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MICROPROPAGATION OF AN OLD SPECIMEN OF COMMON LILAC (*Syringa vulgaris* L.) FROM THE DENDROLOGICAL GARDEN AT PRZELEWICE

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Abstract. The published results on plant propagation *m vitro* culture show the importance of maternal plant age in a successful regeneration process. It is known that initiation effectiveness is low when primary explants are taken from old trees. The aim of this study was an attempt to regenerate *Syringa vulgaris* from an old specimen of Dendrological Garden in *in vitro* culture. April turned out to be the optimal month for collecting explants. The highest percentage of initiated shoots were obtained on MS medium containing 7.5 mg·dm^{-3} BAP and 0.02 mg·dm^{-3} NAA. The highest propagation efficiency was received on MS medium with a double amount of $MgS0_4$ supplemented with 1.0 mg·dm^{-3} BAP. Insignificant effects were observed on the media with cytokinin BAP. The plants with the best-developed root system were obtained on MS medium with reduced to ¼ amount of macro- and micronutrients supplemented with 5.0 mg·dm^{-3} IBA.

Key words: rejuvenation, micropropagation, common lilac, growth regulators

INTRODUCTION

In the ontogenesis the juvenile phase of a plant lasts until the plant reaches the ability of flowering and fructification [Kopcewicz 1998]. In this time the plant possesses a number of morphological features which gradually disappear with the plant reaching the maturity phase. If the material for vegetative propagation is taken from a plant at its juvenile phase, it is going to be difficult to predict which features will appear in the phase of maturity. A similar problem arises with the plants' propagation from seeds. On the other hand, if the material for vegetative propagation is taken from the mature, valuable plant, then its progeny will keep all the features of the maternal organism and in the future they will be equally valuable [Bonga 1987].

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Studies on plants' propagation in *in vitro* cultures emphasize the effect of the maternal plant's age on the regeneration process. That is why it is recommended that the material for *in vitro* cultures should be taken from the youngest plants possible. The shoots that preserve their juvenility throughout the whole life of a plant are those that grow from the stem base of its trunk. Such maturation of particular parts of the tree, which is not simultaneous, is called topophysis [Jankiewicz and Orlikowska 1990, Beck et al. 1998].

The conditions in *in vitro* cultures stimulate the process of tissue rejuvenation. Intensive cutting, passaging, abundance of macronutrients and the optimum temperature favour the growth of axillary and adventitious buds. Additionally, the presence of cytokinins in the media suppresses the apical domination and favours the formation of adventitious merisystems and then the shoots of juvenile character [Jankiewicz and Orlikowska 1990, Orlikowska 1997].

The purpose of the studies was to propagate an 80-year-old specimen of *Syringa vulgaris* from the Dendrological Garden at Przelewice in *in vitro* cultures and obtain the juvenile cuttings of this plant.

MATERIAL AND METHODS

The material for the *in vitro* cultures were fragments of the shoots growing from the lowest part of the trunk of common lilac (*Syringa vulgaris* L.), growing in the collection of the garden. The shoots for the studies were sampled at three dates, at the beginning of February, March and April, and next they were subjected to sterilization. To this aim, the material intended for the culture initiation was rinsed under running water for an hour, and next in the water with an addition of a detergent for 15 minutes. The dried shoots were submerged in 70% ethyl alcohol for 10 minutes and then in 0.2% mercury chloride ($HgCl_2$) for 10 minutes. The disinfected fragments of shoots were rinsed in sterile distilled water three times. The explants were the isolated shoot tips with small fragments of shoots (to 5 mm). The initiation of cultures of common lilac was performed on MS medium [Murashige and Skoog 1962], supplemented with 5.0 and 7.5 $mg \cdot dm^{-3}$ BAP (6-benzylaminopurine) and 0.02 $mg \cdot dm^{-3}$ NAA (α-naphtaleneacetic acid). MS medium, without any growth regulators, was the control medium. Fifteen explants were used for each medium type.

