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IN VITRO PROPAGATION OF *SYRINGA VULGARIS* L.

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Abstract

Actively growing root suckers from 4-year old shrubs of seed origin were collected in the beginning of spring (April 20, 2012) from open field grown shrubs. Softwood, apical and nodal explants (3–4 cm) were used as initial explants. For the induction of axillary shoots formation, the explants were cultured on Murashige and Skoog (1962) basal medium, supplemented with 0, 0.5 mg·l^{-1} or 1.0 mg·l^{-1} BAP. The formed axillary shoots were cultivated on PGRs free MS medium, or supplemented with BAP (0.5, 1.0 or 5.0 mg·l^{-1}) or zeatin (0.5 or 1.0 mg·l^{-1} + 0.1 mg·l^{-1} IBA) for multiplication. For evaluation of the effect of subculture on the multiplication coefficient, the formed axillary shoots (≈ 30 mm) were transferred 3 times on same but fresh medium and cultured for 14 or 21 days. The subcultures did not affected the multiplication rate and maximal number of axillary shoots was reached when the MS medium was supplemented with 5.0 mg·l^{-1} BAP (3.8 ± 0.4). The following variants of an inductive, half-strength MS medium were used for the rooting: free of auxin (variant 1), supplemented with 1.0 mg·l^{-1} and 5.0 mg·l^{-1} IBA (variant 2), supplemented with 5.0 mg·l^{-1} IBA for 48, 72, and 168 h (variant 3), and supplemented with 7.5 mg·l^{-1} IBA for 24, 48, and 72 h (variant 4) and then on half-strength MS medium without auxin. For comparison of the results, the shoots from variant 1 and 2 were rooted on the same medium without transfer to an expressive medium. All pulse treatments with high concentration of IBA (7.5 mg·l^{-1}) improved significantly the rooting (between 76.6 ± 8.0 % and 82.2 ± 4.4 %) in comparison with all other treatments. These results demonstrated that the type of initial plant material, the balance between the concentration of the used auxin and the duration of inductive phase are critical for the rooting rate and quality of the root system.

Key words: auxin pulse treatment, common lilac, rejuvenation, root suckers.

Introduction

Common lilac (*Syringa vulgaris* L.) is famous for its great diversity of ornamental cultivars and hybrids (Krüssmann 1986, Dirr and Heuser 1987, Dirr 1998, Fiala 2008, Rudolf 2008) making it very suitable for use in urban areas. However, cloning of ornamental genotypes in a generative way is impossible because of the heterozygosity of this species (Fiala 2008). That is why the seed progeny is used for selection and hybridization or for production of rootstocks for grafting (Dirr and Heuser 1987). *Syringa vulgaris* cuttings are generally considered difficult to root (Waldenmaier and Bünemann 1991, Howard and Ridout 1992, Howard 1993, Tyatyushkina 2007, Patience and Alderson 1984) and vary between the cultivars (Schmidt 1978, Bassuk et al. 1984, Tyatyushkina 2007), their age

(Waldenmaier and Bünemann 1991), period of cuttings collection (Schmidt 1978), different part of the shoots (Bojarczuk and Jankiewicz 1975, Schmidt 1978). Also, the rooting has been found to increase considerably only during the phase of full bloom (Schmidt 1978, Bojarczuk 1975, 1978, 1979, Dirr and Heuser 1987, Dirr 1998, Hartman et al. 2002, Cameron et al. 2003). For this reason the trade production of its cultivars is performed by grafting on seedlings. However, the production of large quantities of grafts is limited by the season, period duration for rootstock production, and the success depends on the method of grafting (Krüssmann 1986, Dirr and Heuser 1987, Dirr 1998, Fiala 2008). Also, the grafting is labour-consuming and needs large areas.

The success of *in vitro* cloning of woody plants depends on the age of stock plant, explants used, and culture conditions. However, *in vitro* propagation could allow large-scale production of plants and may provide rejuvenated plants with high rooting capacity (Bonga and von Aderkas 1992, Hackett and Murray 1993, Hartmann et al. 2002). For this reason some researchers have focused on *in vitro* propagation of the species and cultivars of genus *Syringa* (Hilderbrandt and Harney 1983, Pierik et al. 1988, Gabryszewska 1989, Waldenmaier and Bünemann 1991, Pinker et al. 1993, Refouvelet et al. 1998, Liu et al. 2013).

