# EFFECT OF VARIOUS LEVELS OF SUCROSE, NITROGEN SALTS AND TEMPERATURE ON THE GROWTH AND DEVELOPMENT OF *Syringa vulgaris* L. SHOOTS *IN VITRO*

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A B S T R A C T

The influence of sucrose (5, 10, 20, 30 g $l^{-1}$), nitrogen salts – $KNO_3$, $NH_4NO_3$ (25%, 50%, 100% in relation to the MS medium) and temperature (15 °C, 20 °C) on the growth of the main shoot and the activation and development of axillary buds in *Syringa vulgaris in vitro* was investigated. Different ratios of sucrose/nitrogen salts in the MS medium had a limited effect on the length of the main shoot of lilac plantlets. Also, the concentration of sucrose and nitrogen salts in the medium did not significantly affect the formation of nodes on the main or axillary shoots. The outgrowth of axillary shoots depended on the sucrose and nitrogen salts concentrations and temperature. Among the various sucrose/nitrogen salts relations, the highest number of axillary shoots (4.2) was found in the plantlets growing at a temperature of 20 °C, on a medium with a low level of sucrose (5 g $l^{-1}$) and 100% strength of $KNO_3$ and $NH_4NO_3$. Increased levels of sucrose in the medium significantly reduced the development of axillary buds in lilac plantlets growing at either temperature. By contrast, high levels of sucrose increased the fresh weight of lilac shoots. Different levels of nitrogen salts in the medium containing the same level of sucrose had no significant effect on the fresh weight of lilac shoots. On the other hand, at all levels of sucrose, the increased strength of nitrogen salts in the culture medium significantly enhanced the emergence and growth of axillary shoots. Increased strength of nitrogen salts in the medium appeared to counteract, at least partially, the inhibitory effect of a high sucrose level on the growth of axillary buds in *Syringa vulgaris*. There was clearly an interaction between the levels of sucrose and nitrogen salts such that a medium with a low sucrose to nitrogen ratio promoted axillary branching, whereas a medium with a high sucrose to nitrogen ratio inhibited the growth of axillary shoots. The different ratios of sucrose/nitrogen salts in the MS medium and the temperature affected the morphology of lilac plantlets. Increased supply of sucrose strongly stimulated leaf

surface area, but the levels of nitrogen salts had a limited effect on leaf size. The plantlets cultured at a temperature of 15 °C had bigger leaves than the plantlets at 20 °C. Low-sucrose treatments, irrespective of the level of nitrogen salts, induced a compact and branched habit of shoots and inhibited root formation. Increasing sucrose content in the medium resulted in a spontaneous formation of roots on the plantlets cultured in the presence of low levels of nitrogen salts.

**Key words**: lilac, sucrose, nitrogen salts, main and axillary shoots, micropropagation

**Abbreviations**: C – carbon, N – nitrogen, ABA – abscisic acid, $GA_3$ – gibberellic acid, IBA – indole-3-butyric acid, BAP – 6-benzylaminopurine, 2iP – isopentenyladenine, 2iPA – isopentenyladenosine, MS – Murashige and Skoog medium (1962)

## INTRODUCTION

*Syringa* is a genus of about 27 wild species of flowering woody plants of the family Oleaceae, native to woodland and scrub from south-eastern Europe to eastern Asia and commonly cultivated in temperate areas. Among the many species, *Syringa vulgaris* L. (lilac) is one of the most popular ornamental shrubs. More than 2000 cultivars have been developed to date. Lilac is conventionally propagated by vegetative methods, such as grafting onto seedling stocks, air layering and cuttings. Micropropagation has opened up new areas of research and propagation allowing the problems of conventional methods to be overcome and enabling rapid multiplication of plants on a commercial scale. For more than two decades tissue cultures have been used for the propagation of lilac (Hildebrandt and Harney, 1983; Einset and Alexander, 1985; Gabryszewska, 1989; Gabryszewska and Warabieda, 1992; Waldenmaier and Bünemann, 1991; Skrzypczak, 1992; Refouvelet et al., 1998; Refouvelet and Daguin, 2000; Scholten, 1998; Popowich and Filipenya, 2000; Charlebois and Richter, 2004; Jacobsone et al., 2006; Nestorowicz et al., 2006; Oprea and Duta, 2008; Cui et al., 2009). The proliferation rate of shoots is a decisive step for the micropropagation of lilac in mass commercial production. Two main methods of *in vitro* propagation of lilac can be distinguished: propagation by axillary branching, in which axillary buds are activated using high levels of cytokinins (Hildebrandt and Harney, 1983; Gabryszewska, 1989; Waldenmaier and Bünemann, 1991; Cui et al., 2009), and the single-node method, in which relatively low cytokinin levels activate an axillary bud and induce stem elongation (Einset and Alexander, 1985; Welander, 1987; Pierik et al., 1988; Waldenmaier and Bünemann, 1991; Gabryszewska and Warabieda, 1992; Charlebois and Richter, 2004). In both methods, cytokinins play an important role, but the effectiveness of multiplication depends on a number of factors (Charlebois and Richter, 2004; Nestorowicz et al., 2006). The propagation of lilac by axillary branching will increase the multipli-