Lilac shoots initiated for growth were propagated on three types of media. They were prepared on the basis of MS medium, with a double content of chosen macroelements. Medium I contained twice as much $MgSO_4$ as in MS, medium II – twice as much KH_2PO_4, and medium III – twice as much $CaCl_2$. MS medium was the control in the experiment. All media were supplemented with cytokinin BAP in the quantity 5.0 $mg \cdot dm^{-3}$.

On the basis of preliminary results, medium I was selected for the further stage of propagation. Five combinations of this medium were prepared, depending on the amount of cytokinin BAP (from 0 to 5 $mg \cdot dm^{-3}$). The control medium did not contain cytokinin. For propagation stage 0.5 cm fragments of shoots with axillary buds were used as explants.

The next stage of regeneration of common lilac was the rooting of shoots. It was performed on 6 types of media prepared on the basis of MS medium with a ¼ of the quantity of macro- and microelements supplemented with differentiated (0.5–5.0 $mg \cdot dm^{-3}$) content of IBA (indole-3-acetic acid). The control medium did not contain auxin. The apical fragments of shoots (1 cm) with two leaves were used as explants in this phase.

All media were solidified with agar in the quantity of 7 $g \cdot dm^{-3}$. Their pH was adjusted at 5.7 before autoclaving. Each of the discussed stages of lilac regeneration lasted 4 weeks. The cultures were placed in a growth chamber with the light intensity of 50 $\mu mol\ m^{-2}\ s^{-1}$, the day's length of 16 h and the temperature of 24 ±1°C. At the stage of propagation and rooting twenty explants were taken for each treatment and each experiment was repeated once.

The mean values of measurements at proliferation (shoot length, numbers of: leaves, internodes, axillary shoots, shoot weight) and rooting stage (plant height, number of leaves, root length, root number) obtained in the experiments are presented in tables. The results were statistically analyzed. The significance of differences was determined by means of variance analysis and Tukey's test, at the level of significance of $\alpha = 0.05$.

RESULTS

The studies on common lilac show that the optimum date of obtaining the explants was the beginning of April (tab. 1). The highest percentage of initiating shoots were obtained from the explants taken at that date (40%). The best results of initiation – 48% were obtained on medium II, which contained 7.5 $mg \cdot dm^{-3}$ BAP and 0.02 $mg \cdot dm^{-3}$ NAA (photo 1). 33% of explants were initiated on the control medium. The initiation of lilac plants at earlier dates of taking the explants ended in failure due to a big number of infections and dying out explants. In February the frequency of initiation was at zero level, while in March it was 7%.

Table 1. Percent (%) of initiated, infected and dead explants of *Syringa vulgaris* depending on media composition and date of plant material collection

Tabela 1. Częstotliwość (%) eksplantatów lilaka zwyczajnego inicjujących wzrost, zakażonych i zamarłych w zależności od składu pożywki i terminu pozyskiwania materiału roślinnego

Month Miesiąc	Medium – Pożywka $mg \cdot dm^{-3}$	% of explants – % eksplantatów		
		initiated inicjujące wzrost	infected zakażone	dead zamarłe
February Luty	control – kontrola	0	27	73
	I – 5.0 BAP 0.02 NAA	0	27	73
	II – 7.5 BAP 0.02 NAA	0	33	67
	mean – średnia	0	29	71
March Marzec	control – kontrola	0	53	47
	I – 5.0 BAP 0.02 NAA	7	73	20
	II – 7.5 BAP 0.02 NAA	13	33	54
	mean – średnia	7	53	40
April Kwiecień	control – kontrola	33	40	27
	I – 5.0 BAP 0.02 NAA	40	27	33
	II – 7.5 BAP 0.02 NAA	48	26	26
	mean – średnia	40	31	29

Photo 1. Lilac explant at initiation stage on MS medium supplemented with 7.5 mg dm^{-3} BAP and 0.02 mg dm^{-3} NAA

Fot. 1. Eksplantat lilaka podczas etapu inicjacji na pożywce MS z dodatkiem 7,5 mg dm^{-3} BAP i 0,02 mg dm^{-3} NAA

Photo 2. Lilac shoots propagated on MS medium with increased $MgSO_4$ content, supplemented with 1.0 mg dm^{-3} BAP

Fot. 2. Pędy lilaka namnażane na pożywce MS o zwiększonej zawartości $MgSO_4$ uzupełnionej 1,0 mg dm^{-3} BAP

The mineral composition of propagation media used in the preliminary experiment had a significant effect on the development of common lilac explants (tab. 2). The shoots growing on medium I, with an increased content of $MgSO_4$ were characterized by the best length (1.49 cm) and the greatest number of internodes (6.50), as well as a high number of leaves (13.30) and axillary shoots (2.10). However, they did not differ significantly from the plants from medium II with double amount of KH_2PO_4.

