DOI: 10.21005/AAPZ2017.42.2.09

FOLIA POMERANAE UNIVERSITATIS TECHNOLOGIAE STETINENSIS
Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech. 2017, 334(42)2, 79–88

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COMPARISON OF PROPAGATION METHOD IN *IN VITRO* AND *IN VIVO* CONDITION OF *Lonicera caerulea* L.

PORÓWNANIE METODY ROZMNAŻANIA *Lonicera caerulea* L. W WARUNKACH *IN VITRO* I *IN VIVO*

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Streszczenie. Celem badań było określenie efektywności ukorzeniania jagody kamczackiej 'Wojtek' w kulturach *in vitro.* Ponadto określono wpływ sposobu cięcia sadzonek zdrewniałych i zielnych na ukorzenianie w różnych podłożach w warunkach *in vivo*. Decydującym etapem przygotowania eksplantatów do namnażania jest etap dezynfekcji. Jeśli chodzi o użyte w doświadczeniu środki dezynfekujące, najlepsze efekty uzyskano po zastosowaniu 10% podchlorynu sodu (NaOCl). Najlepszą pożywka do inicjacji i namnażania eksplantatów pędowych jagody kamczackiej 'Wojtek' była pożywka według składu Murashige i Skoog (MS). Natomiast nie zaobserwowano pozytywnego wpływu cytokininy BAP na proces inicjacji i namnażania pędów *in vitro*. Brak roślinnych regulatorów wzrostu w pożywce ukorzeniającej miał hamujący wpływ na procent ukorzenionych roślin jagody kamczackiej. Natomiast dodatek auksyny IBA do pożywki MS dodatnio wpływał na wysokość roślin, długość i liczbę korzeni. W warunkach szklarniowych największy procent ukorzenionych roślin otrzymano z 4-pędowych zdrewniałych sadzonek. Najlepszym podłożem do ukorzeniania okazał się perlit w połączeniu z torfem. Najgorsze efekty obserwowano w przypadku pędów ukorzenianych w piasku.

Key words: blue honeysuckle, herbaceous and lignified cuttings, micropropagation, PGRs.
Słowa kluczowe: jagoda kamczacka, sadzonki zielne i zdrewniałe, mikrorozmnażanie, PGR.

INTRODUCTION

Blue honeysuckle (*Lonicera caerulea* L.) belongs to *Caprifoliaceae* family, in which approximately 200 species can be found (Svarcova et al. 2007; Miyashita et al. 2009). It is a long-term species (Thompson and Barney 2007) found in circumpolar boreal forests, and also occurs in the Alps (Sato 1985) on wetland and bogs (Bors 2009).

Lonicera caerulea L. is one of the most respectable small fruit trees. Fruits are rich in bioactive compounds (Plekhanova 2000), polyphenols, and anthocyanins (Skupień et al. 2007) and they are of growing interest of growers. They also exhibit anti-inflammatory and bacteriostatic properties (Svarcowa et al. 2007; Smolik et al. 2010; Jurikova et al. 2012).

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Additionally, shrubs are characterized by low soil requirements, resistance against diseases, and water deficit. Honeysuckle is resistant to cold temperatures (Plekhanova and Streltsyna 1998) reaching up to –40°C (Ochmian et al. 2012). Full blooming flowers do not freeze even at a temperature of –8°C (Svarcova et al. 2007; Szot and Lipa 2013). Shrubs are characterized by a short growing season and early-maturing fruits. Depending on the cultivar, blooming is observed between the period of March and April/May.

In Europe, there is a noticeable increase of interest in the cultivation of blue honeysuckle berries in recent years. In response to the growing demand for fruits, new orchards are assumed. You can choose from many cultivars from different breeding centers in Europe, which are characterized by high genotypic variability (Smolik et al. 2010). The high diversity of genotypes has an impact on reproductive efficiency of different blue honeysuckle population. Traditionally, berry is propagated by cutting off semi-hardwood and hardwood stems from the parent plant. This method though generally successful is long-lasting and labor-intensive and largely depends on the individual genotype, age of the stock plant, and vegetation period (Hui et al. 2012). In contrast, a generative manner of propagating by seed, does not guarantee to uniform, true-to-type plants. However, seeds of *Lonicera* species have the problem of low germination rate, low orderliness, and long seedling time. The rapidness of tissue culture techniques can potentially multiply selected cultivars more rapidly than traditional methods (Dziedzic 2008; Hui et al. 2012; Krupa-Małkiewicz and Ochmian 2014). Micropropagation is one of the most promising methods for the production of more plants in a shorter period with less labor and at lower production costs. The tissue culture techniques can be advantageous for the preservation of sufficiently large range of plant variation for future breeding programs. The effectiveness of micropropagation of *Lonicera* species depends on media and PGRs (Plant Growth Regulators), and is genotype specific (Debnath 2007; Sedlák and Paprstein 2007; Hui et al. 2012; Krupa-Małkiewicz and Ochmian 2014).