cation rate, but it will also significantly reduce the rooting potential. Additionally, the axillary branching activated by a high concentration of cytokinins might induce some epigenetic and genetic variations (Waldenmaier and Bünemann, 1991). Lilac tissue culture combined with cold storage has been a useful method for long-term storage of germoplasm with minimal space and cost (Refouvelet et al., 1998; Jacobsone et al., 2006).

Among the many environmental factors, carbon (C) and nitrogen (N) are crucial for plant growth and development. Both C and N nutrients are essential for the building blocks of the cell and for various cellular functions. Current knowledge indicates that plants possess an interactive regulatory machinery that coordinates the capacity of nitrogen assimilation with carbon metabolism, nutrient availability and other environmental factors. Also, carbon and nitrogen are two signals that influence plant growth and development. It has been stated that plants have a carbon/nitrogen sensing/regulatory mechanism (CN-responsive genes) which is different from that expected on the basis of the expression values derived separately from carbon and nitrogen treatments (Coruzzi and Bush, 2001; Coruzzi and Zhou, 2001; Starck, 2006; Zheng, 2009; Nunes-Nesi et al., 2010).

Sugars and nitrogen salts and their interaction with plant growth regulators play an important role in morphogenesis, growth and development of plants *in vitro*. High levels of nitrogen supply promoted shoot regeneration from protoplasts of *Nicotiana plumbaginifolia*, but high levels of hexoses inhibited this process (Caboche, 1987). By contrast, a reduction in nitrogen concentration in the medium increased shoot formation rate in various species of *Cymbidium* propagated *in vitro* (Shimasaki and Uemoto, 1990; Ogura and Okubo, 2003; Huang and Okubo, 2005; Ogura-Tsujita and Okubo, 2006). Lowering the strength of nitrogen salts (1/2 MS) and sucrose (10 g $l^{-1}$) in the medium strongly stimulated axillary shoot formation in *Clematis pitcheri* cultured *in vitro* (Gabryszewska et al., 2008). Increasing the concentration of sucrose, in the presence of the same level of nitrogen, stimulated axillary shoot formation in *Paeonia lactiflora* on a medium with a high level of cytokinins and gibberellin. Renewal bud formation on herbaceous peony explants was promoted by a high level of glucose and low concentrations of exogenous growth regulators (kinetin, IBA, $GA_3$) in the medium (Gabryszewska, 2009, 2010). Recently, it has been stated that tissue culture plantlets are constantly intoxicated with high concentrations of sucrose and nitrogen in the medium (Desjardines et al., 2009). These high concentrations of sucrose and nitrogen salts are supra optimal and stressful for many species, especially woody and perennial plants. For instance, a high concentration of sucrose in the medium was detrimental for the photosynthetic activity in strawberry and other

plants propagated *in vitro* (Hdider and Desjardines, 1994; Jones et al., 1996; Kilb et al., 1996; Serret et al., 1997).

The aim of the experiment was to investigate the influence of various levels of sucrose and nitrogen salts, and temperature on the growth of the main shoot and the activation and development of axillary buds in *Syringa vulgaris in vitro*.

