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The use of tissue cultures in the mass production of *Heuchera* 'Silver Scrolls'

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Abstract: *The use of tissue cultures in the mass production of* Heuchera '*Silver Scrolls*'. *Heuchera* is one of the most important decorative perennials, present in garden, city and park plantings. In recent years, it has also gained importance as a plant for pot compositions, and as a floristic material. The aim of this work is to present an effective protocol for the intensification of the production of *Heuchera* 'Silver Scrolls' in tissue cultures. The effect of the macroelement composition from three types of medium: MS (Murashige and Skoog), QL (Quoirin and Lepoivre), and DKW (Driver and Kuniyuki Walnut) was checked, while the microelements and vitamins in all the treatments were according to the MS recipe. The effect of four concentrations (0.1, 0.5, 1.0, 2.0 mg·L^{-1}) of cytokinins: benzyladenine (BA) and meta-Topolin (mT) on the number and length of shoots was evaluated. Also the effect of different concentrations of auxins: IBA (indole-3-butyric acid) or NAA (1-naphthylacetic acid) on the percentage of rooted cuttings as well as the number and length of roots was determined. The last culture phase was the acclimatization in a greenhouse. The media and the growth regulators significantly affected the rate of propagation in the coral bells. The highest shoot number was obtained on the modified QL with BA, while NAA gave better results of rooting than IBA. Optimal conditions for micropropagation allow to obtain approx. 10,000 microplants from a single explant during the three months of culture.

Key words: *in vitro*, micropropagation protocol, parennials, coral bells, cytokinins, auxins

INTRODUCTION

Tissue cultures are widely used for the propagation of the highly demanded new cultivars. Nowadays, approx. 156 ornamental species are propagated *in vitro* in commercial laboratories all over the world, which makes micropropagation one of the most important sources of plant material for the market. The main producers of plants *in vitro* are: the Netherlands (33%), Japan (24%), Italy (11%), USA (12%), and Thailand (10%), while other countries provide 14%. Recently, Poland has gained importance in this field. Out of 140 commercial *in vitro* laboratories in Europe, 20 are situated in Poland, producing 70–100 million plants annually, of which 90% are ornamentals (Kulus 2015).

Micropropagation involves four phases: culture initiation, proliferation, shoot rooting and acclimatization. Each phase is important and needs a proper – experimentally verified – choice of all the culture parameters: starting from explant sampling (leaves, shoots or other plant parts being in the best physiological condition possible) till providing a culture

medium appropriate for each phase and containing suitable growth regulators. Each plant has its own requirements, and therefore it is necessary to adjust the conditions of all the micropropagation phases to each species – and sometimes the cultivar – in order to develop an efficient and profitable propagation method (Rout et al. 2006). Because of a great demand for its decorative foliage and multitude of cultivars, *Heuchera* is one of the most often micropropagated species. Its use as a cover plant in the urban green areas is increasing, vertical walls included.

The simplest and the cheapest method of the propagation of perennials is seed sowing. In coral bells, only a few cultivars are able to produce fertile seeds, while most of the cultivars do not produce seeds at all. Propagation *in vitro* remains, then, the only reliable method which allows to preserve unique cultivar features and to obtain quickly high amounts of planting material (Sąkol 2011). Nowadays, investigations on micropropagation concentrate on the effects of different growth regulators on plant regeneration.

Plant hormones are essential in tissue cultures, especially cytokinins, stimulating the development of side shoots. One of the most popular cytokinins used in propagation is benzyladenine (BA) (Teixeira da Silva 2012). Another cytokinin applied in micropropagation is meta-Topoline, suggested as an alternative for BA by Strnad et al. (1997). Gentile et al. (2017) showed that the most abundant proliferation of *Corylus colurna* shoots occurred on a culture medium supplemented with 2.0 $mg \cdot L^{-1}$ mT, which later stimulated as well the shoot growth. During micropropagation, cytokinins act synergistically with other growth regulators, such as, for example, auxins, which are indispensable in rhizogenesis (Faisal et al. 2018). Martin (2003) compared the efficiency of naphtylacetic acid (NAA), indolil-3-butyric acid (IBA) and indolil-3-acetic acid (IAA) in the *in vitro* rooting of *Rotula aquatica* Lour. and found that NAA was the best, as 95% of the explants were rooted, producing six roots per explant on the average.