Nesterowicz et al. (2006) and Skrzypczak (1992) considered April as optimal period for cuttings collection. Tomsone et al. (2007) have indicated the beginning of flowering as the best period. It has been reported that *in vitro* culture of common lilac could be established by axillary buds (Hildebrandt and Harney 1983, Waldenmaier and Bünemann 1991), nodal explants (Einest and Alexander III 1985, Pierik and Steegmans 1985, Pierik et al. 1988, Tomsone et al. 2007), lateral buds with a small part of the stem (Gabryszewska 1989), and shoot tips (Einest and Alexander III 1985). It was shown that *in vitro* propagation depends on the cultivar (Pinker at al. 1993, Tomsone et al. 2007) and age of the stock plant (Pierik et al. 1988). However, some of the publications did not report for the age of stock plant (Einest and Alexander III 1985, Tomsone et al. 2007) and the type of induced shoots (Tomsone et al. 2007).

The aim of this work was to study the effect of the type of explants, their disinfection, and the effect of type, concentration, and duration of application of plant growth regulators (PGRs) for *in vitro* multiplication and rooting of common lilac.

Material and Methods

Plant material and disinfection

Four-year old shrubs of seeds origin, growing in the nursery “Sequoia” Ltd. in Sofia were used as stock plants. It was earlier demonstrated that the highest percentage of bud development can be induced when the donor plant is in the end of physiological dormancy or begins active growth (Iliev 1996, Nesterowicz et al. 2006). Therefore, actively growing root suckers were collected in the beginning of spring (April 20, 2012) from open field grown shrubs (Fig. 1).

Softwood, apical and nodal explants (3–4 cm) were used as initial explants. All explants were rinsed for 5 min under running tap water with 2–3 drops of detergent. Then they were disinfected for 2 min

with 0.1 % solution of $HgCl_2$. After being rinsed 3 times in sterile distilled water, the ends (≈ 2 mm) of explants were aseptically removed.

Culture establishment

For the induction of axillary shoots formation, the explants were cultured on Murashige and Skoog (1962) basal medium, supplemented with 0.5 mg·l^{-1} or 1.0 mg·l^{-1} 6-benzylaminopurine (BAP). The results were compared with control (plant growth regulators (PGRs) free medium). Each treatment contained 20 explants and was repeated 3 times. The percentage of developed explants was calculated from the aseptic explants, after 14 days.

Multiplication of the axillary shoots

The formed axillary shoots were cultivated on MS medium, supplemented with BAP (0.5 or 1.0 mg·l^{-1}) or (6-[4-Hydrooxy-3-methil-but-2-enylamino]purine (zeatin, 0.5 or 1.0 mg·l^{-1} + 0.1 mg·l^{-1} IBA) for multiplication. The results were compared with control (PGRs free medium). For evaluation of the effect of subculture on the multiplication coefficient, the induced axillary shoots (≈ 30 mm) were transferred 3 times on same but fresh medium and cultured for 14 or 21 days. Each treatment contained three replicates and for each of them 10 explants were used. The number and length (mm) of the formed new axillary shoots were calculated after each subculture.

Rooting of the axillary shoots

After the multiplication stage, shoots that were longer than 20 mm were transferred to

Fig. 1. Actively growing root suckers.

the following variants of an inductive, half-strength MS rooting medium: free of auxin (variant 1), supplemented with 1.0 mg·l^{-1} and 5.0 mg·l^{-1} IBA (variant 2), supplemented with 5.0 mg·l^{-1} IBA for 48, 72, and 168 h (variant 3), and supplemented with 7.5 mg·l^{-1} IBA for 24, 48, and 72 h (variant 4) and then on an expressive rooting medium (half-strength MS medium without auxins). For comparison of the results, the shoots from variant 1 and 2 were rooted on the same medium without transfer to an expressive medium. Three replications, each containing 15 explants, were cultured in each variant. The percentage of rooted shoots was determined weekly in duration of 5 weeks and the number and length of induced roots of first and second order were determined after 5 weeks. The length of second order roots was determined as a mean of the rooted shoots.

Culture conditions and statistical analysis

All variants of the medium contained 50 mg·l^{-1} NaFeEDTA and 8.0 g·1^{-1} agar. The pH was adjusted to 5.5 before autoclaving at 118 kPa and temperature 120 ºC for 20 min. The cultures were

grown in cultivation chamber at 23 ± 1 ºC with 14 h cool white fluorescent light at photon flux density 40 μmol·m^{-2}·s^{-1}, daily.