In Poland, there is a lot of interest in domestic breeding cultivars. One of the most popular is the 'Wojtek' cultivar. Therefore there is a growing demand for cuttings of that cultivar. Hence, the purpose of this study was to compare propagation method in *in vitro* and *in vivo* of *L. caerulea* L. 'Wojtek'.

MATERIAL AND METHODS

In the experiment, the rooting efficiency of blue honeysuckle 'Wojtek' cultivar *in vitro* and lignified or herbaceous cuttings in five soil substrates (peat, sand, perlite, peat + sand, and peat + perlite) was evaluated.

In vitro. Vigorously growing shoot tips (200–300 mm) of 4-year-old Polish blue honeysuckle 'Wojtek' were collected in May from the experimental orchard of the Pomology Department in West Pomeranian University of Technology in Szczecin. The leaves were removed prior to soaking the shoots for 15 minutes in water with a detergent (Ludwik washing-up liquid), then immersed in 70% (v/v) ethanol solution for 30 s. After the preliminary disinfection, the explants were disinfected with 10% (v/v) solution of sodium hypochlorite (NaOCl) for 10 minutes and with 0.2% (v/v) solution of mercury sulfate ($HgSO_4$) for 10 minutes. Under a sterile laminar flow hood, the shoot tips were rinsed three times with sterile distilled water. After surface-

-disinfection, the explants of *L. caerulea* were excised to approximately 10–20 mm and cultured on initiation MS (Murashige and Skoog 1962) medium with 1.0, 2.0 and 4.0 mg · dm^{-3} 6-benzyloaminopurine (BAP). One explant per Erlenmayer flask was inserted into each of the 94 culture flask. The contamination rate and survival of the explants after sterilization were analyzed.

After 4 weeks, uncontaminated explants were subcultured onto fresh proliferation MS medium with different concentration of micro- and macroelements (full strength, ½ and ¼ micro- and macroelements) and supplemented with BAP at concentrations of 1.0 and 2.0 mg · dm^{-3}. In each combination of the medium 5 replications (25 explants per medium) were tested. The subculturing procedures were repeated four times every four-week intervals.

Shoots (10–20 mm length) derived from the proliferation medium were placed on rooting MS media (full strength and ½ micro- and macroelements) supplemented with auxins: indole-3-butyric acid (IBA) at the concentrations of 2.0 and 2.5 mg · dm^{-3} and indole-3-acetic acid (IAA) at the concentration of 5.0 mg · dm^{-3}. Each combination included 25 shoots (5 shoots per flask) in 5 series. After four weeks the number of *in vitro* rooted plants was recorded.

Explants placed on MS medium without the addition of growth regulators was the control at all stages of the experiment. All the media were supplemented with 8.0 g · dm^{-3} agar (Biocorp, Poland), 30 g · dm^{-3} sucrose and 100 mg · dm^{-3} inositol, pH was adjusted to 5.7 by adding 0.1M of NaOH or HCl and autoclaved at 121ºC (0.1 MPa) for 19 minutes. The cultures were maintained in a growth room at a temperature of 24 ± 1ºC under 16h photoperiod from a fluorescent lamp (photosynthetic photon flux density 40 µmol · m^{-2} · s^{-1}).

The mean values of measurements at proliferation stage (shoot length, number of new shoots and leaves per explant), and at the rooting stage (plant height, the longest root length, number of roots and leaves) obtained in the experiments are presented in tables.

In vivo. The effectiveness of rooting of lignified and green cuttings was examined. Five combinations of different soil substrates (pH 5.5–6.0) were used – peat, sand (granulation 1–2.5 mm), perlite EP 180 (granulation 0.2–3.2 mm), peat + sand (1 : 1), peat + perlite (1 : 1). Fifty cuttings were prepared in triplicate for each object (150 pieces). Three types of cuttings were used – herbaceous, two-node lignified, four-node lignified.