## MATERIAL AND METHODS

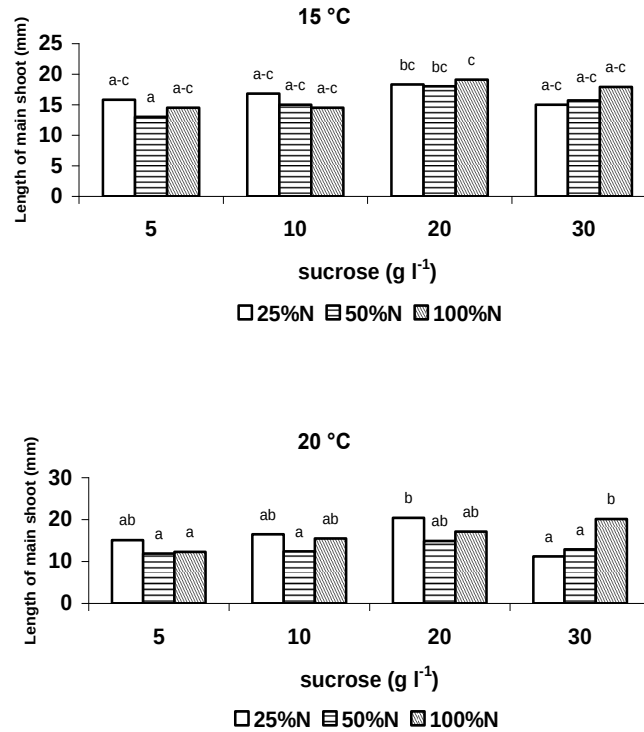
Mother plants (1-2 years old) of *Syringa vulgaris* L. were cultivated in a greenhouse. Excised shoot tips were isolated as explants. The initial explants and the subsequent subcultures of single-node culture were performed on the MS basal medium containing 0.5 mg $l^{-1}$ 2iP, 20 g $l^{-1}$ sucrose and 6 g $l^{-1}$ agar. After the seventh subculture, the elongated shoots were cut into segments with the apical bud and two-nodes and used in the experiment.

The influence of sucrose (5, 10, 20, 30 g $l^{-1}$), nitrogen salts – $KNO_3$, $NH_4NO_3$ (25%, 50%, 100% in relation to the MS medium) and temperature (15 °C, 20 °C) on the growth of the main shoot and the activation and development of axillary buds *in vitro* on the MS medium with 0.5 mg $l^{-1}$ 2iP was investigated.

Each treatment consisted of 3 jars with 5 explants. The experiment was repeated twice (2 series). Culture conditions: photoperiod – 16 h of light provided by cool-white fluorescent lamps (Philips TLD 36W/95) at 80 µmol $m^{-2}s^{-1}$, temperature – 15 °C or 20 °C. The observations and measurements were recorded after 10 weeks of culturing. The number of axillary shoots and the number of nodes per main/axillary shoots were recorded. Also, the fresh weight of shoots and the length of the main and axillary shoots were determined. Morphological examinations of lilac plantlets were carried out. The data were statistically analysed and the means compared using Duncan's multiple range t-test.

## RESULTS AND DISCUSSION

The activation of axillary buds plays a main role in both methods of *Syringa vulgaris* micropropagation. Shoot branching is the process by which axillary buds (dormant), located on the axil of a leaf, develop and form new branches (axillary shoots). Bud outgrowth is regulated by the interaction of environmental signals and endogenous ones, such as plant hormones. Hormones known to have a major influence are auxin, cytokinin, and a novel hormone, as yet chemically undefined (Ongaro and Leyser, 2008). Auxin (transported basipetally) and the novel hormone (moved acropetally) inhibit bud outgrowth. In contrast, cytokinins (transported acropetally) promote axillary shoot growth (Ongaro and Leyser, 2008). In most of the studies on *in vitro* propagation of lilac, the axillary branching was promoted by BAP or a combination of BAP with zeatin (Hildebrandt and

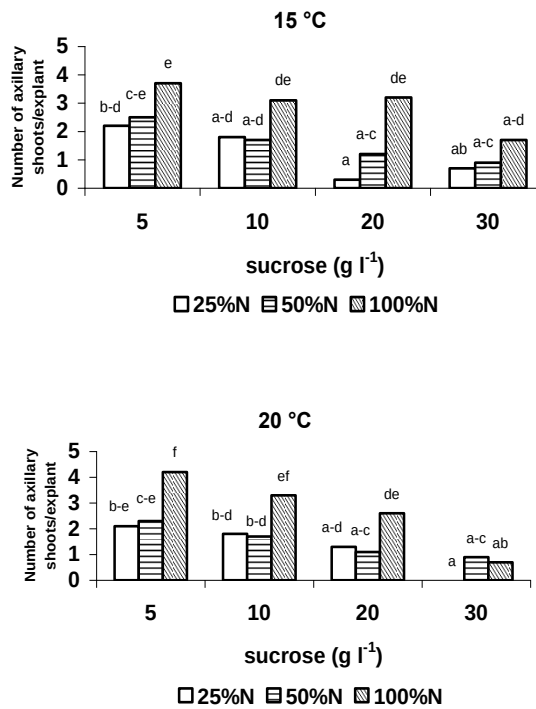



Explanation: Means followed by the same letter do not differ at p = 0.05 according to Duncan's multiple range t-test. Analysis of significance was done separately for each temperature

