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In vitro propagation of *Syringa vulgaris* L. cultivars

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The propagation of lilac (*Syringa vulgaris* L.) sorts *in vitro* was studied in order to improve the propagation protocol. It has been established that a convenient time for single node explant isolation from young shoots is the period when generative buds blossom out. Microcutting type (single node or tip) and cutting density in the cultivation vessel had a little effect on microshoot development during propagation. The media containing 150% Murashige–Skoog (MS) macrosalts stimulated shoot proliferation better than did MS 100% or Anderson's (1984) macrosalt composition. Cytokinins BAP or 2iP (1; 3 mg/l) together with NAA 0.05 mg/l and IAA 0.15 mg/l improved shoot growth during micropropagation. The *ex vitro* acclimatization and rooting took a month; the efficiency was approximately 99%. The growth of the plantlets transferred to open field was stimulated fertilizing them with NH_4NO_3 and KH_2PO_4 . The fertilization had no effect on the concentration of photosynthetic pigments.

Key words: *Syringa*, lilac, micropropagation, fertilization

INTRODUCTION

Syringa vulgaris varieties are widely used ornamental plants. Their propagation by grafting or using other traditional vegetative propagation methods is less productive than by *in vitro* technique. However, there are not much data to present the peculiarities of lilac micropropagation. The influence of cytokinins, rooting and *ex vitro* acclimation has been reported [1–3]. In this study, the obtained experience in shoot culture establishment, micropropagation, rooting and *ex vitro* acclimation, as well as fertilization of plantlets during the first season of vegetation in the open field is reported with the aim to improve the lilac propagation protocol.

MATERIALS AND METHODS

Plant material and culture initiation

Single node cuttings from young shoots of *Syringa vulgaris* L. ('Charles Sargent', 'Decaisne', 'Condorcet', 'Ogni Donbassa') were sampled six times from the beginning of lilac florescence until the fade (May – beginning of June) from the open fields of the Botanic Garden of the University of Latvia. The shoots were washed with antibacterial soap (Safequard, Procter & Gamble, Amiens, France), then the surface sterilised in diluted commercial sodium hypochlorite (0.75%) for 15 min and rinsed three times in sterile distilled water. Individual single node cuttings were planted vertically in a medium containing Anderson's (1984) [4] and Murashige–Skoog's (1962) [5] macrosalts (7 : 3), Anderson's (1984)

microsalts, 20 g l⁻¹ sucrose, 10 g l⁻¹ glucose, 100 mg l⁻¹ inositol, 1.2 mg l⁻¹ thiamine, 0.3 mg l⁻¹ pyridoxine, 1.5 mg l⁻¹ nicotinic acid, 6.1 g l⁻¹ agar (Kräuter-Mix GmbH, Abtswind, Germany), 0.2 mg l⁻¹ 6-benzylaminopurine (BA), 0.2 mg l⁻¹ N6-[2-isopentenyl]adenine (2iP), 0.15 mg l⁻¹ indole-3-acetic acid (IAA), 0.05 mg l⁻¹ α -naphthaleneacetic acid (NAA). The medium pH was adjusted to 5.8 prior to autoclaving. The cultures were incubated under cool white fluorescent lamps (35–50 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with a 16-h photoperiod at 25 \pm 2 °C. For each sampling, 10 to 20 explants per cultivar were used.

Shoot multiplication

'Primrose' and 'Königin Luise' microshoot cutting type and their density influence on shoot multiplication were tested using the above medium. Shoot development was examined comparing cuttings with shoot tip or single node (five cuttings per vessel). The effect of cutting density was observed cultivating 5, 10 or 15 cuttings per vessel. Each treatment contained 5 vessels (250 ml).

The influence of inorganic salts on 'Liega' and 'Dobeles sapņotājs' shoot multiplication was tested varying macrosalt composition: Murashige–Skoog (1962) 100%, 150%, Anderson (1984) 100% or Anderson together with Murashige–Skoog (7 : 3). Each treatment contained 20 replicates, one cutting per vessel.

The influence of auxins and cytokinins on 'Primrose' was examined using the above medium as the base and varying the concentration and composition of BA, 2iP, IAA, NAA. Each treatment contained 20 replicates, one cutting per vessel.

