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The effect of plant growth regulators and sucrose on the micropropagation of common lilac (*Syringa vulgaris* L.)

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Abstract: *The effect of plant growth regulators and sucrose on the micropropagation of common lilac (*Syringa vulgaris *L.).* Common lilac (*Syringa vulgaris* L.) is an attractive plant with colourful and fragrant flowers, often used in urban areas. Although the main method of propagation of the shrub is budding or grafting, it is also propagated by tissue culture. The aim of the study was to determine the effect of the presence and concentration of IBA or NAA on rhizogenesis in 'Katherine Havemeyer' and 'Sensation' lilac microcuttings. Shoot proliferation was dependent on the type and concentration of cytokinin in the medium. For both cultivars the highest shoot numbers were obtained on medium supplemented with *meta*-topolin: 5.0 mg·dm^{-3} for 'Katherine Havemeyer' and 2.5–7.5 mg·dm^{-3} for 'Sensation'. The addition of 30 g·dm^{-3}of sucrose to the medium improved regeneration and stimulated shoot growth in the cultivar 'Katherine Havemeyer'. The largest number of roots was obtained on medium supplemented with 1.0 mg·dm^{-3} IBA for 'Katherine Havemeyer' and with 0.5–2.0 mg·dm^{-3} IBA for 'Sensation'. The use of NAA resulted in dieback of microcuttings.

Key words: cytokinins**,** IBA, NAA, microcuttings, rhizogenesis, shoot regeneration

INTRODUCTION

The genus *Syringa*, belonging to the olive family (*Oleaceae*), includes about 30 species originating from Asia and South Eastern Europe. The most common species widely planted in parks and gardens is the common lilac (*Syringa vulgaris* L.) – a shrub or small tree which can grow up to 7 m high and 3–4 m wide. Lilacs are suitable for formed hedges since they tolerate pruning. Due to the numerous root suckers (secondary shoots) the shrub expands rapidly, and hence it can be successfully used for the reforestation of slopes, roadsides and banks of reservoirs. Lilacs are also cultivated for cut flowers, because they can easily be forced [Latocha 2006].

Lilac cultivars are generally propagated vegetatively to maintain genetic stability. In practice, nurserymen reproduce the common lilac only by budding or grafting. However, the production of large quantities of grafts is limited by the season and the long period needed for rootstock production, and success of propagation depends on the method of grafting. Therefore, *in vitro* propagation is highly useful for rapid multiplication of this species. In recent years, tissue cultures have been used for the propagation of lilac [Concioiu et al. 2012, 2013, Lyubomirova and Iliev 2013]. The regeneration rate of shoots is a crucial step for the micropropagation of lilac in com-

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mercial production. In lilac micropropagation, axillary branchings [Gabryszewska 1989, Cui et al. 2009] and/or single-node explants [Welander 1987, Gabryszewska and Warabieda 1992, Charlebois and Richter 2004] might be used. Axillary buds are activated by cytokinins, which play an important role in both methods [Charlebois and Richter 2004, Nesterowicz et al. 2006]. 6-benzylaminopurine (BA) and 6-(γ,γ-dime thylallylamino)purine (2iP) have been the most frequently used cytokinins in lilac regeneration by axillary branching. They increase the multiplication rate, but also reduce the rooting potential of microcuttings [Gabryszewska 1989]. The use of BA analogues, such as [6-(3-hyd roxybenzylamino)purine] (*meta*-topolin, *m*T), could be an alternative way to avoid such side effects.

Sucrose is the main source of carbon for *in vitro* cultures of many plants, including lilacs. Plant cells and tissues in a culture medium lack autotrophic ability and therefore need an external carbon source for energy. The addition of an external sucrose dosage to the medium enhances cell proliferation and shoot regeneration. The optimal sucrose concentration in a medium should be sufficient to satisfy the basic energy requirements for cell differentiation without imposing any negative osmotic effects on shoot formation. This indicates that sucrose acts not only as a carbon energy source in a medium, but also as an osmoticum [Nowak et al. 2004] and that different sucrose concentrations are one of the factors controlling the regeneration and growth of shoots [Gibson 2000]. A number of studies indicate the positive impact of 30 $g \cdot dm^{-3}$ sucrose on the plant culture, as it induces the maximum number of shoots as well as being beneficial for shoot elongation in *S. vulgaris* [Refouvelet et al. 1998, Gabryszewska 2011].