So far, several works have been completed on the propagation of *Heuchera* by tissue cultures. Stapfer and Heuser (1986) described the method of reproduction of coral bells by initiating culture from the shoot tip, and then proliferation on a Woody Plant Medium (WPM) (Lloyd and McCown 1981), which was generally developed for the propagation of woody plants. Miura et al. (1995) managed to induce a random proliferation of shoots on the MS medium most frequently cultivated during micropropagation (Murashige and Skoog 1962) with a reduced composition, and cultures were initiated from the leaves and petioles.

In spite of the great popularity of coral bells, the number of scientific papers concerning its micropropagation is limited. This paper describes a production cycle of coral bells starting from the culture initiation, through recipes for culture media in the proliferation and rooting phase, till acclimatization to the *ex vitro* conditions.

MATERIAL AND METHODS

The experimental materials were shoots taken from the three-year old plants of *Heuchera* ‘Silver Scrolls’ grown in an

ornamental nursery. The cultivar, resulting from the crossing of *Heuchera americana*, *H. pubescens* and *H. villosa* f. *purpurea*, is considered outstanding for silvery colour of the upper leaf surface and dark wine-red colour of the lower surface and leaf petioles. The cultivar flowers profusely between May and August, producing white flowers with pink petal bases. The plant's height is 20 cm, reaching up to 55 cm during flowering (Sąkol 2011).

Initiation

Shoots of coral bells, 6 cm long, were taken from stock plants and the leaf bases were removed to uncover the buds (Fig. 1). Next, they were cut into smaller portions and underwent sterilization: 10 min rinsing in water with a detergent, 30 s being soaked in 70% ethanol, and 20 min in 2% sodium or calcium hypochlorite (NaOCl, $Ca(OCl)_2$) with a few drops of Tween 20. Finally, the stem fragments were rinsed in sterilized distilled water, and this was done in sterile conditions under a chamber with laminar air flow. Next the material was cut into smaller parts, usually single buds which were placed on the initial MS medium (Murashige and Skoog 1962) with 30 $g \cdot L^{-1}$ sucrose and pH 5.8. After one week, a visual inspection was performed and the bacteria-infected but alive explants were again sterilized 30 s in 70% ethanol, 10 min in the 1% water solution of colloidal silver, and again 30 s in 70% ethanol.

FIGURE 1. Shoot of *Heuchera* 'Silver Scrolls' – devoid of petioles, with visible buds, after the first stage of decontamination (bar = 1 cm)

Proliferation phase part 1 – different macroelement compositions

Two weeks after the culture initiation, the visually clean explants (Fig. 2) were transferred on to the proliferation medium MS supplemented with 30 $g \cdot L^{-1}$ sucrose, pH 5.8, with 1 $mg \cdot L^{-1}$ BA and 0.01 $mg \cdot L^{-1}$ NAA (1-naphthylacetic acid). In all the experiments, microelements and vitamins were added according to the composition of the MS medium.

Over a period of one month a sufficient amount of material was obtained to begin experiment 1, in which the effect of the macroelement composition from three types of medium on the regeneration of coral bells was compared: MS (Murashige and Skoog 1962), QL (Quoirin and Lepoivre 1977) and DKW (Driver and Kuniyuki 1984). Vitamins and microelements were added to all the treatments according to the MS recipe. Each medium was enriched with 1 $mg \cdot L^{-1}$ BA and 0.01 $mg \cdot L^{-1}$ NAA, 30 $g \cdot L^{-1}$ sucrose, pH 5.8 (before autoclaving), and the medium was solidified with Difco® Bacto® Agar 8 $g \cdot L^{-1}$. The media were portioned into jars of 450 ml