**Figure 1**. The influence of sucrose (5, 10, 20, 30 g $l^{-1}$), nitrogen salts (25%, 50%, 100% in relation to the MS medium) and temperature (15 °C, 20 °C) on the length of the main shoot in *Syringa vulgaris in vitro*

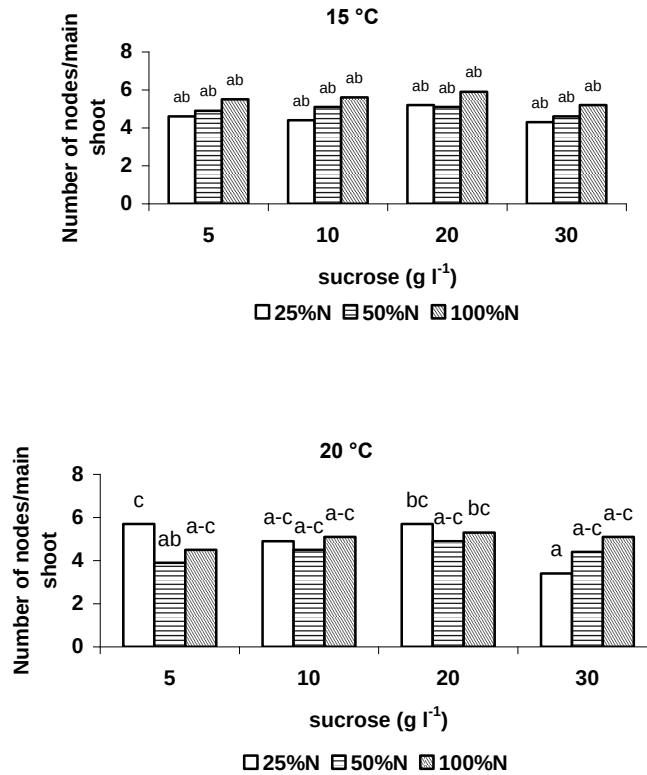
Harney, 1983; Gabryszewska, 1989; Waldenmaier and Bünemann, 1991; Refouvelet et al., 1998; Popowich and Filipenya, 2000; Nestorowicz et al., 2006; Oprea and Duta, 2008; Cui et al., 2009). Bud outgrowth and elongation of shoots from nodal explants were affected by low concentrations of 2iP or 2iPA (Pierik et al., 1988; Gabryszewska and Warabieda, 1992; Scholten, 1998; Charlebois and Richter, 2004). In the present study, a relatively low level of 2iP (0.5 mg $l^{-1}$) was used to activate the axillary buds and induce stem elongation in *Syringa vulgaris* from segments with the apical bud and two-nodes. The different ratios of sucrose/nitrogen salts in the MS medium had a limited effect on the length of the main shoot (Fig. 1). At 15 °C, the longest main shoots (from 18.3 to 19.0 mm) were observed on the medium containing 20 g $l^{-1}$ sucrose in combination with the different levels of nitrogen salts. In the

case of shoot cultures growing at 20 °C, the increased strength of nitrogen salts in the medium significantly stimulated the elongation of the main shoot (from 11.2 to 20.1 mm), but only on the medium with the highest sucrose level (30 g $l^{-1}$). By contrast, in *Ceratonia siliqua* shoot culture, a strong reduction in nitrogen salts in the medium (5%, 10% of full-strength MS) stimulated the elongation of the main shoot (Vinterhalter et al., 2007). The concentration of sucrose and nitrogen salts in the medium did not significantly affect node formation on the main or axillary shoots (Fig. 3 and 4). Only in the lilac shoot culture maintained at 20 °C did the decrease in nitrogen nutrition (25% concentration of $KNO_3$ and $NH_4NO_3$) apparently stimulate node formation on the main shoots growing in the presence of the low level of sucrose (5 g $l^{-1}$) as compared to the high level of sucrose (30 g $l^{-1}$) (Fig. 3).




