the propagation rate of *L. edulis*, but more alkaline medium causes shortening of shoots by reducing the length of internodes. Our results indicate that the pH of the nutrient medium has a significant effect on rhizogenesis of *L. edulis* microshoots. Two optima were found for the rooting capacity parameter (%): pH 3.8–4.3 (56.5–60.1 %) and pH 6.3–6.8 (53.8–61.1 %). Moreover, shoot height and number of leaves at the rooting stage did not differ significantly between pH levels. *In vitro* culture experiments confirm the tolerance of *L. edulis* to a wide pH range (3.8–8.3). This *in vitro* laboratory screening should be confirmed by future field trials aimed at evaluating the long-term effects of stress induced by different pH levels and at assessing the phenotypic stability of the blue honeysuckle sample analysed.

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