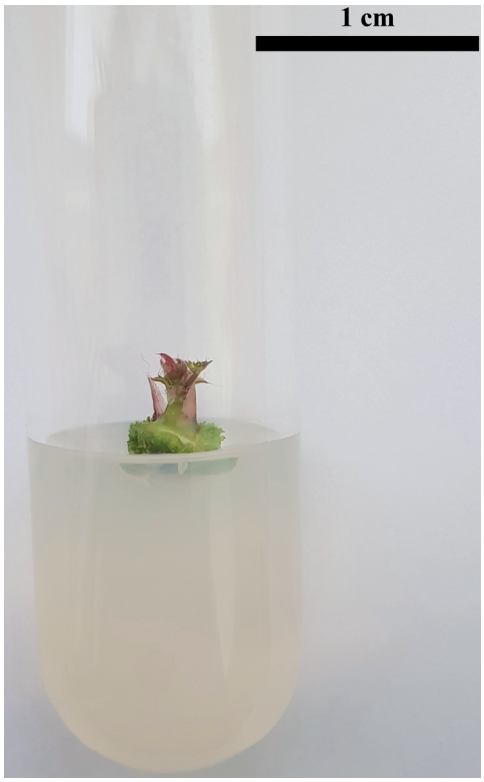

FIGURE 2. Regenerating bud after a week from the culture initiation (bar = 1 cm)

volume (v/v), 50 ml into one jar. After autoclaving the jars filled with media, six explants (min. 0.5 cm long) were placed into each jar. There were six treatments in the experiment, each consisting of 10 jars, i.e. 60 explants. The jars were place in a phytotron at 23°C, under the conditions of a 16-hour day with light provided by fluorescent lamps, and a photosynthetic photon flux of 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$. After three weeks the percentage of regenerating explants was determined, the number of regenerated shoots was counted, and the shoot length measured.

Proliferation phase part 2 – type and concentration of cytokinins

In the second experiment, the effect of the concentration of two cytokinins on the regeneration of coral bells was compared. The cytokinins used were: benzyladenine (BA) and meta-Topoline (mT), and regeneration took place on the modified QL medium enriched with 0.01 $mg \cdot L^{-1}$ NAA (Table 1).

TABLE 1. The concentrations of the cytokinins BA and mT present on the QL medium with 0.01 $mg \cdot L^{-1}$ NAA

Treatment	Culture medium QL
1	Control
2	0.1 $mg \cdot L^{-1}$ BA
3	0.5 $mg \cdot L^{-1}$ BA
4	1 $mg \cdot L^{-1}$ BA
5	2 $mg \cdot L^{-1}$ BA
6	0.1 $mg \cdot L^{-1}$ mT
7	0.5 $mg \cdot L^{-1}$ mT
8	1 $mg \cdot L^{-1}$ mT
9	2 $mg \cdot L^{-1}$ mT

Rooting phase

The aim of the experiment was to check the effect of the auxin IBA (indole-3-butyric acid) and NAA on the rooting of the coral bells microcuttings. The microshoots produced in the second experiment were placed on the modified QL medium with either IBA or NAA in the following concentrations of 0.01, 0.5, 1.0, 2.0, 4.0 $mg \cdot L^{-1}$ while the control treatment was without auxin. The method of preparing QL and the experimental conditions were as described above. There

were 10 replicates, each containing six microshoots. The plantlets were evaluated after four weeks: the percentage of rooted cuttings was determined, roots counted and their length measured (cm).

Acclimatization

All the rooted plants were taken out of the jars, and after the culture medium was rinsed off their roots they were planted into boxes filled with the mixture of peat with perlite 1 : 1 (v/v). The plants were split into two parts: one was sprayed with distilled water, while the other half was treated with the 1% ABA water solution, which was supposed to hasten the acclimatization. The boxes were covered with transparent plastic panes and placed in the phytotron (Fig. 3) at 21°C under 16-hour daylight. After one week, the plants started to be acclimatized by gradually opening the covers. After a period of seven successive days, the covers were removed. Several days later the plants were potted into P9 pots filled with the mixture of neutralised peat (pH 5.5–6.0) and perlite 1 : 1 (v/v) and placed in a greenhouse.

Statistical analysis

Arcsine transformation was performed for all the experimental data taken in percentages before subjecting them to statistical analysis (Snedecor and Cochran 1967). To compare the means, the percentages of rooted cuttings were transformed according to Bliss (Laudański and Mańkowski 2007). All the experimental data (the number of shoots per explant and shoot length, and the number of roots per explant and root length) were subjected to a one-way factorial ANOVA followed by Tukey test at $\alpha = 0.05$ (Wójcik and Laudański 1989).