Explanation: see Figure 1

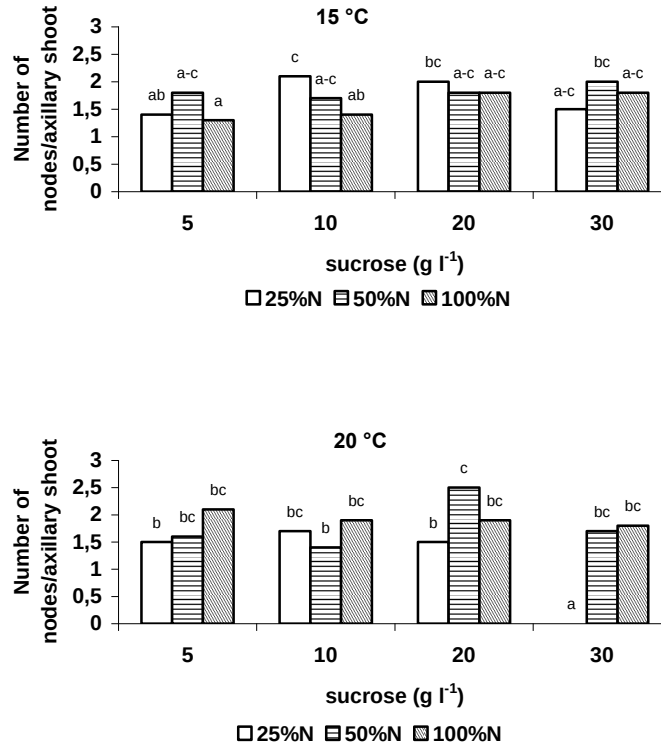
**Figure 2**. The influence of sucrose (5, 10, 20, 30 g $l^{-1}$), nitrogen salts (25%, 50%, 100% in relation to the MS medium) and temperature (15 °C, 20 °C) on the number of axillary shoots in *Syringa vulgaris in vitro*




Explanation: see Figure 1

**Figure 3**. The influence of sucrose (5, 10, 20, 30 g $l^{-1}$), nitrogen salts (25%, 50%, 100% in relation to the MS medium) and temperature (15 °C, 20 °C) on the number of nodes on the main shoot in *Syringa vulgaris in vitro*

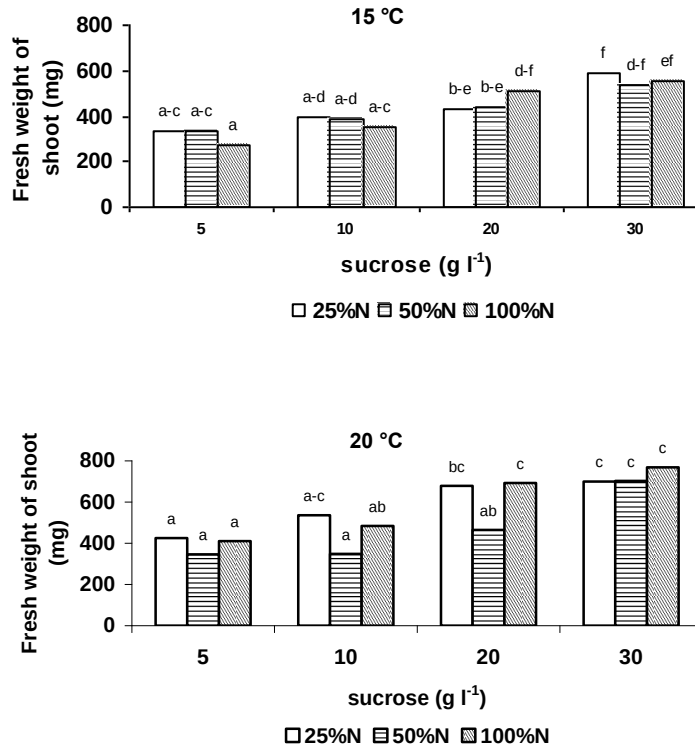
The development of axillary shoots occurred on the MS medium containing a low level of 2iP (0.5 mg $l^{-1}$) and depended on the concentration of sucrose and nitrogen salts and temperature (Fig. 2 and 4). When the medium was supplemented with the standard level of sucrose (30 g $l^{-1}$) and 100% strength of $KNO_3$ and $NH_4NO_3$, the activation of axillary buds was very weak (0.03-0.7) (Fig. 2). Among the various sucrose/nitrogen salts relations, the highest number of axillary shoots (4.2) was found in the plantlets growing at 20 °C, on the medium with the lowest sucrose level – 5 g $l^{-1}$ and 100% strength of $KNO_3$ and