Lignified and herbaceous cuttings were collected in March and at the beginning of June, respectively. The lignified stems of healthy annuals stems were cut into 3–4 cuttings with the appropriate number of nodes. Herbaceous cuttings were formed from the apical part of the plant, characterized by a length of 50–60 mm, with 3–4 nodes and 2–3 apical leaves. Plastic containers were filled with soil substrate up to 150 mm layer followed by a gentle tamp and watering. The shoots were first dipped in rooting medium (herbaceous – IBA 0.3%, benomyl 0.1%, captan 1.0%; lignified – NAA 0.3%, IBA 0.05%, benomyl 0.1%, activated charcoal 5.0%) for 3 sec., and then planted at one-third of their length. The containers were placed on shaded tables in the greenhouse. The temperature was maintained at 18–20ºC during the rooting of lignified cuttings, and 22–24ºC for herbaceous cuttings. For the first two weeks the plants were automatically fogging and in the next stage of rooting soil moisture was maintained at pF < 2.5. After the rooting period, the percentage of survival rate of cuttings and the number and the length of the longest roots were specified as well.

The results were statistically analyzed. The significance of differences was determined by means of analysis of variance (ANOVA) and Tukey's test, significant at $\alpha = 0.05$ level (Table 2–5). If the values are expressed in percentages, the Bliss transformation $y = \arcsin\sqrt{x}$ was used. The t-Student test was used to compare two means (Table 1).

RESULT AND DISCUSSION

In vitro propagation

For disinfection the initial explants, chemical solutions such as mercuric chloride (Sedlák and Paprstein 2007), mercuric sulfate (Krupa-Małkiewicz and Ochmian 2014), sodium hypochloride (Karhu 1997; Osburn et al. 2009), and calcium hypochloride (Dziedzic 2008) are used. In our study, two disinfectants, mercuric sulfate and sodium hypochloride were applied. Of the 94 explants of blue honeysuckle 'Wojtek' collected, only 28.1% were contaminated with fungal infection after sterilization with 10% NaOCl (Table 1). In turn, after application of 0.2% $HgSO_4$ solution, 62.9% infected explants were obtained. These explants were later discarded. Sedlák and Paprstein (2007) used 0.15% mercuric chloride ($HgCl_2$) solution for disinfection and observed that 50% of initial uncontaminated explants of cultivar Altaj died after the sterilization procedure. According to Debnath (2007) the most important and necessary step *in vitro* propagation of *Vaccinium* genus is regeneration from primary explants.

Table 1. Surface disinfection with 70% alcohol followed by 10% NaOCl and 0.2% $HgSO_4$ for 10 min
Tabela 1. Dezynfekcja powierzchniowa eksplantatów w 70% alkoholu oraz 10% NaOCl i 0,2% $HgSO_4$ przez 10 min

Disinfection solution Roztwór dezynfekujący	Contaminated explants Zakażone eksplantaty [%]	Uncontaminated explants Eksplantaty niezakażone	
		explants that developed shoots eksplantaty, które rozpoczęły wzrost [%]	explants that did not develop shoots eksplantaty, które nie rozpoczęły wzrostu [%]
10% NaOCl	28.1	28.1	43.8
0.2% $HgSO_4$	62.9	32.3	4.8
t-Student test	**	n.s.	**

n.s. – non significant or significant at $p = 0.01$ (**) – różnice nieistotne lub istotne na poziomie $p = 0,01$ (**).

Addition of BAP cytokine to MS medium at concentrations of 1.0, 2.0, and 4.0 mg · dm^{-3} showed no statistically significant effect on the initiation of shoots of blue honeysuckle Wojtek cultivar (Table 2). The number of new shoots per explant was at the same level (1.50). The shoot length of blue honeysuckle on MS media supplemented with 1.0 and 4.0 mg · dm^{-3} was similar to that of the control group (47.50 mm), while the explants on MS medium supplemented with BAP at a concentration of 2.0 mg · dm^{-3} were slightly lower (33.80 mm). According to Karhu (1997), Sedlák and Paprstein (2007), Dziedzic (2008) as well Krupa-Małkiewicz and Ochmian (2014) the best initiation medium to obtain the largest number of new microshoots is MS supplemented with BAP at concentrations of 1.0 and 2.0 mg · dm^{-3}. Osburn et al. (2009) for the initiation of Japanese and 'Amur' honeysuckle applied three kinds of media: DKW (Driver and Kuniyuki 1984), MS, and WPM (Lloyd and McCown 1981). Japanese honeysuckle produced statistically more microshoots on DKW basal medium than

on MS or WPM, while explants of 'Amur' honeysuckle proliferated more on DKW and MS media than on WPM. According to Sedlák and Paprstein (2007), proliferation rate varied with the genotype and different kinds of media.