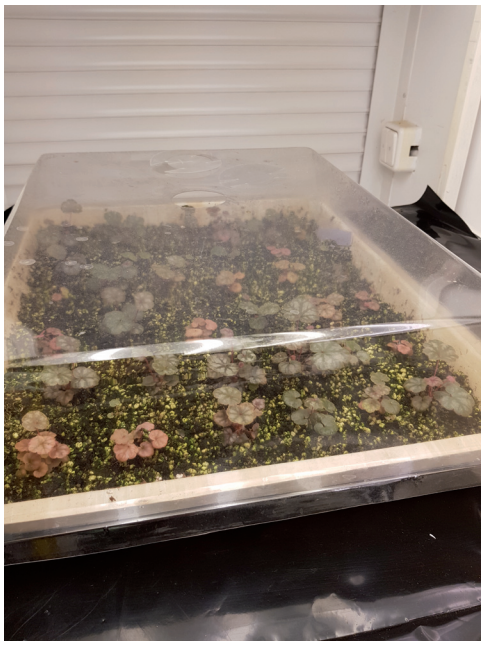

FIGURE 3. Acclimatization of plants to the *ex vitro* conditions in the phytotron, in plastic cells filled with the substrate

RESULTS

Initiation

The 2% calcium hyperchlorite concentration was the most efficient solution for the sterilization of the *Heuchera* stem, and after the first week of culture 90% of the explants were visually clean. The subsequent sterilization of the infected explants with the ethanol and silver solution allowed to obtain more clean explants, showing no signs of damage.

Proliferation phase part 1 – different macroelement compositions

The analysis of variance did not show a significant effect of the medium type on the percentage of regenerating explants of *Heuchera* 'Silver Scrolls': all of them regenerated, 100%. However, the culture medium affected the mean number of shoots per explant and their length. The highest number of shoots was obtained on QL supplemented with BA and NAA. An abundant proliferation was also observed on the MS medium with the same growth regulators. The least numbers of explants were produced on MS and DKW devoid of BA and NAA. The longest shoots were obtained on MS and DKW without growth regulators: 1.2 cm, while the shortest – 0.7–0.8 cm – on MS, QL and DKW supplemented with 0.01 mg·L^{-1} NAA and 0.5 mg·L^{-1} BA (Table 2).

Proliferation phase part 2 – type and concentration of cytokinins

The concentration of each of the two cytokinins added to the QL medium significantly affected the percentage of regenerating explants, the average shoot number regenerated per explant and the shoot length. The presence of mT gave 100% regeneration. Significantly less explants regenerated in the control treatment (without cytokinin). In the presence of BA, regeneration was less abundant than in the case of mT, but only the presence of 2.0 mg·L^{-1} BA significantly lowered it relative to mT (Table 3).

The highest shoot number was produced on the medium containing 0.5 mg·L^{-1} BA – approx. 12 per explant on the average – while on the con-

TABLE 2. The effect of the macroelement composition and growth regulators on regeneration in *Heuchera* 'Silver Scrolls'

Parameter	Medium					
	MS Control	MS with 0.01 mg·L^{-1} NAA + 0.5 mg·L^{-1} BA	DKW Control	DKW with 0.01 mg·L^{-1} NAA + 0.5 mg·L^{-1} BA	QL Control	QL with 0.01 mg·L^{-1} NAA + 0.5 mg·L^{-1} BA
Percentage of regenerating explants (%)	100 ±0.01a	100 ±0.01a	100 ±0.01a	98.3 ±2.19a	100 ±0.01a	100 ±0.01a
Number of shoots per explant	5.9 ±0.66b	9.7 ±1.59d	4.6 ±0.67a	7.1 ±1.33c	7.9 ±0.67c	10.7 ±1.18e
Shoot length (cm)	1.2 ±0.26c	0.4 ±0.08a	1.2 ±0.24c	0.52 ±0.08a	0.87 ±0.12b	0.37 ±0.05a

Means in a row ±*SD* followed by the same letter do not differ significantly at $\alpha = 0.05$.