Explanation: see Figure 1

**Figure 4**. The influence of sucrose (5, 10, 20, 30 g $l^{-1}$), nitrogen salts (25%, 50%, 100% in relation to the MS medium) and temperature (15 °C, 20 °C) on the number of nodes on axillary shoots in *Syringa vulgaris in vitro*

$NH_4NO_3$ (Fig. 2). Also, the same composition of the medium stimulated axillary shoot production (3.7) on the explants growing at 15 °C. Increased sucrose levels in the medium significantly reduced axillary bud development in lilac plantlets growing at either temperature (Fig. 2). The decrease was gradual, and on the medium with the highest level of sucrose (30 g $l^{-1}$) the number of axillary shoots was the lowest (0.03 or 0.7). By contrast, high levels of sucrose increased the fresh weight of lilac shoots (Fig. 5). The cultures on the medium with the highest sucrose concentration (30 g $l^{-1}$) maintained at 20 °C produced greater shoot fresh weight (699-768 mg) than the cultures at 15 °C (536-591 mg) (Fig. 5). The different levels of nitrogen salts in the medium containing the same level of sucrose had no significant effect on the fresh




Explanation: see Figure 1

**Figure 5**. The influence of sucrose (5, 10, 20, 30 g $l^{-1}$), nitrogen salts (25%, 50%, 100% in relation to the MS medium) and temperature (15 °C, 20 °C) on the fresh weight of *Syringa vulgaris* shoots *in vitro*

weight of lilac shoots (Fig. 5). On the other hand, at all levels of sucrose, the increased concentration of nitrogen salts in the culture medium significantly enhanced the emergence and growth of axillary shoots (Fig. 2). Increased strength of nitrogen salts in the medium appeared to counteract, at least partially, the inhibitory effect of high sucrose levels on axillary bud outgrowth in *Syringa vulgaris*. There was clearly an interaction between sucrose and nitrogen salts such that a medium with a low sucrose-to-nitrogen ratio promoted axillary branching, whereas a medium with a high sucrose-to-nitrogen ratio inhibited axillary bud outgrowth. Similar results were obtained for apical stem culture of *Clematis pitcheri* in which sucrose at 10 g $l^{-1}$ and $KNO_3$ and $NH_4NO_3$ at 50% strength stimulated axillary branching, but high levels of sucrose (30 g $l^{-1}$) inhibited

the growth of axillary buds (Gabryszewska et al., 2008). In shoot culture of *Ceratonia siliqua*, a strong reduction in nitrogen salts in the medium (5%, 10% of full-strength MS), but the same level of sucrose – 20 g $l^{-1}$, significantly decreased axillary branching and multiplication rate (Vinterhalter et al., 2007). A reduction in sucrose level to 15 g $l^{-1}$ and low strength (25%) of MS salts stimulated the growth of axillary buds from nodal segments of *Ilex dumosa* (Luna et al., 2003). On the other hand, a high sucrose concentration increased axillary shoot proliferation in *Vaccinium corymbosum* and the production of shoots in a culture of *Eucomis autumnalis* (Cao et al., 2003; Taylor and Van Staden, 2001). In the case of *Paeonia lactiflora* shoot cultures, a high concentration of sucrose (60-90 g $l^{-1}$) inhibited the growth and development of axillary shoots on the medium with cytokinins, but stimulated axillary bud outgrowth and multiplication in the presence of cytokinins and $GA_3$ added together (Gabryszewska, 2009). It is known that sugar alone, or through interaction with different phytohormones (GA, cytokinins, ABA, ethylene), can induce or suppress the response of many growth-related genes in higher plants (Smeekens, 2000; Ciereszko, 2002; Gibson, 2005; Rolland et al., 2002; 2006). Also, the nitrogen signal can influence many genes in higher plants and nitrogen signalling appears to interact with phytohormone signalling (Coruzzi and Zhou, 2001; Starck, 2006). The interaction between nitrogen and cytokinin in the regulation of metabolism and plant development has been demonstrated (Sakakibara et al., 2006). Also, it has been found that elevated nitrogen levels increase the endogenous levels of gibberellins (Rajagopal and Rao, 1974; Jang et al., 2008). In contrast, sugars have been shown to inhibit gibberellin signalling and suppress cell division and growth in several different plant systems (Perata et al., 1997; Chao et al., 2000). Gibberellin is suspected 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 recent years, it has also been shown that GAs promote photosynthesis and nitrogen utilization (Iqbal et al., 2011). The role of gibberellins in the axillary branching of *Syringa vulgaris* has not been investigated. It seems that the interaction of sugar and nitrogen and different phytohormones, especially gibberellins and cytokinins, plays an important role in axillary bud activation (control of apical dominance) in lilac. As a consequence, the optimal C/N ratio could enhance the levels or activity of endogenous hormones and the subsequent outgrowth of axillary shoots.