Table 2. Initiation of *Lonicera caerulea* L. 'Wojtek' shoots on various MS media
Tabela 2. Inicjacja *Lonicera caerulea* L. 'Wojtek' na różnych pożywkach MS

Medium Pożywka	Shoot length Długość pędu [mm]		Number of new shoots per explant Liczba nowych pędów na 1 eksplantacie		Number of leaves per 1 shoot Liczba liści na 1 pędzie	
MS	47.5 ± 0.9	a	1.5 ± 0.6	a	7.0 ± 1.1	b
MS + 1.0 mg · dm^{-3} BAP	50.0 ± 1.1	a	1.5 ± 0.6	a	18.5 ± 1.9	a
MS + 2.0 mg · dm^{-3} BAP	33.8 ± 0.7	a	1.2 ± 0.5	a	14.5 ± 3.4	a
MS + 4.0 mg · dm^{-3} BAP	50.0 ± 1.1	a	1.5 ± 0.6	a	16.0 ± 2.3	a
Mean – Średnia	45.3		1.4		14.0	

Means in the same column followed by the same letter are not significantly different at $\alpha < 0.05$ according to Tukey test; ± SD – standard deviation.
Średnie w kolumnach oznaczone tymi samymi literami alfabetu nie różnią się według testu Tukeya na poziomie istotności $\alpha < 0,05$; ± SD – odchylenie standardowe.

The results of this study show that shoots proliferated on MS medium containing 100% and 50% mineral salts supplemented with 1.0 or 2.0 mg · dm^{-3} BAP were shorter but the number of new shoots in comparison to that of control was similar (Table 3).

Table 3. Proliferation of shoot tips of *Lonicera caerulea* L. 'Wojtek' cultured on various combination of MS media supplemented with BAP
Tabela 3. Namnażanie pędów wierzchołkowych *Lonicera caerulea* L. 'Wojtek' na różnych kombinacjach pożywki MS uzupełnionej BAP

Medium Pożywka	Shoot length Długość pędu [mm]		Number of new shoots per explant Liczba nowych pędów na 1 eksplantacie		Number of leaves per 1 shoot Liczba liści na 1 pędzie	
MS	47.5 ± 0.9	a	3.0 ± 0.5	a	7.0 ± 1.5	a
MS + 1.0 mg · dm^{-3} BAP	29.0 ± 1.4	b	2.7 ± 1.3	a	9.8 ± 5.9	a
MS + 2.0 mg · dm^{-3} BAP	20.0 ± 0.2	b	3.0 ± 0.2	a	10.0 ± 0.5	a
½MS + 1.0 mg · dm^{-3} BAP	28.6 ± 1.1	b	2.0 ± 1.0	a	7.0 ± 2.4	a
½ MS + 2.0 mg · dm^{-3} BAP	25.0 ± 0.6	b	2.1 ± 0.8	a	7.5 ± 3.8	a
¼ MS + 1.0 mg · dm^{-3} BAP	23.6 ± 0.7	b	2.5 ± 0.7	a	6.5 ± 4.0	a
¼ MS + 2.0 mg · dm^{-3} BAP	24.4 ± 1.2	b	1.9 ± 0.8	a	9.0 ± 4.4	a
Mean – Średnia	46.2		2.5		8.1	

Explanations see Table 1 – Objaśnienia zob. tab. 1.

According to Karhu (1997), there is a linear relationship between the concentration of BAP and the efficient production of high-quality microshoots of *L. caerulea* f. *caerulea* and *L. caerulea* f. *edulis*. Sedlák and Paprstein (2007) obtained the highest multiplication rate for blue honeysuckle genotype 20/1 on the MS medium supplemented with 2.0 mg · dm^{-3} BAP. In turn, Dziedzic (2008) achieved the satisfactory growth of biomass and high number of new shoots for 'Czelabinka' and 'Duet' on MS medium containing 75% salt supplemented with BAP at concentrations of 1.0 and 2.0 mg · l^{-3}. Osburn et al. (2009) reported higher number of microshoots of Japanese honeysuckle and 'Amur' honeysuckle which were produced on DKW medium containing 2.5 μM BAP with or without 1.25 μM IBA. They suggested that addition of IBA did not enhance the effect of BAP.