TABLE 3. The effect of the cytokinin type and concentration on regeneration in *Heuchera* 'Silver Scrolls' cultured on the modified QL medium

Treatment	QL medium with 0.01 NAA and cytokinins ($mg \cdot L^{-1}$)	Percentage of regenerating explants (%)	Average number of shoots/explants	Average shoot length (cm)
1	Control	93.0 ±11.50a	4.2 ±0.72a	0.31 ±0.07a
2	0.1 BA	94.9 ±8.20ab	7.6 ±1.20e	0.37 ±0.09ab
3	0.5 BA	96.6 ±7.20ab	11.9 ±2.20f	0.43 ±0.10bc
4	1.0 BA	99.3 ±1.40b	7.3 ±1.10de	0.41 ±0.10bc
5	2.0 BA	93.2 ±8.80a	6.5 ±1.10e	0.45 ±0.11bc
6	0.1 mT	100 ±0.01b	5.4 ±0.71b	0.53 ±0.09e
7	0.5 mT	100 ±0.01b	5.6 ±1.10bc	0.56 ±0.08e
8	1.0 mT	100 ±0.01b	7.3 ±0.70e	0.56 ±0.11e
9	2.0 mT	100 ±0.01b	6.9 ±2.30de	0.47 ±0.09cd

Means in a column ±*SD* followed by the same letter do not differ significantly at $\alpha = 0.05$.

trol medium only approx. four microshoots were produced on each explant (Table 3). The addition of the cytokinin mT in the concentration of 1.0–2.0 $mg \cdot L^{-1}$ resulted in the production of almost seven microshoots, comparably to their number obtained with 0.1 $mg \cdot L^{-1}$ BA. The shoot length was the most affected by the medium supplemented with 0.1–1.0 $mg \cdot L^{-1}$ mT, where it attained almost 0.5 cm while in the control treatment the shoots were the shortest having approx. 0.3 cm (Table 3).

Rooting phase

The statistical analyses confirmed a significant effect of both auxins: IBA and NAA, on the parameters of rhizogenesis in the *Heuchera* 'Silver Scrolls'. The highest percentage of rooted shoots (almost 90%) was obtained on the medium with 1.0 $mg \cdot L^{-1}$ NAA, while 1.0 $mg \cdot L^{-1}$ IBA gave 80% rooting. The results on other media were comparable with that obtained in the control treatment and ranged between 64 and 71% (Table 4). The highest root number was also obtained on the medium with 1.0 $mg \cdot L^{-1}$ NAA – over four roots produced per explant. The lowest root number was found in the control treatment and in the media supplemented with NAA or IBA in their lowest concentration of 0.1 $mg \cdot L^{-1}$. The medium enriched with 1.0 $mg \cdot L^{-1}$ NAA (Fig. 4) was also the best for root

FIGURE 4. Rooted explants of *Heuchera* 'Silver Scrolls' on the QL medium supplemented with 1 $mg \cdot L^{-1}$ NAA, after five weeks from the start of the experiment (bar = 1 cm)

TABLE 4. The effect of concentration and type of auxins used in the experiment on *Heuchera* 'Silver Scrolls' rooting on the modified QL medium

Parameter	QL medium						
	Control	0.1 mg·L^{-1} IBA	0.5 mg·L^{-1} IBA	1.0 mg·L^{-1} IBA	0.1 mg·L^{-1} NAA	0.5 mg·L^{-1} NAA	1.0 mg·L^{-1} NAA
Percentage of rooted shoots (%)	64.0 ±11.60a	65.0 ±10.90a	65.2 ±10.90a	82.6 ±6.10b	63.3 ±11.80a	71.3 ±7.70a	89.7 ±8.90c
Root number per cutting	1.5 ±0.50a	1.5 ±0.50a	1.3 ±0.40a	2.5 ±0.30b	1.3 ±0.40a	3.5 ±0.90c	4.4 ±1.00d
Root length (cm)	0.26 ±0.05ab	0.25 ±0.08a	0.31 ±0.08b	0.20 ±0.03b	0.25 ±0.05a	0.34 ±0.07bc	0.47 ±0.17d

Means in a row ±*SD* followed by the same letter do not differ significantly at $\alpha = 0.05$.

elongation, where roots nearly 0.5 cm long were produced. On the media supplemented with IBA in three concentrations and NAA in its lowest dosage, the root length was comparable to the value found in the control treatment (0.2–0.3 cm) – Table 4.