The above cultivation conditions were used. The duration of cultivation in each experiment was 9 to 11 weeks.

Rooting and *ex vitro* acclimation

The rooting and *ex vitro* acclimation were performed in a single step – in a peat substrate. Plantlets were grown under plastic bags to maintain a high relative humidity in the air. Rooted plantlets were exposed to the normal glasshouse environment until they grew 2–5 cm in length.

Plantlet growth in the open field

The influence of N, P, K nutrition on plantlet development just after their transfer to the open field was evaluated fertilizing ‘Liega’ and ‘Königin Luise’ with NH₄NO₃ and KH₂PO₄. The plantlets (2–5 cm in length) were transplanted into 1 l pots (single plant per pot) with SIA “Latflora” Kaiġu peat KKS-U (the best substrate for lilac propagation in comparison with Olaine peat or garden soil, unpublished data), pH 5.96, N 90.0 mg l⁻¹, P 94.0 mg l⁻¹, K 300 mg l⁻¹ (colorimetrically tested [6] amounts of nutrition elements in a substrate before the experiment). Plantlets were transferred in the open field and fertilized first after two weeks and after three weeks for the second time with a nutrition solution: N 35 mg, P 20 mg, K 90 mg per plantlet. After the fourth and fifth weeks N was 17.5 mg, P 20 mg and K 90 mg per plantlet. The last measurement of the shoot length was made in the tenth week.

Assessing the results of plantlet development *in vitro* and *ex vitro*, their length was measured and in some experiments shoots number counted.

Chlorophyll analysis

The content of chlorophyll a and b and carotinoides was measured spectrophotometrically (CФ-26) using the method de-

scribed by Gavrilenko [7] in fresh leaves (0.1 g) in a solution of 96% ethanol.

The results presented were means ± standard deviations.

RESULTS AND DISCUSSION

It was found that the best time for explant isolation was from the beginning of lilac florescence. The explants isolated during the fade produced callus and their capacity of shoot production decreased.

During shoot multiplication, single node microcuttings produced shoots approximately in the same amount and length as did shoot tip microcuttings (Fig. 1). Usually one shoot developed from a lilac microcutting, therefore the number of shoots was not used as an indicator in further experiments. The density of microcuttings had a little effect on shoot length (Fig. 2). Murashige–Skoog macrosalt mixture (150%) stimulated the growth of lilac shoots as indicated by Pierik [2]. Our results supported these findings: 150% MS was more efficient than a lower macronutrient content or Anderson’s mixture (Fig. 3). A better shoot development under the effect of 2iP influence had been shown [1, 2]. However, our results indicated that shoot growth was stimulated by cytokinins together with auxins. The shoots

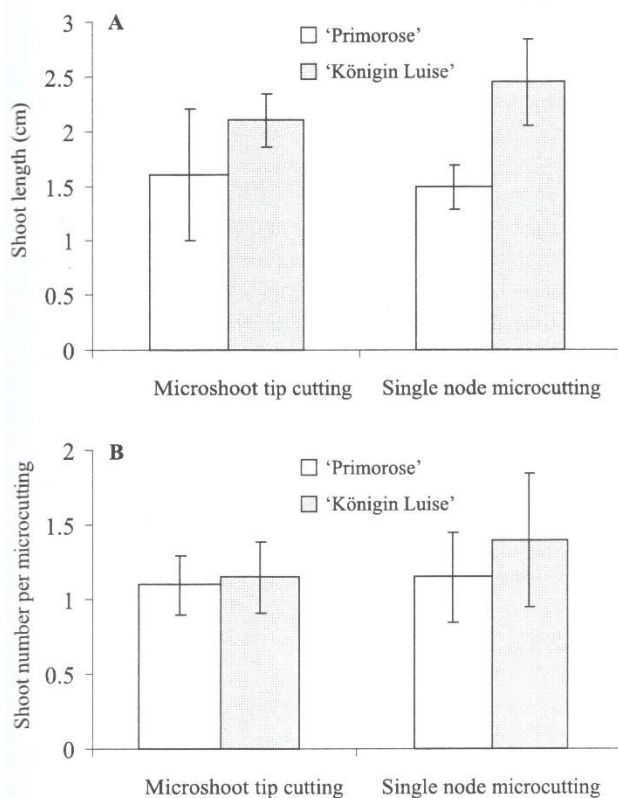


Fig. 1. Shoot growth from different microcuttings during multiplication stage (results after 9 weeks of cultivation). A – mean shoot length, B – mean shoot number per microcutting