The different ratios of sucrose/--nitrogen salts in the MS medium and the temperature affected the morphology of lilac plantlets (Fig. 6). Increased supply of sucrose strongly stimulated leaf surface area, but the levels of nitrogen salts had a limited effect on leaf size. The plantlets

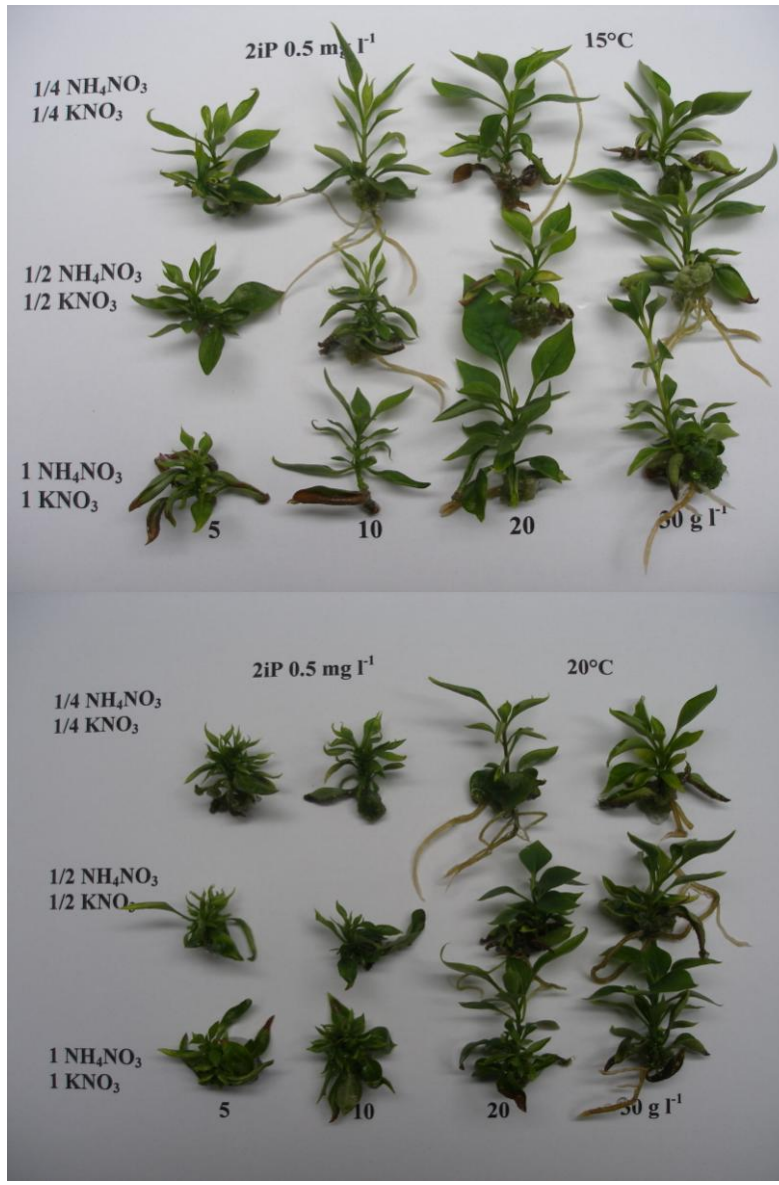



**Figure 6**. The growth of *Syringa vulgaris* shoots on the MS medium with different levels of sucrose (5, 10, 20, 30 g $l^{-1}$) and nitrogen salts (1/4 $KNO_3$, $NH_4NO_3$ – 25%; 1/2 $KNO_3$, $NH_4NO_3$ – 50%; 1 $KNO_3$, $NH_4NO_3$ – 100% in relation to the MS medium) at a temperature of 15 °C and 20 °C

cultured at 15 °C had bigger leaves than the plantlets at 20 °C. Low-sucrose treatments (5-10 g $l^{-1}$), irrespective of the level of nitrogen salts, induced a compact and branched habit of shoots and inhibited root formation, especially in the plantlets cultured at 20 °C. The increase in the sucrose content in the medium resulted in a spontaneous formation of roots on the plantlets cultured in the presence of low levels of nitrogen salts. It has been reported that sucrose also strongly stimulated the development of leaf surface area in *in vitro* grown *Ceratonia siliqua*, but a decrease in the level of nitrogen salts in the medium rapidly reduced leaf size (Vinterhalter et al., 2001). Carbohydrates and nitrogen have long been reported as having an influence on adventitious root initiation. The greatest rate of root initiation on rose shoots cultured *in vitro* occurred on the media with a high sucrose-to-nitrogen ratio (Hyndman et al., 1982). Increasing sucrose concentration up to 70 g $l^{-1}$ enhanced rooting on apple shoots propagated *in vitro* (Calamar and De Klerk, 2002).