The results were analysed by One-Way ANOVA followed by a post hoc LSD test at $p < 0.05$, using SPSS 20.0 for Windows. Percentage values were transformed using arcsine square root $(\sqrt{P})$ (Compton 1994) to normalize error distribution prior variance analysis.

Results

Disinfection of the initial explants

The results from disinfection demonstrated that nodal explants contained more contaminations than apical explants. However, they are more resistant to the action of $HgCl_2$ i.e. less of them were damaged from the treatment and significantly more aseptic and viable explants were obtained (Table 1).

Culture establishment

Callus induction was not noticed on the explants, cultured on cytokinin free medium (control). The first signs of callus formation were detected on the base of the explants 7 days after culture establishment on the variants of the medium, containing BAP and in the end of the experiment its amount was small. Simultaneously, axillary shoot formation was observed on the explants. The level of elongated shoots was higher in the control and low concentration of BAP and after 14 days reached 95.8 ± 4.2 % and 96.7 ± 3.3 %, resp., but there was not found any statistical difference in comparison with the other treatments. It was found that the concentration of BAP does not affect the percentage of explants with bud dormancy breaking and number of the formed shoots. However, the lower concentration of BAP (0.5 mg·l^{-1}) had statistically significant effect on the rate of growing buds (55.0 ± 5.0), but the shoots were statistically longer on cytokinin free medium (10.0 ± 1.5 mm) (Table 2).

Multiplication of the axillary shoots

After 14 days of cultivation and the first subculture there was no significant difference in the rate of formed axillary shoots between all treatments. After the second subculture the rate of axillary shoots increased when the explants were cultured on highest concentrations of BAP (1.0 and 5.0 mg·l^{-1}) but remained statistically identical after the third subculture. The serial number of subculture did not affect significantly the number of formed axillary shoots when the explants were cultured on medium supplemented with zeatin. Also, the equal concentrations

Table 1. Disinfection of the initial explants.

Type of the explants	Contaminated explants, %	Damaged explants, %	Aseptic explants, %
Softwood apical explants	32.3 ± 4.8 a	35.9 ± 5.0 b	31.0 ± 7.1 a
Softwood nodal explants	48.6 ± 5.8 b	8.0 ± 4.5 a	43.4 ± 8.7 b

Note: The means (M) ± standard error (SE) within a column followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$.

of BAP and zeatin induced statistically identical effect (Table 3).

After 21 days of cultivation and the first subculture statistical difference between the control and lowest concentration of BAP (0.5 mg·l^{-1}) was not observed. The multiplication rate increased with the increasing of the concentrations of BAP. Maximal number of shoots was reached when the medium was supplemented with 5.0 mg·l^{-1} BAP (3.8 ± 0.4). Similar tendency was observed after the second and third subculture (Fig. 2). The zeatin was more effective after first and second subculture. However, zeatin was more effective than BAP when these cytokinins were used in concentration of 1.0 mg·l^{-1} (Table 3).

Table 2. Effect of BAP concentration on the formation of axillary shoots.

Concentration of BAP, mg·l^{-1}	Explants with bud dormancy breaking, %	Growing buds, %	Number of the formed shoots	Length of the shoots, mm
Control	95.8 ± 4.2 a	45.4 ± 4.4 a	2.1 ± 0.3 a	10.0 ± 1.5 b
0.5	96.7 ± 3.3 a	55.0 ± 5.0 b	2.5 ± 0.3 a	6.3 ± 1.0 a
1.0	80.6 ± 10.0 a	42.5 ± 3.2 a	1.9 ± 0.2 a	6.3 ± 1.0 a

Note: The means (M) ± standard error (SE) within a column followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$

However, it should be indicated that cytokinin treatments resulted in curling of some leaves in all shoots (Fig. 3).

The number of the formed shoots depended on the type of cytokinin (F = 8.629, $p < 0.05$), its concentration (F = 90.660, $p < 0.05$), and duration of cultivation (F = 69.886, $p < 0.05$). However, the results indicate that prolonged subculturing of the axillary shoots could not lead to increasing of their multiplication rate (F = 2.185, $p < 0.05$) (Table 4).