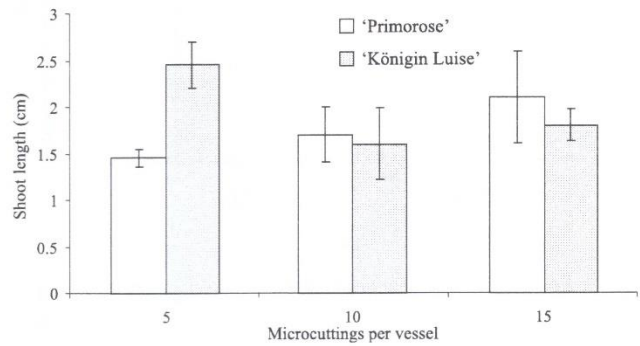


Fig. 2. Effects of microcutting density on shoot growth during multiplication (results after 9 weeks of cultivation)

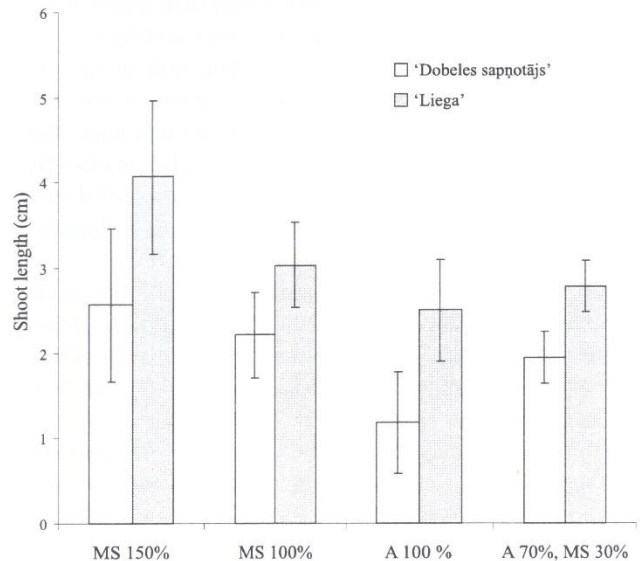


Fig. 3. Effects of macrosalts on shoot growth during multiplication (results after 9 weeks of cultivation)

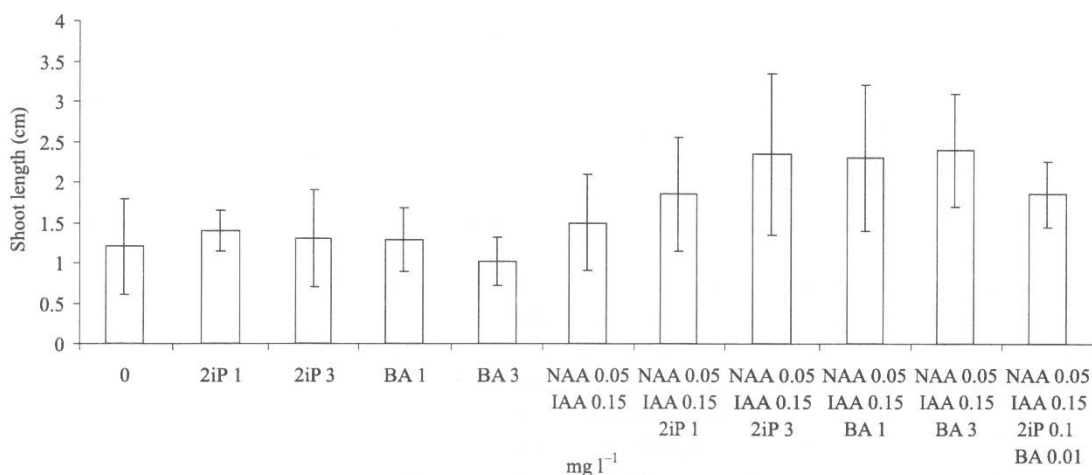


Fig. 4. Effects of auxins and cytokinins on 'Primrose' shoot growth during multiplication (results after 10–11 weeks of cultivation)