Factors affecting adventitious root formation include the type and concentration of natural or synthetic auxin in the rooting medium [De Klerk 2002]. Use of auxin is one of the most common and effective ways to enhance rooting of plants. For the rooting of lilac, various auxins have been used, in particular indole-3-butric acid (IBA) [Gabryszewska and Warabieda 1992, Nesterowicz et al. 2006, Oprea and Concioiu 2012, Lyubomirova and Iliev 2013, Parvanova et al. 2015], 1-naphthaleneacetic acid (NAA) [Oprea and Concioiu 2012] and indoleacetic acid (IAA) [Charlebois and Richter 2004], although the type and concentration requirements of each of these varied between cultivars.

The aim of the present experiment was to investigate the influence of various types and levels of cytokinins and sucrose concentrations on the proliferation rate and growth of shoots in *S. vulgaris*, *in vitro*. The rooting potential of regenerated microshoots was also evaluated.

MATERIAL AND METHODS

Plant material and culture conditions

The source plants of common lilac (*Syringa vulgaris* L.) 'Katherine Havemeyer' and 'Sensation' for tissue culture were originally collected in February/ /March from three-year-old shrubs growing in a nursery. For establishment of

a culture, young vegetative shoots of 30 cm length were collected and washed under running tap water for 5 min to remove any surface dirt. Then the stems were inserted into beakers with distilled water and placed in a phytotron. After four weeks the shoots (2–3 cm long) grown from buds were collected as a source of primary explants. Next, the leaves were removed and the stems were cut into nodal segments approx. 0.5 cm long. They were surface-disinfected with 70% ethanol for 1 min, and further disinfected with 1.5% solution of sodium hypochlorite (NaOCl). After disinfection the explants were placed on basal MS [Murashige and Skoog 1962] medium supplemented with 5.0 mg·dm^{-3} 6-benzyladenine (BA) (Sigma-Aldrich), 0.02 mg·dm^{-3} 1-naphthaleneacetic acid (NAA) (Sigma-Aldrich) and 20 g·dm^{-3} sucrose, and solidified with 8.0 g·dm^{-3} Bacto™ Agar (Becton, Dickinson and Company, USA). The pH was adjusted to 5.8 with 1 N NaOH and 1 N HCl before autoclaving at 121°C at 110 kPa for 20 min. This medium has been used for the micropropagation of other lilac genotypes [Nesterowicz et al. 2006]. For 'Sensation' the concentration of $MgSO_4$ in the MS medium was doubled (740 mg·dm^{-3}) due to chlorosis appearing on the leaf blades (own research – unpublished data).

The cultures were incubated in a growth chamber at 23 ±1°C with a 16 h light/8 h dark photoperiod. The light intensity was 35 µmol·m^{-2}·s^{-1} from cool white fluorescent tubes.

Effect of type and concentration of cytokinins on shoot regeneration

To promote axillary and adventitious shoot regeneration, nodal explants 0.5 cm long were cultured on a medium supplemented with the following growth regulators: 0.02 mg·dm^{-3} NAA in combination with 6-benzylaminopurine (BA), kinetin (KIN) 6-(γ,γ-dimethylall ylamino)purine (2iP) (Sigma-Aldrich) in concentrations of 1.25, 2.50 and 5.0 mg·dm^{-3}, or [6-(3-hydroxybenzylam ino)purine] (meta-topolin, mT) (Duchefa) in concentrations of 1.25, 2.5, 5.0 and 10.0 mg·dm^{-3}. The control medium did not contain plant growth regulators. After eight weeks the following data were recorded: percentage of regenerated plants, total number of regenerated shoots per explant, and shoot length (cm).

Effect of sucrose concentration on shoot regeneration

One of the aims of the experiment was to determine the effect of sucrose concentration on shoot regeneration. Single-node shoot fragments 0.5 cm long were placed on MS medium supplemented with sucrose at concentrations of 0, 5, 10, 20, 30, 40 g·dm^{-3}. For all treatments 0.02 mg·dm^{-3} NAA and 5.0 mg·dm^{-3} BA were added. After eight weeks the percentage of regenerated plants, total number of regenerated shoots per explant and shoot length (cm) were recorded.