Table 3. Factors affecting the number of formed axillary shoots.

Duration of the inductive phase (days)	Type and concentration of the PGRs (mg·l^{-1})	Subculture I	Subculture II	Subculture III
	Control (PGRs-free)	1.1 ± 0.1 a A	1.3 ± 0.1 abc A	1.2 ± 0.1 a A
	0.5 BAP	1.2 ± 0.1 abc A	1.1 ± 0.1 ac A	1.2 ± 0.1 ac A
14 days	1.0 BAP	1.6 ± 0.1 bc A	2.1 ± 0.2 de B	1.9 ± 0.1 b–f AB
	5.0 BAP	1.5 ± 0.2 abc A	2.6 ± 0.4 ef B	2.1 ± 0.2 def AB
	0.5 zeatin + 0.1 IBA	1.5 ± 0.2 a–d A	2.0 ± 0.3 c–f A	2.0 ± 0.3 c–f A
	1.0 zeatin + 0.1 IBA	1.4 ± 0.3 abc A	1.6 ± 0.2 a–d A	1.6 ± 0.2 a–f A
	Control (PGRs-free)	1.3 ± 0.1 ab A	1.2 ± 0.1 ac A	1.4 ± 0.1 abc A
	0.5 BAP	1.4 ± 0.1 abc A	1.7 ± 0.1 bcd A	2.6 ± 0.1 ef B
21 days	1.0 BAP	1.8 ± 0.1 c A	2.0 ± 0.2 de A	1.6 ± 0.1 a–d A
	5.0 BAP	3.8 ± 0.4 f A	3.5 ± 0.2 g A	3.5 ± 0.2 g A
	0.5 zeatin + 0.1 IBA	2.7 ± 0.4 e B	2.1 ± 0.2 de AB	2.0 ± 0.2 df A
	1.0 zeatin + 0.1 IBA	2.4 ± 0.2 de AB	2.8 ± 0.2 f B	2.1 ± 0.1 f A

Note: The means (M) ± standard error (SE) within a column followed by the same small letter and in the rows followed by the same capital letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$.

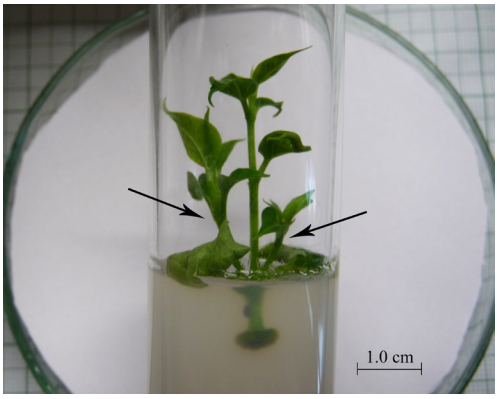

Fig. 2. Axillary shoot formation during the multiplication stage.

After 14 days of cultivation and the first subculture the length of shoots was higher in shoots on PGRs free medium (control) but after 21 days of cultivation there were no differences in the shoot length between the used treatments. However, after the second and third subculture, independently of the duration of cultivation, highest length of the shoots was observed on medium supplemented with 1.0 mg·l^{-1} BAP (12.0 ± 1.0 mm and 14.6 ± 1.1 mm; 17.5 ± 1.3 mm and 24.8 ± 2.6 mm, resp.) and the increased concentration of BAP induced depressive effect. The comparison of the equal concentrations of used cytokinins demonstrated that zeatin stimulated statistically significantly the elongation of the shoots after the third subculture, only. In comparison with the lower concentration of zeatin, the higher one (1.0 mg·l^{-1}) had stimulating effect after the third subculture, only (Table 5). Key factor for the elongation of the shoots was duration of cultivation (F = 138.862, p < 0.05). The length of the shoots depended on all other investigated factors (F = 5.953, 24.536, 20.999 resp.; p < 0.05) and the interaction between concentration of cytokinin and number of

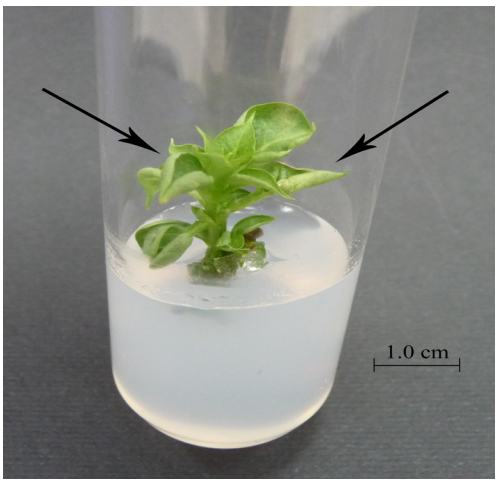

Fig. 3. Shoots with curling leaves.