In our studies, microcuttings rooted differently depending upon propagation methods and medium composition (Table 4, Fig. 1). Addition of auxins to MS medium showed stimulatory effect on the length of roots of blue honeysuckle, and on their structure. The longest roots (52.0 mm) were observed on 50% mineral salt MS medium supplemented with 2.0 mg · dm^{-3} IBA and 5.0 mg · dm^{-3} IAA. The highest number of roots (4.0) developed shoots on 50% mineral salt MS with addition of 2.5 mg · dm^{-3} IBA. Lack of PGRs in MS medium affected negatively the rooting of the plants of blue honeysuckle, only 8% of the explants being rooted. The number of rooted cuttings significantly increased with the use of IBA auxin. The addition of IBA and IAA to MS medium caused 100% rooting of honeysuckle explants. Sedlák and Paprstein (2007) also obtained 100% of rooted shoots of genotype 20/1 and 'Altaj' on MS medium supplemented with high concentration of IBA (2.0 mg · dm^{-3}).

Table 4. The influence of MS and ½MS medium supplemented with IBA and IAA on rooting of *Lonicera caerulea* L. 'Wojtek'
Tabela 4. Wpływ pożywki MS i ½MS uzupełnionej IBA i IAA na ukorzenianie pędów *Lonicera caerulea* L. 'Wojtek'

Medium Pożywka	Shoot length Długość pędu [mm]		Root length per shoot Długość korzeni na 1 pędzie [mm]		Number of roots per shoot Liczba korzeni na 1 pędzie		Number of leaves per 1 shoot Liczba liści na 1 pędzie	
MS	18.3 ± 0.8	b	5.0 ± 0.2	b	0.1 ± 0.4	b	7.5 ± 1.6	a
½MS + 2.5 mg · dm^{-3} IBA	25.0 ± 1.5	b	33.0 ± 2.6	ab	4.0 ± 2.5	a	6.9 ± 3.2	a
MS + 2.5 mg · dm^{-3} IBA	36.7 ± 3.3	ab	37.0 ± 3.3	ab	2.5 ± 2.4	ab	10.0 ± 2.9	a
½ MS + 2.0 mg · dm^{-3} IBA + + 5.0 mg · dm^{-3} IAA	40.0 ± 1.2	ab	52.0 ± 1.6	a	3.4 ± 2.6	ab	10.4 ± 4.3	a
MS + 2.0 mg · dm^{-3} IBA + + 5.0 mg · dm^{-3} IAA	58.3 ± 2.6	a	46.0 ± 1.8	ab	2.8 ± 1.7	ab	13.1 ± 3.5	a
Mean – Średnia	35.7		34.6		2.6		9.6	

Explanations see Table 1 – Objaśnienia zob. tab. 1.