Table 2. Mean values (x) of morphological traits *Syringa vulgaris* propagated on media with differentiated values of mineral salts
Tabela 2. Średnie wartości cech morfologicznych lilaka zwyczajnego namnażanego na pożywkach o zróżnicowanym składzie mineralnym

Medium Pożywka	Shoot length Długość pędów cm	Number of leaves Liczba liści	Number of internodes Liczba międzywęźli	Number of axillary shoots Liczba pędów bocznych	Shoots weight Masa pędów g
Control (MS) – Kontrola (MS)	plants got brown and died – rośliny zbrązowiałe i zamarłe				
I – 2x $MgSO_4$	1.49a	13.30a	6.50a	2.10a	0.12a
II – 2x KH_2PO_4	1.06ab	13.70a	6.30a	2.10a	0.18a
III – 2x $CaCl_2$	0.66b	5.20b	2.30b	1.00b	0.13a
$LSD_{0.05}$ – $NIR_{0.05}$	0.47	5.67	3.20	1.09	0.11

a, b – mean values followed by the same letters are not significantly different at p = 0.05
a, b – wartości oznaczone tą samą literą nie różnią się między sobą istotnie przy p = 0,05

Table 3. Mean values (x) of morphological traits *Syringa vulgaris* propagated on MS medium with increased $MgSO_4$ content, supplemented with differentiated values of BAP
Tabela 3. Średnie wartości cech morfologicznych lilaka zwyczajnego namnażanego na pożywce MS o zwiększonej zawartości $MgSO_4$ i zróżnicowanej zawartości BAP

BAP mg dm^{-3}	Shoot length Długość pędów cm	Number of leaves Liczba liści	Number of internodes Liczba międzywęźli	Number of axillary shoots Liczba pędów bocznych	Shoot weight Masa pędów g
0.0 (Control – Kontrola)	1.28b	7.86b	3.43b	1.43b	0.05b
0.5	1.69ab	13.14ab	5.93ab	2.50ab	0.21ab
1.0	2.42a	18.30a	8.45a	3.72a	0.38a
3.0	2.03ab	18.89a	8.39a	3.60a	0.30ab
5.0	1.54ab	16.53a	7.63a	3.11ab	0.15ab
$LSD_{0.05}$ – $NIR_{0.05}$	1.00	8.59	3.33	2.04	0.27

a, b – mean values followed by the same letters are not significantly different at p = 0.05
a, b – wartości oznaczone tą samą literą nie różnią się między sobą istotnie przy p = 0,05

The medium with an increased content of $CaCl_2$ proved to be the least advantageous at this stage of studies. The shoot length on this medium was 0.66cm and its weight 0.13 g. Those shoots formed few leaves (5.20) and axillary buds (1.00). The control medium also proved to be unfavourable to the regeneration of lilac. After two weeks the shoots growing on it began to turn yellow, then brown and next they died out.

The results concerning lilac propagation at the second stage showed that the content of cytokinin (BAP) in the medium had a significant effect on its course (tab. 3). The longest shoots (2.42 cm), with the greatest weight (0.38 g) and the highest number of internodes (8.45) and axillary shoots (3.72) were obtained on the medium supplemented with 1.0 $mg \cdot dm^{-3}$ BAP (photo 2). On the remaining media containing BAP insignificant lower results were obtained. The increase of the content of cytokinin in the medium was followed by decreased values of all the analyzed morphological features of the examined shoots. The lack of BAP in the medium had an inhibiting effect on the development of common lilac.