Table 4. Significance of the studied factors and their combinations on the mean number of the axillary shoots estimated by a post hoc LSD test.

Factors	*F*	Level of significance
TC	8.629	0.003
C	90.660	0.000
D	69.886	0.000
S	2.185	0.113
TC × C	3.782	0.023
TC × D	2.036	0.154
TC × S	1.137	0.321
C × D	19.152	0.000
C × S	1.336	0.238
D × S	3.117	0.045
TC × C × D	6.230	0.002
TC × C × S	0.646	0.629
TC × D × S	2.504	0.082
C × D × S	2.025	0.059
TC × C × D × S	1.815	0.123

Note: R Squared = 0.345 (Adjusted R Squared = 0.331), p < 0.05.
Legend: TC = Cytokinin, C = Concentration of the cytokinin, D = Duration of cultivation, S = Number of the subcultures.

Table 5. Factors affecting the length (mm) of formed axillary shoots.

Duration of the inductive phase, days	Type and concentration of the PGRs, mg·l^{-1}	Subculture I	Subculture II	Subculture III
14 days	Control (PGRs-free)	10.2 ± 0.9 c A	10.0 ± 0.9 bcd A	9.1 ± 0.7 a A
	0.5 BAP	4.9 ± 0.5 ab A	5.2 ± 0.5 ab A	5.0 ± 0.5 a A
	1.0 BAP	8.7 ± 0.6 abc A	12.0 ± 1.0 d–g B	14.6 ± 1.1 d B
	5.0 BAP	4.3 ± 0.4 a A	6.4 ± 0.7 a A	6.3 ± 0.6 a A
	0.5 zeatin + 0.1 IBA	6.1 ± 2.2 abc A	6.6 ± 1.1 abc A	6.0 ± 1.1 a A
	1.0 zeatin + 0.1 IBA	6.1 ± 1.2 abc A	9.3 ± 1.2 a–g A	9.2 ± 1.5 ac A
21 days	Control (PGRs-free)	10.6 ± 1.1 c A	13.9 ± 1.1 g A	18.5 ± 1.7 e B
	0.5 BAP	11.0 ± 1.0 c A	13.7 ± 1.1 efg A	13.3 ± 1.0 cd A
	1.0 BAP	10.5 ± 0.8 c A	17.5 ± 1.3 h B	24.8 ± 2.6 f C
	5.0 BAP	10.1 ± 0.6 c A	13.7 ± 0.6 fg B	13.1 ± 0.5 bcd B
	0.5 zeatin + 0.1 IBA	9.7 ± 0.7 bc A	9.2 ± 0.7 a–d A	13.1 ± 0.9 bcd B
	1.0 zeatin + 0.1 IBA	11.1 ± 1.4 c A	10.9 ± 0.9 c–g A	20.9 ± 1.3 e B

Note: The means (M) ± standard error (SE) within a column followed by the same small letter and in the rows followed by the same capital letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$.

subcultures, and duration of cultivation and number of subculture ($F = 4.605$ and 12.412, resp; $p < 0.05$) (Table 3 and 6).

Rooting of the axillary shoots

In the absence of IBA (control) the rooting rate was low and the emergence of new roots progressed slowly (Table 7). Very small amount of callus was observed on the base of the shoots in the end of the rooting period cultured on all variants of the medium, supplemented with IBA (Fig. 4).

On medium without transfer of the shoots, the beginning of adventitious root formation was observed after 21 days. Rooting of the shoots was observed after 28 days in all rooting variants of the medium. Statistical difference in the rooting rate after longer period of cultivation (35 days) was found on PGRs free medium, only. The addition of IBA increased rooting response up to 55.6 ± 12.4 %, but statistical significant differences were not observed between investigated concentrations of IBA (Table 7).