Effect of auxin on rooting of microcuttings

One of the aims of the experiment was to determine the optimal concentration and type of auxin in the rooting medium. Apical shoot fragments 1.5 cm long were placed on MS medium supplemented with NAA or IBA in concentrations of 0.5, 1.0, 2.0 mg dm^{-3}. The control medium did not contain any growth regulators. After eight weeks the rooting rate, number of roots, root length and length of shoots (cm) were recorded.

Experimental design and statistics

Experiments were conducted in a completely randomized design. Each treatment consisted of 60 explants/microcuttings (three replications, each containing 20 objects). The arcsin transformation of regeneration rate percentages was performed [Wójcik and Laudański 1989]. The data were subjected to one factorial analysis of variance using SPSS. Multiple comparisons among means were made using Duncan's test at $p \leq 0.05$.

RESULTS

Effect of type and concentration of cytokinin on shoot regeneration

The influence of the type and concentration of cytokinin on the percentage of regenerating explants was significant (Table 1). On all media, single-node explants of the cultivar 'Katherine Havemeyer' regenerated at a rate between 75 and 100%. Single-node shoot fragments of the cultivar 'Sensation' regenerated at a rate of 75–95%.

The type and concentration of this cytokinin affected the number of shoots per explant and their length (Table 1). In both cultivars the greatest number of shoots per explant was obtained on the medium with *m*T (four–five shoots). For the best proliferation of both tested varieties the optimal concentration of cytokinin in the medium was 5 mg·dm^{-3} (more than five shoots per explant). Increasing the concentration of *m*T in the medium resulted in the formation of a smaller number of short shoots. The longest shoots were recorded for 'Katherine Havemeyer' on medium supplemented with 2.5 mg·dm^{-3} 2iP, and for 'Sensation' on medium supplemented with 1.25 mg·dm^{-3} *m*T. In 'Sensation' explants placed on the medium supplemented with BA, 2iP or KIN, from 0.8 to 1.2 shoots per explant were obtained. For 'Katherine Havemeyer' the lowest number of small shoots (from 1.3 to 1.6 cm in length) were obtained on the medium supplemented with KIN, regardless of its concentration.

Effect of sucrose concentration on shoot regeneration

The effect of sucrose concentration on the percentage of regenerated explants was significant (Table 2). Shoots placed on a medium without sucrose regenerated at a much lower rate than in the other treatments. A significantly higher degree of regeneration in both cultivars of common lilac (over 90%) was obtained on the medium supplemented with 20.0–40.0 g·dm^{-3} sucrose. In the presence of 40.0 g·dm^{-3} sucrose leaf chlorosis was observed.

TABLE 1. Effect of type and concentration of cytokinin on proliferation rate of *Syringa vulgaris* shoots

Plant growth regulators (mg·dm^{-3})					Cultivar					
					'Katherine Havemeyer'			'Sensation'		
BA	2iP	KIN	*m*T	NAA	Regeneration rate (%) **	Number of shoots	Length of shoots (cm)	Regeneration rate (%) **	Number of shoots	Length of shoots (cm)
0	0	0	0	0	98.3 b*	1.1 ab	1.8 bc	78.3 ab	0.9 a	1.5 ab
1.25	0	0	0	0.02	85 ab	1.2 ab	1.7 ab	95.0 b	1.1 a	2.8 e
2.50	0	0	0	0.02	100 b	1.6 bc	2.8 f	75.0 a	0.9 a	1.9 b-e
5.00	0	0	0	0.02	93.3 ab	2.1 cd	1.5 ab	80.0 ab	1.0 a	2.2 c-e
0	1.25	0	0	0.02	100 b	1.7 bc	2.5 d-f	78.3 ab	0.9 a	2.4 c-e
0	2.50	0	0	0.02	100 b	2.3 d	3.2 g	85.0 ab	1.0 a	2.7 de
0	5.00	0	0	0.02	78.3 a	2.0 cd	2.3 de	81.7 ab	0.9 a	2.6 de
0	0	1.25	0	0.02	93.3 ab	0.9 a	1.6 ab	75.0 a	0.8 a	2.1 b-e
0	0	2.50	0	0.02	85.0 ab	0.9 a	1.3 a	93.3 ab	1.2 a	1.8 a-c
0	0	5.00	0	0.02	75.0 a	1.0 a	1.5 ab	88.3 ab	0.9 a	2.4 c-e
0	0	0	1.25	0.02	100 b	4.3 e	2.2 c-e	88.3 ab	3.6 b	4.6 g
0	0	0	2.50	0.02	100 b	4.4 e	2.6 ef	93.3 ab	5.3 cd	3.7 f
0	0	0	5.00	0.02	100 b	5.3 f	2.4 d-f	90.0 ab	5.8 d	2.3 c-e
0	0	0	7.50	0.02	100 b	4.6 e	2.4 d-f	93.3 ab	5.3 cd	2.1 b-e
0	0	0	10.00	0.02	100 b	4.1 e	2.1 cd	95.0 b	4.6 c	1.9 a-d