Table 4. Mean values (x) of morphological traits of *Syringa vulgaris* rooted on media with differentiated values of IBA

Tabela 4. Średnie wartości cech morfologicznych lilaka zwyczajnego ukorzenianego na pożywkach o zróżnicowanej zawartości IBA

IBA mg dm^{-3}	Plant height Wysokość roślin cm	Number of leaves Liczba liści	Root length Długość korzeni cm	Root number Liczba korzeni	Plant weight Masa roślin g
0.0 (Control – Kontrola)	1.87a	11.78a	5.37ab	2.89a	0.16ab
0.1	1.77a	8.90b	3.69bc	1.19bc	0.12bc
0.5	1.93a	7.80b	3.33bc	1.35bc	0.09bc
1.0	2.04a	12.21a	4.70ab	2.29ab	0.16ab
3.0	1.47a	7.22b	1.49c	0.56c	0.08c
5.0	2.03a	11.16a	7.17a	3.00a	0.22a
$LSD_{0.05} – NIR_{0,05}$	0.75	3.35	3.19	1.38	0.07

a, b – mean values followed by the same letters are not significantly different at p = 0.05
a, b – wartości oznaczone tą samą literą nie różnią się między sobą istotnie przy p = 0,05

The studies revealed a significant effect of the level of IBA, used in the rooting media, on the number of formed leaves and roots, the length of the longest root and the weight of plants (table 4). The content of IBA in the medium had no significant effect only on the height of the studied plants. The longest (7.17 cm) and the most numerous (3.00) roots were formed by shoots growing on the medium with 5.0 $mg \cdot dm^{-3}$ IBA. Those plants also belonged to the group with the biggest number of leaves. The smallest number and the shortest roots were observed in the case of plants obtained on the medium containing 0.1, 0.5 and 3.0 $mg \cdot dm^{-3}$ IBA.

DISCUSSION

In vitro cultures make use of the plants' ability to regenerate. The effectiveness of this process depends on a number of factors, the most important of which include the genotype and the age of the maternal plant [Bonga 1987, Rakoczy-Trojanowska and Malepszy 1990]. Topophysis observed in woody plants makes it possible to obtain juvenile buds from the trunk base of aged plants. Quraishi and Mishra [1998] compared the course of the regeneration process in *in vitro* cultures of a 15-year-old *Cleistanthus collinus*, depending on the origin of the primary explant. The studies showed that the shoots initiated to grow from explants taken from the trunk base were considerably higher than those initiated from the top parts of the tree crown. On the other hand, at the propagation stage the length and number of the formed axillary buds were bigger in plants initiated to grow from the top part of the crown. Those differences were visible up to the 7^{th} passage. A similar relation was observed by Kaur et al. in *Anogeissus sericea* [1992, quoted after Quraishi and Mishra 1998].

Beck et al. [1998] drew attention to the relation between the age of acacia *Acacia mearnsii* and the effectiveness of disinfection and efficiency of the initiation phase in *in vitro* cultures. The smallest number of infections were obtained by those authors among the explants taken from the youngest acacia trees. The effect of explant disinfection dropped with the age of the maternal plant. It was the lowest when 6-year-old plants were the maternal plants. The effectiveness of disinfection increased again when 8-year--old and older acacia trees were used for initiation.

The time of obtaining primary explants is also of importance. In Skrzypczak's studies [1992], the optimum date for the shoots initiation of common lilac growing in the collection turned out to be the period between the middle of April and the middle of May. When the explants were taken later, it resulted in a big number of infections, strong lignification of the shoots or dormancy of the maternal plants. The best period to obtain primary shoots of common lilac from the collection of the Dendrological Garden at Przelewice was the month of April, which is in accordance with the studies conducted by Skrzypczak [1992].