The beginning of rooting process for all pulse treatments was observed earlier in comparison with experiments without pulse treatment i.e. after 7 days when the shoots were pulse treated with 7.5 mg·l^{-1} IBA for 72 h and after 14 days in all other durations of inductive phase. The same tendency was found about the finishing of rooting process in comparison with the experiments without transfer of the shoots. All pulse treatments with high concentra-

Table 6. Significance of the studied factors and their combinations on the mean length of axillary shoots estimated by a post hoc LSD test.

Factors	*F*	Level of significance
TC	5.953	0.015
C	24.536	0.000
D	138.862	0.000
S	20.999	0.000
TC × C	5.305	0.005
TC × D	0.891	0.345
TC × S	0.855	0.425
C × D	0.766	0.513
C × S	4.829	0.000
D × S	12.412	0.000
TC × C × D	0.912	0.402
TC × C × S	0.746	0.560
TC × D × S	1.271	0.281
C × D × S	1.804	0.094
TC × C × D × S	0.515	0.725

Note: *R* Squared = 0.135 (Adjusted *R* Squared = 0.124), $p < 0.05$.
Legend: TC = Cytokinin, C = Concentration of the cytokinin, D = Duration of cultivation, S = Number of the subculture.

tion of IBA (7.5 mg·l^{-1}) improved significantly the rooting (between 76.6 ± 8.0 % and 82.2 ± 4.4 %) in comparison with all pulse treatments with lower concentration of IBA (Table 7). However, our results showed that there is not statistical difference in the rooting rate between pulse treatments with 5.0 mg·l^{-1} IBA or between pulse treatments with 7.5 mg·l^{-1} IBA. Also, the roots in some shoots appeared above the callus formed (Fig. 5) and in others shoots through the callus (Fig. 6).

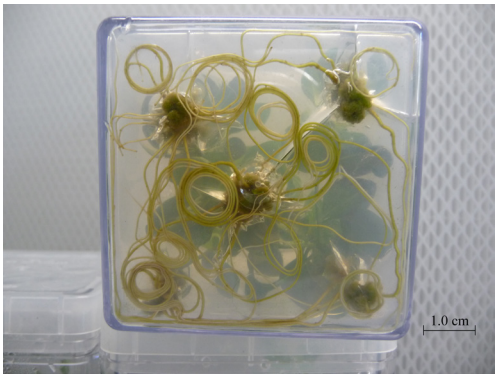

Fig. 4. Callus formation on the basal part of the shoots in the end of rooting experiments.

Our results demonstrated that the percentage of rooting depended on all investigated factors (Table 8).

The number of first order roots was higher after the cultivation of the shoots for 48 and 72 h on inductive medium, supplemented with 7.5 mg·l^{-1} IBA, and after cultivation without transfer on medium, containing 5.0 mg·l^{-1} IBA (2.8 ± 0.2, 3.9 ± 0.5 and 3.9 ± 0.5, resp.). Statistical difference between them was not found. The percentage of shoots, originated from PGRs free medium formed significantly more roots of second order (40.8 ± 12.3 %). However, their number was not strongly affected by the different treatments. The first order roots reached highest length after the cultivation of shoots for 48 and 72 h on inductive medium, supplemented with 7.5 mg·l^{-1} IBA (48.4 ± 2.0 mm and 45.4 ± 1.4 mm, resp.). Similar tendency was observed for the second order roots. However, their length was significantly lower (Table 9).

Discussion

It is well known that *in vitro* propagation of woody plants is strongly affected by the age of stock plant (Bonga 1982, Bonga and von Aderkas 1992, Hartmann et al. 2002). One of the most juvenile plant material are the root suckers (Bonga 1982, Hackett 1985, Waldenmaier and Bünemann 1991, Bonga and von Aderkas 1992, Hackett and Murray 1993,

Table 7. Factors affecting the dynamics of rooting process, %.