* Means followed by the same letter are not significantly different at $p \leq 0.05$.
** 100% was 60 explants.

TABLE 2. Effect of sucrose concentration in medium on shoot proliferation

Sucrose ($g \cdot dm^{-3}$)	Cultivar					
	'Katherine Havemeyer'			'Sensation'		
	Regeneration rate %**	Number of shoots	Length of shoots (cm)	Regeneration rate %**	Number of shoots	Length of shoots (cm)
0	48.3 a*	0.6 a	0.5 a	23.3 a	0.2 a	0.3 a
5	76.7 b	2.4 b	1.5 b	55.0 b	0.6 b	0.8 b
10	93.3 b	2.2 b	1.7 b	68.3 b	1.2 c	1.3 c
20	96.7 b	2.1 b	1.5 b	95.0 c	1.0 c	2.6 e
30	98.3 b	3.3 c	2.7 c	91.7 c	1.0 c	4.1 f
40	91.7 b	2.0 b	1.7 b	90.0 c	1.2 c	2.1 d

* Means followed by the same letter are not significantly different at $p \leq 0.05$.
** 100% was 60 explants.

The effect of sucrose concentration on the number and length of shoots was also significant (Table 2). The highest multiplication rate and the longest shoots in both cultivars were obtained on the medium supplemented with 30.0 $g \cdot dm^{-3}$ sucrose. For 'Katherine Havemeyer' three shoots per explant, 2.7 cm long, were formed, while for 'Sensation' there was one shoot 4.1 cm long.

Effect of auxin on rooting of microcuttings

A significant effect of auxin on the percentage of rooted microcuttings was observed (Table 3). Microcuttings placed on the medium supplemented with IBA rooted at a rate of almost 100% in the case of 'Katherine Havemeyer' and 75–88% in the case of 'Sensation'.

For both cultivars the effect of IBA on the number of roots and their average length was significant. The greatest number of roots was obtained on medium supplemented with 1.0 $mg \cdot dm^{-3}$ IBA for 'Katherine Havemeyer'. However, their length did not differ significantly from the plants rooted on medium with 2.0 $mg \cdot dm^{-3}$ IBA. With the presence of auxin in the medium (0.5–2.0 $mg \cdot dm^{-3}$)

TABLE 3. Effect of the auxin IBA on rooting in two lilac cultivars

IBA ($mg \cdot dm^{-3}$)	Cultivar							
	'Katherine Havemeyer'				'Sensation'			
	Rooting rate (%) **	Number of roots	Length of roots (cm)	Length of shoots (cm)	Rooting rate (%) **	Number of roots	Length of roots (cm)	Length of shoots (cm)
0	78.3 a*	0.6 a	1.1 a	1.6 a	61.7 a	0.4 a	0.7 a	1.5 a
0.5	100 b	2.1 b	1.2 a	2.3 b	81.7 b	2.6 b	1.5 b	1.5 a
1.0	100 b	3.0 c	2.1 b	2.2 b	88.3 b	2.7 b	2.5 c	1.8 a
2.0	98.3 b	2.4 b	2.1 b	2.9 b	75.0 b	2.5 b	2.1 b	1.8 a

* Means followed by the same letter are not significantly different at $p \leq 0.05$.
** 100% was 60 explants.

the 'Sensation' microshoots formed significantly more roots than those placed on a medium lacking growth regulators. The longest roots were produced by microshoots placed on the medium supplemented with 1.0 mg·dm^{-3} IBA.