Acclimatization

The acclimatization of *Heuchera* 'Silver Scrolls' was relatively successful. Plants watered with pure water survived only in 78% of cases and the main problem was browning and drying – first of the leaves, finally of the whole plants. After the foliar ABA application, the plants were better adapted to the *ex vitro* conditions and they were not losing water as easily as the control plants sprayed with water. Abscisic acid increased the survival rate to 94%, which may be regarded as a satisfactory result.

DISCUSSION

The effect of the different macroelements composition in three culture media on regeneration in *Heuchera* 'Silver Scrolls' was evaluated. The MS and DKW are the media most frequently used for the micropropagation of perennials, while QL was included into these trials due to the promising results of our preliminary studies on the micropropagation of several coral bells cultivars (unpublished data). This medium developed by Quoirin and Lepoivre (1977) contains less nitrogen, especially in its ammonium form, than the medium MS, and is usually used for the micropropagation of fruit crops (Zimmerman and Swartz 1994). The coral bells responded equally

well to the above three media and no statistical differences in the percentage of regenerating explants were found.

However, where the number of shoots developed by a single explant is considered, the modified medium QL (microelements and vitamins as in MS) proved the best. This parameter is decisive for the efficiency and profitability of the propagation method. On the QL medium supplemented with 0.01 mg·L^{-1} NAA and 0.5 mg·L^{-1} BA, over 10 new shoots with a leaf rosette were produced per explant. Also Bhoomsiri and Masomboon (2003) working on *Rosa damascena* found that the best shoot proliferation and rooting was on QL enriched with growth regulators – 100% regeneration occurred in the presence of 0.1 mg·L^{-1} NAA and 4.0 mg·L^{-1} BA. Thus, after eight weeks of culture the authors obtained nearly 60 new shoots from a single explant.

Apart from the culture medium, also the growth regulators are important in micropropagation, especially cytokinins – which affect cell divisions and differentiation, i.e. they control many processes occurring in plants such as their growth, the development of conductive tissues, branching and the response to environmental stresses (Feng et al. 2017). Here two cytokinins were compared: benzyladenine and meta-Topoline. The latter (in all the concentrations used: 0.1–2.0 mg·L^{-1}) gave 100% explant regeneration and the longest shoots. Also the application of BA resulted in a high regeneration – over 90% for all the concentrations – but the results of rooting were less favourable than in the presence of mT. Comparable results were reported by Zhao et al. (2017) for the micropropagation of *Heuchera villosa* 'Caramel' on MS containing NAA and BA or TDZ. The highest shoot regeneration (90.6%) was obtained for 0.1 mg·L^{-1} BA and 0.01 mg·L^{-1} NAA. On the medium with BA, shoots developed normally; while in the presence of TDZ, symptoms of hyperhydricity appeared. Bairu et al. (2007) in their work on the micropropagation of *Aloe polyphylla* tried to find an alternative for benzyladenine and zeatin, including as well meta-Topoline into their experiments. The highest percentage of regenerating explants and the highest new shoot numbers were found in the presence of 5.0 μM meta-Topoline.

During the rooting phase, the auxin type and concentration was checked. Production of a good root system during this phase is essential for the success of subsequent acclimatization and this may be achieved due to the choice of a right auxin (Mirani et al. 2017). Equally important is its concentration, as an excess of auxin may result in rhizogenesis inhibition (Kulpa et al. 2018). Here two auxins were used: IBA (indole-3-butyric acid) or NAA (1-naphthylacetic acid) in the concentration range from 0.1 to 1.0 mg·L^{-1}. The best effects were obtained with 1.0 mg·L^{-1} NAA, which gave almost 90% rooted plantlets with well developed, long roots. Caraballo et al. (2010) tried to micropropagate *Agave fourcroydes* Lem. For rooting they used the following auxins: 2,4-D (2,4-dichlorophenoxyacetic acid), IBA and NAA. The application of the auxins in the concentration of 0.5 or 0.75 mg·L^{-1} resulted in 100% rooting, with 9–11 roots produced by a single shoot. For the rooting of *Heuchera villosa* 'Caramel' Zhao et al. (2017) used NAA, IBA and IAA (indolo-3-acetic acid). They obtained results

even better than in this work, as 95% of the microcuttings were rooted on the MS, with the macronutrients reduced by half and supplemented with 0.2 $mg \cdot L^{-1}$ NAA. Also in *Heuchera sanguine* 95% of the microcuttings were rooted, when the medium was enriched with 4.9 μM IBA (Hosoki and Kajino 2003).