Concentration of IBA, mg·l^{-1}	Duration of the inductive phase, h	7th day	14th day	21st day	28th day	35th day
Control (PGRs-free)	Without transfer	0.0 ± 0.0 a A	0.0 ± 0.0 a A	0.0 ± 0.0 a A	2.1 ± 2.1 a A	22.2 ± 11.8 a B
1.0		0.0 ± 0.0 a A	0.0 ± 0.0 a A	8.9 ± 4.4 ab AB	20.0 ± 3.9 ab BC	35.6 ± 11.1 ab C
5.0		0.0 ± 0.0 a A	0.0 ± 0.0 a A	22.2 ± 5.9 bcd B	42.2 ± 5.9 b C	55.6 ± 12.4 bcd C
5.0 (from control)		0.0 ± 0.0 a A	0.0 ± 0.0 a A	15.6 ± 2.2 bc A	44.4 ± 5.9 b B	51.1 ± 9.7 abc B
5.0	48	0.0 ± 0.0 ab A	11.1 ± 4.4 ab AB	13.3 ± 6.7 ab AB	20.0 ± 6.7 ab B	24.4 ± 8.0 a B
5.0	72	0.0 ± 0.0 a A	24.4 ± 2.2 b B	28.9 ± 2.2 cd B	31.1 ± 2.2 ab B	37.8 ± 5.9 ab B
5.0	168	0.0 ± 0.0 a A	8.9 ± 2.2 a A	33.3 ± 6.7d B	42.2 ± 5.9 b BC	51.1 ± 5.9 abc C
5.0 (from control)	72	0.0 ± 0.0 a A	8.9 ± 2.2 a AB	20.0 ± 7.7 bcd BC	35.6 ± 14.6 b C	57.8 ± 12.4 b–e D
7.5	24	0.0 ± 0.0 a A	40.0 ± 7.7 c B	57.8 ± 2.2 e C	73.3 ± 7.7 c CD	76.6 ± 8.0 cde D
7.5	48	0.0 ± 0.0 a A	51.1 ± 9.7 cd B	75.6 ± 2.2 f C	80.0 ± 3.9 c C	82.2 ± 4.4 e C
7.5	72	4.4 ± 4.4 b A	55.6 ± 5.9 d B	68.9 ± 8.0 fe BC	75.6 ± 11.8 c C	77.8 ± 9.7 de C

Note: The means (M) ± standard error (SE) within a column followed by the same small letter and in the rows followed by the same capital letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$.

Hartmann et al. 2002). To date, *in vitro* propagation of common lilac has been reported by using explants from mature or young stock plants (Pierik et al. 1988). It was found that rejuvenation by frequently subculturing of the shoots would be necessary for induction of high rate of multiplication and rooting of the axillary shoots. The number of subcultures depends on the age and genotype of stock plant (Pierik et al. 1988). It is known that one of the limiting factors for the autovegetative propagation of woody plants is physiological status of the stock plants i.e. the season of explants collection. However, we found that root suckers, collected in the beginning of spring in active growth are suitable initial plant material for the establishment of *in vitro* culture. Our results indicated that subculture is not obligatory for the rejuvenation and obtaining of high multiplication rate *in vitro*. Also, the results demonstrated that the subcultures did not decrease the multiplication rate. It was found that BAP in concentration of 1.0 mg·l^{-1} is the best for the induction and elongation of axillary shoots (Jankiewicz and Orlikowska 1990, Nesterowicz et al. 2006). However, our results are in agreement with the findings of Pierik et al. (1988), Gabryszewska (1989), and Refouvelt et al. (1998) that the formation and elongation of axillary shoots requires higher level of BAP.

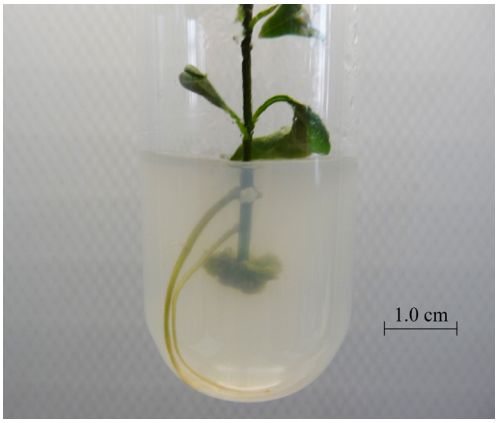

Fig. 5. Roots appeared above the callus formed.

Fig. 6. Roots appeared through the callus.

Common lilac was considered as difficult-to-root species (Waldenmaier and Bünemann 1991, Marks and Simpson 2000, Ford et al. 2002). It was found that rooting ability of common lilac *in vitro* decreased with the increasing of stock plant age (Waldenmaier and Bünemann 1991). The rooting of the shoots depends on their rejuvenation by subculturing (Pierik 1990), their length, and culture origin (Hildebrandt and Harney 1983, Marks and Myers 1994, Marks and Simpson 2000). Our results demonstrated that subculturing of the shoots during the multiplication stage is not necessary if root suckers are used as initial plant material.