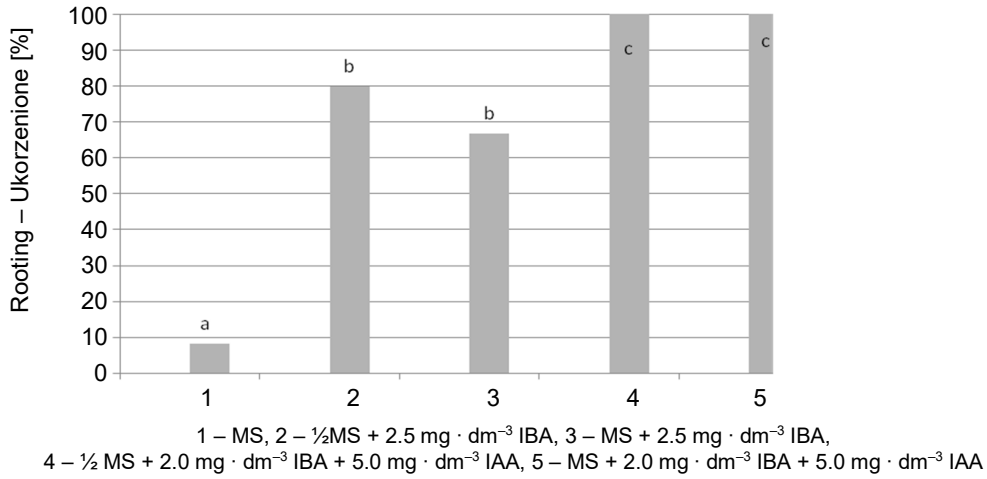

Fig. 1. Percentage of rooting explants depending on the different combination of MS media
Explanations see Table 1.
Ryc. 1. Procent ukorzenionych eksplantatów w zależności od zastosowanych kombinacji pożywki MS
Objaśnienia zob. tab. 1.
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According to Dziedzic (2008), the most effective for inducing the roots of 'Czelabinka' (96% rooted shoots) and 'Duet' (92% rooted shoots) was WPM medium supplemented with 2.0 mg · dm^{-3} IBA and 2.0 mg · dm^{-3} IAA rather than MS. Osburn et al. (2009) achieved 87% of Japanese honeysuckle and 76% of 'Amur' honeysuckle rooted microcuttings treated with 980 μM IBA, which was less than microcutting treated with water.

In vivo propagation

During *in vivo* propagation, percentage of rooted cuttings was lower as compared to *in vitro* conditions (Fig. 2). Four-node lignified cuttings in a mixture of peat and perlite (90%) and in the perlite alone (88%) were rooted with the highest performance (Fig. 3). For comparison, the percentage of highbush blueberry seedlings' reception in the same soil substrate was very small-up to 25% (Giroux et al. 1999). The effectiveness of rooting was influenced by the type of cuttings and soil substrates. Two-node lignified cuttings were rooted with the worst performance (on average 43%). Sand mixed with peat was proved to be an unsuitable medium for rooting. These media were not much effective during the rooting of cuttings of highbush blueberry (Ochmian and Saniewska 2012). Mainland (1993) confirmed the insufficient usefulness of sand as a substrate for rooting.