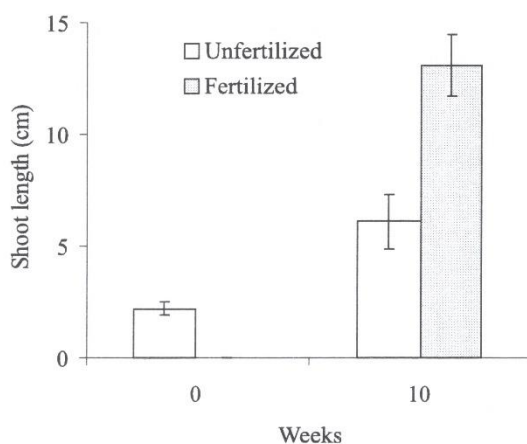


Fig. 5. Influence of N, P, K feeding on 'Königin Luise' plantlet growth in the open field

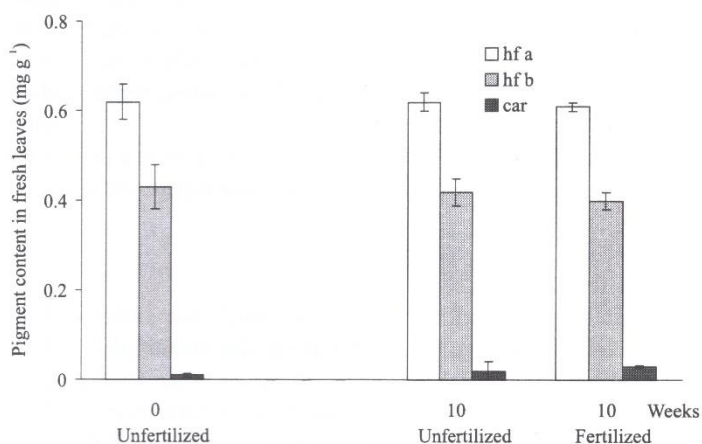


Fig. 6. Influence of N, P, K feeding on the content of photosynthetic pigments in 'Königin Luise' leaves

grown on media with 2iP or BA 1 or 3 mg l⁻¹ together with IAA 0.15 mg l⁻¹ and NAA 0.05 mg l⁻¹ were longer than shoots grown on media supplemented with auxins or cytokinins (Fig. 4).

The rooting and *ex vitro* acclimatization were an essential part of micropropagation. For mass propagation, it was economically profitable to integrate both processes in a single step. *Syringa vulgaris* shoots were rooted *in vitro* [2] or *ex vitro* [1]. Our experience with 28 cultivars showed that a successful rooting performed in a single step together with *ex vitro* acclimatization took approximately a month and the survival was 99%.

Much attention was given to mineral fertilization of plants; particularly important elements for them were nitrogen, phosphorus and potassium. The recommendations for lilac fertilization concern ordinary propagated plant growth [8, 9], but there was no information about effects of fertilization on plantlet growth in the first period in open field after *in vitro* propagation. The results indicate that plantlet growth in the open field was stimulated by N, P and K feeding (Fig. 5). In the first two fertilizations N was 35 mg per plantlet. A twice higher amount of N (70 mg) during this time stimulated the growth at the same level as N 35 mg (unpublished data). In the third and the fourth fertilizations N was decreased to 17.5 mg per plantlet to promote

their maturation. Higher P (40 mg) and K (180 mg) doses than used during the experiment (P 20 mg, K 90 mg per plantlet) were not useful, because P and K were found to accumulate in the soil and plantlets did not use them (unpublished data). Fertilization showed not significant influence on the content of photosynthetic pigments in the leaves of lilac (Fig. 6).

In our experience, in lilac propagation it was possible to assume that growth *in vitro* is influenced mainly by cultivation conditions, however, success in culture initiation and multiplication efficiency greatly depended on the taxon. On the other hand, *ex vitro* acclimatization, rooting and further development were successful for all 28 lilac cultivars tested.

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