Table 8. Significance of the studied factors and their combinations on the dynamic of rooting estimated by a post hoc LSD test.

Factors	*F*	Level of significance
C	103.521	0.000
DIP	4.426	0.001
DR	93.786	0.000
C × DIP	6.780	0.009
C × DR	8.046	0.000
DIP × DR	2.107	0.006
C × DIP × DR	1.659	0.157

Note: *R* Squared = 0.347 (Adjusted *R* Squared = 0.335), $p < 0.05$.
Legend: DR = Duration of rooting, C = Concentration of IBA, DIP = Duration of inductive phase.

It is well known that the type and concentration of auxin has a central role in the induction of adventitious roots (De Klerk 2001, De Klerk et al. 1997, 1999, Kurepin et al. 2011). These factors could lead to variation in the percent of rooting and asynchrony of root expression in Common lilac (Wainwright 1987, Marks and Simpson 2000). Also, it was demonstrated that auxin pulse treatment is critical for the rooting of plants *in vitro* and depends on the duration of inductive phase (De Klerk 1996, 2001, 2002, De Klerk et al. 1999, Mitras et al. 2009, Dancheva 2012). After the inductive phase, once the cells have been determined to root formation, auxin is no longer required for the rooting of *Syringa vulgaris* shoots (Marks and Simpson 2000). The effect of IBA and its concentration is documented in a few studies (Waldenmaier and Bünemann 1991, Nesterowitz et al. 2006), but in some of them the percentage of rooted shoots is not indicated (Nesterowitz et al. 2006). Our results showed that rooting response and number of roots depend on the duration of cultivation on inductive medium. The cultivation of shoots to a root induction medium for shorter period improved the rooting and number of the formed roots. These results demonstrated that the balance between the concentration of the used auxin and the duration of inductive phase are critical for the rooting rate and quality of the root system.

Table 9. Factors affecting the quality of the root system.

Concentration of IBA, mg·l^{-1}	Duration of the nductive phase, h	First order roots		Second order roots		
		Number	Length, mm	Shoots with second order roots, %	Number	Length, mm
Control	Without transfer	1.6 ± 0.3 a	23.4 ± 4.4 abc	0.0 ± 0.0 a	0.0 ± 0.0 ab	0.0 ± 0.0 a
1.0		2.3 ± 0.4 a	26.8 ± 3.2 abc	4.8 ± 4.8 a	0.3 ± 0.3 ab	0.8 ± 0.4 a
5.0		3.9 ± 0.5 bc	20.0 ± 1.3 a	8.5 ± 4.3 a	0.1 ± 0.1 a	0.2 ± 0.24 a
5.0 (from control)		2.3 ± 0.5 a	33.7 ± 2.1 c	40.8 ± 12.3 c	1.2 ± 0.4 bc	5.4 ± 0.9 bc
5.0	48	1.4 ± 0.2 a	19.9 ± 5.1 ab	11.1 ± 11.1 ab	0.6 ± 0.5 abc	3.1 ± 1.2 ab
5.0	72	2.3 ± 0.5 a	21.7 ± 1.8 ab	17.9 ± 9.0 ab	0.8 ± 0.6 abc	2.4 ± 0.7 ab
5.0	168	2.3 ± 0.4 a	43.6 ± 3.2 de	9.7 ± 5.0 a	0.1 ± 0.1 a	1.3 ± 0.8 a
5.0 (from control)	72	2.0 ± 0.2 a	30.2 ± 2.5 bc	32.4 ± 9.0 bc	1.3 ± 0.4 c	7.8 ± 1.1 c
7.5	24	2.7 ± 0.3 ab	41.4 ± 1.9 d	14.6 ± 7.3 ab	0.4 ± 0.2 ab	3.6 ± 1.0 ab
7.5	48	2.8 ± 0.2 abc	48.4 ± 2.0 e	19.3 ± 4.0 abc	0.7 ± 0.3 abc	4.9 ± 0.9 b
7.5	72	3.9 ± 0.5 c	45.4 ± 1.4 de	18.8 ± 5.1 ab	0.8 ± 0.3 abc	5.3 ± 0.9 bc

Note: The means (M) ± standard error (SE) within a column followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$.

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