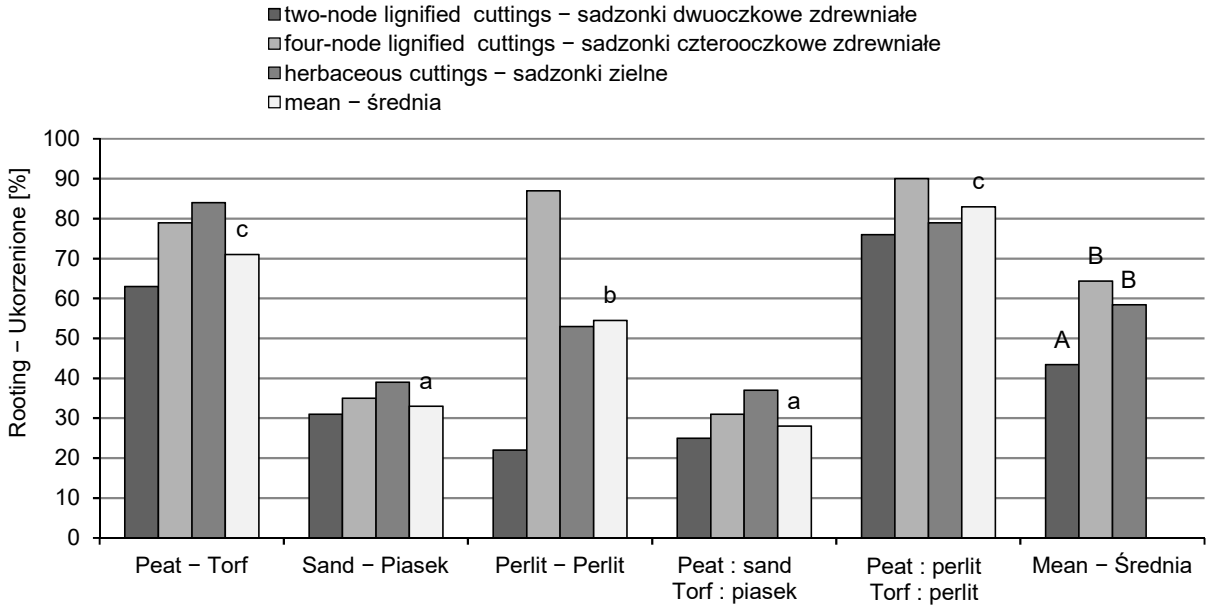

Fig. 2. Percentage of rooting of cuttings depending on the propagation
Means in the same column followed by the same letter are not significantly different at α < 0.05 according to Tukey test. The same small letters indicate mean values for soil substrate, the same capital letters indicate mean values for the cuttings.
Ryc. 2. Procent ukorzenionych sadzonek w zależności od metody ukorzeniania
Średnie w kolumnach oznaczone tymi samymi literami alfabetu nie różnią się według testu Tukeya na poziomie istotności α < 0,05. Średnie oznaczone małymi literami alfabetu oznaczają podłoża, duże litery – wartości średnie dla sadzonek.

Karhu (2003) concluded that to obtain good quality of cuttings, attention should be paid to the quality of rooted plants rather than the root number. However, according to Dziedzic (2008), Osburn et al. (2009) and Hui et al. (2012) acclimatization of blue honeysuckle plants derived from *in vitro* culture depends largely on the genotype of the plant and not on the concentration

of auxins in the medium. Owing to natural habit of woody shrub, its roots form at every node when in contact with soil. Thus, *in vitro* rooting is not necessary for some genotypes of blue honeysuckle. Varieties of cultivated blue honeysuckle derived from different cultivation centers are very diverse in terms of genetic makeup (Smolik et al. 2010).

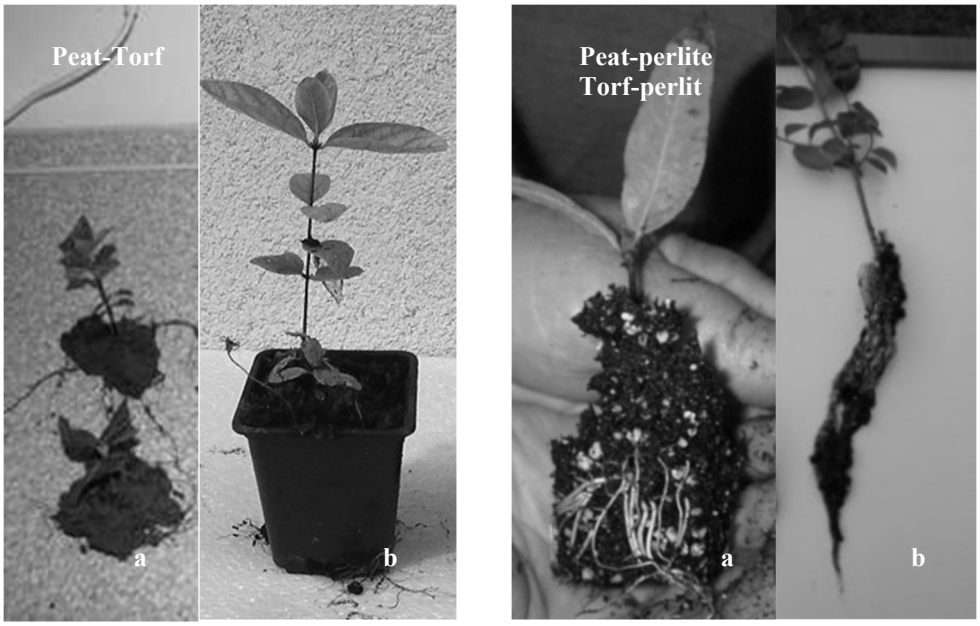

Fig. 3. Lignified cuttings (a) and herbaceous (b) rooted in peat and peat : perlite 1 : 1
Ryc. 3. Sadzonki zdrewniałe (a) i zielne (b) ukorzeniane w torfie i mieszance torf : perlit 1 : 1

Table 5. The influence of soil substrate and the type of cuttings on the number and length of roots
Tabela 5. Wpływ podłoża i rodzaju cięcia na liczbę i długość korzeni