The final phase of micropropagation is acclimatization. The most important problem after transferring microcuttings to the *ex vitro* conditions is the lack of stomata response to environmental *stimuli* (Pospíšilová et al. 2000), for example stomata do not close in response to temperature and light stress. To improve acclimatization by limiting transpiration, microcuttings can be treated with abscisic acid. Foliar application of the water solution of this growth regulator results in the accumulation of H_2O_2 in cells accompanying the stomata, causing closing of the latter (Vilela et al. 2007). A positive effect of such a treatment was confirmed in this work: after the foliar application of 1% ABA (abscisic acid) 94% of the plants became acclimatized and could be potted in the greenhouse. There were 16% more plants which survived relative to the untreated cuttings. Other authors who did not treat microcuttings with ABA reported comparable values: 85% of living plants for *Heuchera villosa* 'Caramel' (Zhao et al. 2017) and 73% for *Heuchera sanguine* (Hosoki and Kajino 2003). It is thus obvious that even a low concentration (1%) of ABA can improve plant's adaptive ability and make microcuttings less sensitive to stress conditions and water loss.

The results of the experiments showed that the culture media, growth regulators and their concentrations significantly affected the micropropagation of coral bells. The modified medium QL was the best for stimulating the number of regenerated shoots and their growth, the use of benzyladenine resulted in the highest shoot number regenerated on a single explant, while meta-Topoline increased the shoot length and the auxin NAA improved the rooting of microcuttings – stimulating the production of numerous and long roots.

The above presented results are a sound base for the protocol which may be recommended for the commercial micropropagation of *Heuchera* 'Silver Scrolls' and a base to develop a set of conditions to micropropagate other coral plant taxa. A possibility to obtain 10,000 microcuttings from a single explant during 12 weeks of culture (four proliferation cycles, each lasting three weeks) may ensure a good profitability of the proposed method.

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Streszczenie: *Wykorzystanie kultur tkankowych w komercyjnej produkcji żurawki* '*Silver Scrolls*'. Żurawka (*Heuchera* sp.) to jedna z najważniejszych bylin ozdobnych, mająca zastosowanie w nasadzeniach ogrodowych, miejskich i parkowych. W ostatnich latach również zyskała na znaczeniu jako roślina do kompozycji w pojemnikach oraz materiał florystyczny. Celem tej pracy jest przedstawienie skutecznego protokołu intensyfikacji produkcji *Heuchera* 'Silver Scrolls' w kulturach tkankowych. Sprawdzono wpływ składu makroelementów w trzech wybranych pożywkach: MS (Murashige i Skoog), QL (Quoirin i Lepoivre) oraz DKW (Driver i Kuniyuki Walnut). Mikroelementy i witaminy we wszystkich kombinacjach dodano zgodnie ze składem MS. W kolejnych doświadczeniu oceniono wpływ czterech stężeń (0,1; 0,5; 1,0; 2,0 $mg \cdot L^{-1}$) cytokinin: benzyloadeniny (BA) i meta-topoliny (mT) na liczbę i długość pędów. Określono również wpływ różnych stężeń auksyn: IBA (kwas indolilo-3-masłowy) lub NAA (kwas 1-naftylooctowy) na procent ukorzenionych sadzonek oraz liczbę i długość korzeni. Ostatnim etapem była aklimatyzacja roślin w warunkach szklarniowych. Pożywka i regulatory wzrostu znacząco wpływały na współczynnik namnażania żurawki. Najwięcej nowych pędów uzyskano na zmodyfikowanej pożywce QL z dodatkiem BA, a auksyna NAA wpływała lepiej na ukorzenianie niż IBA. Optymalne warunki mikrorozmnażania pozwalają na uzyskanie ok. 10 000 roślin z jednego eksplantatu w trakcie trzech miesięcy prowadzenia kultury.

Słowa kluczowe: *in vitro*, protokół mikrorozmnażania, byliny, żurawki, cytokininy, auksyny

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