Microshoots of both cultivars rooted on the medium containing NAA produced a large amount of callus at the shoot bases, regardless of the auxin concentration. After three weeks of culture the plant material died.

DISCUSSION

Microshoot proliferation is a decisive step for the *in vitro* culture of lilacs, since it directly determines the feasibility of mass micropropagation. Proliferation may be achieved through axillary shoot inducement, where cytokinins play an important role. In a wide range of lilac cultivars, BA and 2iP are the cytokinins used to initiate shoot organogenesis. They are used either individually or in combination with NAA. Lyobumirova and Iliev [2013] for *S. vulgaris* used the MS medium with 5.0 mg·dm^{-3} BA and 0.1 mg·dm^{-3} IBA. Nesterowicz et al. [2006] lowered the BA concentration to 1.0 mg·dm^{-3}, replacing the IBA with 0.02 mg·dm^{-3} NAA. Tomsone et al. [2007] obtained the best results in the presence of 1.0–3.0 mg·dm^{-3} 2iP in combination with 0.05 mg·dm^{-3} NAA. Variable sensitivity to the type and concentration of growth regulators is also characteristic for lilac cultivars. For 'Katherine Havemayer' Refouvelet et al. [1998] applied a medium supplemented with 5.0 mg·dm^{-3} BA and 0.01 mg·dm^{-3} NAA, while Tomsone et al. [2007] used BA in combination with 0.1 mg·dm^{-3} IAA. Charlebois and Richter [2004] pointed to the lower sensitivity of 'Katherine Havemayer' explants to the type of cytokinins, compared with the cultivars 'Charles Joly' or 'Madame Florent Stepmann' [Dragt et al. 1992], for which the presence of 2iP riboside in the medium rather than 2iP was more effective.

In this study the activity of *meta*-topolin (*m*T) isolated from poplar (*Populus* ×*canadensis* Moench cv. *Robusta*) was examined [Strnad et al. 1997]. Metatopolin is a hydroxylated analogue of BA with a hydroxyl group attached to its N^6 side chain, which results in the formation of O-glucoside metabolites that can be reversibly sequestrated in planta to produce active cytokinin forms when needed. Results indicate that mT and its derivatives are superior to BA in improving shoot production and reducing tissue culture-induced abnormalities in plant species [Bairu et al. 2007]. Both 'Katherine Havemeyer' and 'Sensation' explants proliferated more profusely on a medium enriched with mT than in other treatments. Kaminek et al. [1987] demonstrated that *m*T is translocated faster than BA in plant tissue. Previous studies suggested that response to cytokinin was mediated by family-specific receptors, which regulate a signal-transduction pathway [Heyl and Schmülling 2003]. Mok et al. [2005] published a study showing that BA and *m*T have different affinity in receptor recognition. The authors found that *m*T and *trans*-zeatin interact with the *Arabidopsis* AHK4 receptor, while BA and thidiazuron (TDZ) interact with the maize ZmHK1 receptor.

Sucrose as a carbon source supports the growth of plant cells in a culture [Nowak et al. 2004]. Sucrose in concentrations from 10 to 50 mg·dm^{-3} is generally used for *in vitro* cultures. The optimum sucrose concentration as an efficient carbon source has been examined in tissue cultures of some species of lilac, such as *Syringa ×hyacinthiflora* [Cui et al. 2009] and *S. josickaea* [Catana et al. 1998], in which 30 mg·dm^{-3} sucrose enhanced shoot development, while for *S. chinensis* Will. cv. Saugeana mannitol at a concentration of 16 mg·dm^{-3} was also suitable [Welander et al. 1987]. In our experiment, a gradual increase in sucrose concentration resulted in an increase in shoot initiation only in 'Katherine Havemayer', while 'Sensation' produced the same number of shoots in the presence of 10–40 g·dm^{-3} sucrose. However, at a concentration of 30 g·dm^{-3} the shoots were significantly longer than at other concentrations. Ahmad et al. [2007] indicated that sugars are perceived by cells as chemical signals *in vitro*, with very high concentrations acting as stressing agents. Perata et al. [2003] reported that high sugar concentrations could inhibit gibberellin signalling and suppress cell division and growth in several different plant systems. Gibberellin is thought to play an important role in the control of cell division and elongation, and in the control of apical dominance (paradormancy) [Horvath et al. 2003]. In turn, Gabryszewska [2011] reported that increased sucrose concentrations (5–30 mg·dm^{-3}) reduced axillary shoot formation in *S. vulgaris*, but a supply of nitrogen salts could overcome the inhibitory effect of sucrose.