The results presented here showed that different sucrose/nitrogen salts ratios in the MS medium exerted pronounced physiological effects that were reflected in the morphology of developing shoots of *Syringa vulgaris in vitro*.

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# WPŁYW RÓŻNEGO POZIOMU SACHAROZY, SOLI AZOTOWYCH I TEMPERATURY NA WZROST I ROZWÓJ PĘDÓW *Syringa vulgaris* L. *IN VITRO*

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## S T R E S Z C Z E N I E

Celem badań było określenie wpływu różnego poziomu sacharozy (5, 10, 20, 30 g $l^{-1}$) i soli azotowych $KNO_3$, $NH_4NO_3$ (25%, 50%, 100% według pożywki MS) oraz temperatury (15 °C, 20 °C) na wzrost pędu głównego i rozwój pędów bocznych *Syringa vulgaris in vitro*. Zróżnicowany poziom sacharozy i soli azotowych w pożywce nie miał istotnego wpływu na wzrost wydłużeniowy pędu głównego lilaka oraz na powstawanie węzłów na pędzie głównym i pędach bocznych. Aktywacja pąków kątowych i wzrost pędów bocznych na pożywce MS zawierającej niskie stężenie 2iP 0,5 mg $l^{-1}$ zależała od stężenia sacharozy i soli azotowych oraz temperatury. Spośród różnych stosowanych proporcji sacharozy/soli azotowych, najwięcej pędów bocznych (4,2) stwierdzono u roślin rosnących w temperaturze 20 °C na pożywce zawierającej najniższy poziom sacharozy – 5 g $l^{-1}$ i 100% stężenie $KNO_3$ i $NH_4NO_3$.

Wzrost stężenia sacharozy w pożywce istotnie hamował aktywację i wzrost pędów bocznych u kultur rosnących w obydwu temperaturach. Z drugiej strony, wysoki poziom sacharozy w pożywce stymulował wzrost świeżej masy pędów. Kultury pędów rosnące w obecności 30 g $l^{-1}$ sacharozy produkowały większą ilość świeżej masy w temperaturze 20 °C niż w 15 °C. Różne stężenia soli azotowych, przy tym samym poziomie sacharozy w pożywce, nie wpływały istotnie na wzrost świeżej masy pędów, natomiast wzrost stężenia soli azotowych, przy wszystkich badanych poziomach sacharozy, istotnie zwiększał aktywację pąków kątowych i rozwój pędów bocznych. Zwiększenie poziomu soli azotowych w pożywce częściowo przeciwdziałało hamującemu wpływowi sacharozy i stymulowało aktywność pąków kątowych oraz wzrost pędów bocznych lilaka. Obserwowano interakcje pomiędzy stosunkiem sacharozy/soli azotowych w pożywce a aktywacją pąków kątowych. Niska relacja sacharozy do soli azotowych stymulowała aktywację pąków i krzewienie pędów, natomiast wysoki stosunek sacharozy/soli azotowych hamował powstawanie pędów bocznych.

Zróżnicowany poziom sacharozy i soli azotowych w pożywce oraz temperatura wpływały na morfologię pędów lilaka. Wzrost stężenia sacharozy w pożywce silnie stymulował wzrost powierzchni blaszki liściowej, natomiast wpływ poziomu soli azotowych był bardzo słaby. Pędy rosnące w temperaturze 15 °C charakteryzowały się większymi blaszkami liściowymi w porównaniu z pędami rosnącymi w 20 °C. Niskie stężania sacharozy, niezależnie od zawartości soli azotowych w pożywce, powodowały zwarty i silnie rozgałęziony pokrój pędów, natomiast wysokie stężenia sacharozy sprzyjały spontanicznemu powstawaniu korzeni w obecności niskiego poziomu azotu.

**Słowa kluczowe**: lilak, sacharoza, sole azotowe, pędy boczne, mikrorozmnażanie