Cuttings Rodzaj sadzonek (A)		Medium – Podłoże (B)					Mean Średnia
		peat torf	sand piasek	perlite perlit	peat – sand torf – piasek 1 : 1	peat – perlite torf – perlit 1 : 1	
		Number of roots per plant – Liczba korzeni					
Lignified Zdrewniałe	2-node 2-pędowe	9.2 ± 1.3 de	2.7 ± 0.3 ab	7.8 ± 0.7 c-e	4.9 ± 0.4 a-d	12.8 ±1.6 f	7.5 B
	4-node 4-pędowe	11.5 ± 1.6 e	3.5 ± 0.4 a-c	9.4 ± 1.1 de	5.3 ± 0.5 a-d	14.2 ± 1.8 f	8.8 B
Herbaceous Zielne		5.8 ± 0.9 a-d	1.9 ± 0.3 a	4.1 ± 0.6 a-c	3.5 ± 0.4 a-c	7.6 ± 1.0 b-e	4.6 A
Mean – Średnia		8.8 B	2.7 A	7.1 B	4.6 A	11.5 C	
		Root length – Długość korzeni [mm]					
Lignified Zdrewniałe	2-node 2-pędowe	67 ± 12 cd	21 ± 4 h	73 ± 13 b-d	51 ± 5 c-g	112 ± 8 a	65 B
	4-node 4-pędowe	62 ± 10 c-e	24 ± 5 gh	75 ± 9 bc	44 ± 6 d-h	95 ± 11 ab	60 B
Herbaceous Zielne		37 ± 11 e-h	22 ± 3 gh	49 ± 7 c-h	31 ± 3 f-h	60 ± 7 c-f	40 A
Mean – Średnia		55 BC	22 A	66 C	42 B	89 D	

Data represented by mean ± SD – odchylenie standardowe. Means having same letter in a column were not significantly different by Tukey's comparison p = 0.05 level – Średnie w kolumnach oznaczone tymi samymi literami alfabetu nie różnią się według testu Tukeya na poziomie istotności p = 0,05.
The same small letters indicate mean values for interaction, the same capital letters indicate mean values for the main effects – Średnie oznaczone małymi literami alfabetu oznaczają interakcję, duże litery – wartości średnie dla grup głównych.

The number of roots obtained during the rooting of cuttings *in vivo* was satisfactory (Table 5, Fig. 2). Most roots (above 11 on average) were formed by cuttings rooted in a mixture of peat and perlite in 1 : 1 ratio. Four-node lignified cuttings rooted in this medium had more than 14 roots, of an average length of 89.0 mm. The worst parameters were observed for cuttings rooted in sand. A small amount of short roots was also found in herbaceous cuttings (Table 5). As observed by Douglas (1996), better results of rooting can be achieved by rooting of lignified cuttings during early springtime in comparison to herbaceous cuttings during summer.

CONCLUSION

In this study, no positive effect of BAP cytokine on initiation and proliferation stage of blue honeysuckle was reported. Addition of auxins to MS medium had a stimulating effect on length, and number of roots *in vitro*, and in the greenhouse. For explants cultured *in vitro* it was observed that rooting efficiency of blue honeysuckle was high. Among cuttings, four-node lignified cuttings were rooted with the highest performance in the soil substrate prepared with perlite and peat. A rapid *in vitro* and *in vivo* shoots of *L. caerulea* L. 'Wojtek' multiplication described in this paper could have an important factor on ability to rapidly proliferate and maintain desirable blue honeysuckle cultivars. To meet the expectations of producers, *in vitro* technique as well as conventional techniques using lignified and herbaceous cuttings produce satisfactory number of new plants.

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Abstract. The aim of the study was to determine the effectiveness of rooting of blue honeysuckle of 'Wojtek' cultivar *in vitro*. Moreover, suitability of different soil substrate for rooting the lignified herbaceous cuttings *in vivo* was evaluated. The process of disinfection was the decisive factor in the process of preparing the explants for the propagation. Among the disinfection solutions used for sterilization of 'Wojtek' microshoots, the best results were obtained after application of 10% NaOCl solution. Initiation and propagation of blue honeysuckle proceed with the highest efficiency in Murashige and Skoog (MS) medium, and no positive effect of BAP cytokine on this process was reported. Proper selection of composition of the medium guaranteed high performance of rooting of explants *in vitro* – lack of plant growth regulators (PGRs) in MS medium led to a decrease in the percentage of rooted plants. The addition of auxins to MS medium had a stimulating effect on plant height, length, and number of roots. In the greenhouse, four-node lignified cuttings in the medium prepared with perlite and peat were rooted with the highest performance. Rooting of lignified herbaceous cuttings in the substrate prepared with peat and perlite stimulated the development of the root system as well. The worst influence for rooted cuttings was observed in sand.

This work was supported by the Polish Ministry of Science and Higher Education under grant No. N N310 205737.