The course of the process of micropropagation is dependent both on the mineral composition of the medium and the kind and concentration of the applied growth regulators. Bonga [2004] induced somatic embryogenesis in explants from a 42-year-old European larch *Larix decidua.* The formation of somatic embryos was related to the mineral composition of the medium. Gabryszewska [1991] recommends MS medium with increased content of macroelements (1.5 MS), supplemented with 1.0–2.0 $mg \cdot dm^{-3}$ 2iP. Whereas Refouvelet et at. [1998] propagated common lilac on MS with the addition of 5.0 $mg \cdot dm^{-3}$ BAP and 0.01 $mg \cdot dm^{-3}$ NAA. Jankiewicz and Orlikowska [1990] state that the high level of cytokinins in the medium caused rejuvenation of the explants and this effect lasts some time after planting them to the ground. The authors' own studies showed that the high level of cytokinin did not have a stimulating effect on the growth of lilac shoots. The medium containing the low concentration of BAP (1.0 $mg \cdot dm^{-3}$) turned out to be the best. Similar results, both for explants taken from the trunk base and from the tree crown, were obtained by Quraishi and Mishra [1998].

Rooting is a critical phase of tree micropropagation process especially in the case ofcultures derived from explants taken from old tree specimens [Pierik et al. 1997]. Skrzypczak [1992] obtained the best results of rooting 'Madam Florent Stepman' and 'Madame Lemoine' on liquid media (1/4 MS) with perlite supplemented with 0.5–1.0 $mg \cdot dm^{-3}$ IBA. Marks and Simson [2000], in their studies on the rooting of common lilac, considered to be a plant difficult to root, applied a high concentration of IBA auxin (2.0–5.0 $mg \cdot dm^{-3}$ IBA) with the aim of initiating the process of root formation. Gabryszewska [1991] emphasises a stimulating effect of high auxin concentration on the rooting of common lilac. In her experiments she used the media containing 4.0–5.0 $mg \cdot dm^{-3}$ and reduced macroelement content by half in comparison MS. The authors' own studies confirmed the effect of high concentrations of IBA in the process of the rooting of common lilac.

CONCLUSIONS

1. It was found that for an 80-year-old common lilac *Syringa vulgaris* growing in the Dendrological Garden at Przelewice the best date to obtain the shoots for establishing a culture was the month of April.

2. The medium on which the growth was initiated on the highest percentage of explants was MS medium containing 7.5 $mg \cdot dm^{-3}$ cytokinin BAP and 0.02 $mg \cdot dm^{-3}$ auxin NAA.

3. The shoots of common lilac propagated the best on MS medium with a double content of $MgSO_4$, supplemented with 1.0 $mg \cdot dm^{-3}$ cytokinin BAP.

4. The optimum medium for the rooting of lilac shoots turned out to be MS medium with a ¼ content of macro- and microelements, supplemented with 5.0 $mg \cdot dm^{-3}$ auxin IBA.

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MIKROROZMNAŻANIE STAREGO OKAZU LILAKA ZWYCZAJNEGO (*Syringa vulgaris* L.) Z OGRODU DENDROLOGICZNEGO W PRZELEWICACH

Streszczenie. W badaniach nad rozmnażaniem roślin w kulturach *in vitro* podkreśla się wpływ wieku rośliny matecznej na powodzenie procesu regeneracji. Efektywność inicjacji kultur ze starych okazów drzew jest niska. Celem badań była próba regeneracji w kulturach *in vitro* lilaka zwyczajnego *Syringa vulgaris* z okazu mającego 80 lat. Optymalnym terminem pozyskiwania eksplantatów z kolekcji był kwiecień. Najlepsze wyniki na etapie inicjacji otrzymano na pożywce MS uzupełnionej 7,5 mg·dm^{-3} BAP i 0,02 mg·dm^{-3} NAA. Najwyższy współczynnik rozmnożenia roślin otrzymano na pożywce MS z podwojoną ilością $MgSO_4$ i uzupełnionej 1,0 mg·dm^{-3} BAP. Nieistotnie niższe wyniki otrzymano na pozostałych pożywkach zawierających BAP w swoim składzie. Najlepiej rozwinięty system korzeniowy obserwowano u roślin ukorzenianych na pożywce zawierającej ¼ makro- i mikroelementów pożywki MS, uzupełnionej 5,0 mg·dm^{-3} IBA.

Słowa kluczowe: odmładzanie, mikrorozmnażanie, lilak zwyczajny, regulatory wzrostu

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