It is well known that the type and concentration of auxin play a central role in the induction of adventitious roots [De Klerk 2002]. These factors could lead to variation in the percentage of rooting in common lilac microcuttings. For rooting lilac microcuttings, the most commonly used auxin is IBA. Lyobumirova and Iliev [2013] obtained the best results on the MS medium enriched with 1.0–5.0 mg·dm^{-3} IBA, while Nesterowicz et al. [2006] considered a higher concentration of that growth regulator to be appropriate. In turn, Parvanova et al. [2015] obtained 75––100% of rooted microcuttings by raising the concentration of IBA to 7.5 mg·dm^{-3}. In our experiment the appropriate IBA concentration was 1.0 mg·dm^{-3}, since in the case of both cultivars those microcuttings produced the largest number of roots. Also Oprea and Concioiu [2012] observed better responses of microcuttings to lower concentrations of auxin: 'Madame Lemoine' produced roots in the presence of 0.5 mg·dm^{-3} of NAA, and 'Sensation' did so in the presence of 0.6 mg·dm^{-3} IBA. The results obtained in this study do not confirm those results, since on the medium enriched with NAA, microshoots of both lilac cultivars produced a significant amount of callus tissue at the bases and then died. NAA produced toxic effects when used in all tested concentrations.

CONCLUSIONS

The presence of *meta*-topolin in the medium and 30 mg·dm^{-3} of sucrose contributed to better regeneration of shoots, regardless of the cultivar. In both 'Katherine Havemayer' and 'Sensation', IBA

was found to have a positive impact on root formation. The presence of NAA in the medium resulted in callusing of microcuttings and their dieback.

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Streszczenie: *Wpływ regulatorów wzrostu i sacharozy na mikrorozmnażanie lilaka pospolitego (*Syringa vulgaris *L.).* Lilak pospolity (*Syringa vulgaris* L.) jest atrakcyjną rośliną, o barwnych i przyjemnie pachnących kwiatach, często wykorzystywaną w przestrzeni miejskiej. Główną metodą rozmnażania tego krzewu jest okulizacja lub szczepienie. Często rozmnażany jest również w kulturach tkankowych. Celem badań było określenie wpływu obecności w pożywce MS różnego stężenia cytokininy w połączeniu z NAA oraz sacharozy na regenerację pędów oraz stężenia IBA lub NAA na ryzogenezę mikrosadzonek lilaka 'Katherine Havemeyer' i 'Sensation'. Proliferacja pędów uzależniona była od rodzaju oraz stężenia zastosowanej w pożywce cytokininy. W przypadku obu odmian najwięcej pędów uzyskano na pożywce wzbogaconej w *meta*-topolinę w stężeniu 5,0 mg·dm^{-3} dla odmiany 'Katherine Havemeyer' i 2,5–7,5 mg·dm^{-3} dla 'Sensation'. Zastosowanie 30 mg·dm^{-3} sacharozy w pożywce skutkowało lepszą regeneracją pędów oraz stymulowało ich wzrost u odmiany 'Katherine Havemeyer'. Największą liczbę korzeni uzyskano na pożywce z dodatkiem 1,0 mg·dm^{-3} IBA w przypadku odmiany 'Katherine Havemeyer' i 0,5–2,0 IBA mg·dm^{-3} dla 'Sensation'. Zastosowanie NAA powodowało zamieranie mikrosadzonek.