* VEGETATIVE PROPAGATION OF SYRINGA VULGARIS L. IN VITRO

R.L.M. Pierik, H.H.M. Steegmans, A.A. Elias, O.T.J. Stiekema, and A.J.van der Velde.
Department of Horticulture
Agricultural University
P.O.Box 30
6700 AA Wageningen
The Netherlands

Abstract

Excised shoot tips from adult S y r i n g a v u l g a r i s L. plants were rejuvenated by repeated subculturing in vitro. The number of subcultures required to rejuvenate the shoots was strongly dependent on the age and genotype of the plant material. Three rootstocks (K8, A2 and A3) and 5 cultivars (Mademoiselle Marie Legray, Madame Florent Stepman, Maréchal Foch, Hugo Koster and Herman Eilers) were studied in vitro.

The basic culture medium for isolation, subculture and shoot elongation contained: Murashige and Skoog (MS) macro-salts 1.5 strength, MS micro-salts (except Fe), NaFeEDTA 50 mg/l, saccharose 3.5%, 2iP 0.8 mg/l, Difco Bacto-agar 0.8%, pH 6.0 and distilled water. The cultures were incubated in a growth room at 21°C and a day/night schedule of 16 h fluorescent light/8 h darkness.

After rejuvenation, cloning was realized by repeated single-node culture. The application of a cytokinin and low irradiance (4-5 W m^{-2}) induced stem elongation from pre-formed buds in the axils of the leaves. Zeatin(riboside) was most effective for stem elongation, followed by 2iP. The cytokinins, BA and kinetin, were not very effective in inducing stem elongation. Stem elongation was dependent on the genotype used. High cytokinin levels (2-5 mg/l) induced axillary branching but hardly any stem elongation and the leaves were strongly curled. Single-node culture and stem elongation in a medium containing 1.0 mg/l 2iP was definitely the best method of propagating lilac in vitro.

Shoots were easy to root on a cytokinin-free medium with auxin or on an artificial substrate (rock wool) after an auxin dip. Acclimatization of in vitro rooted shoots to greenhouse conditions was successful when at low irradiance and high relative humidity for the first 2 weeks after transfer.

Introduction

In horticulture S y r i n g a v u l g a r i s L. cultivars are normally propagated by budding (also called bud-grafting) or grafting on seedling rootstocks because taking of cuttings is often unsuccessful. Lilacs for forcing in winter, are in most cases budded on S y r i n g a v u l g a r i s from seedling origin because other S y r i n g a species are not very suitable (shoot production from the roots, bad root ball formation); S y r i n g a v u l g a r i s seeds are imported from East European countries. One of the most troublesome characteristics of lilac rootstocks is genetic variability, which can

* Publication 538

be seen in root ball development, shoot diameter and growth, shoot formation on the roots, and flowering behaviour, etc. (van den Berg, personal communication).

The variability within rootstock material has been the principal reason for developing micropropagation methods for lilacs. The primary aim was to select excellent rootstocks from a seedling population and to clone them in vitro. Another reason for cloning lilac cultivars in vitro, was to eliminate the rather expensive budding and grafting technique required to clone cultivars (Pierik and Steegmans, 1985; Pierik et al., 1986). Efficient cloning methods are very important in the Netherlands because it has a vast production area (87 ha) of lilacs (van den Berg, 1986).

A very restricted number of publications can be found on lilac propagation in vitro. Hildebrandt and Harney (1983) developed a shoot multiplication method by axillary branching, whereas Pierik and Steegmans (1985), Pierik et al. (1986), Einset and Alexander III (1984) and Welander (1987) successfully propagated lilac by applying the single-node method.

We choose to propagate lilacs using the single-node method because axillary branching with high cytokinin levels in the medium resulted in strongly curled and distorted leaves (Pierik and Steegmans, 1985)

2. Material and methods

Actively growing shoot tips were selected from greenhouse-grown shrubs of various ages. Three rootstocks (of seed origin) were chosen: K8 (1.5 year old, selected from 10 rootstocks), A2 and A3 (both from 50-60 year old shrubs, selected by a commercial lilac forcer in Aalsmeer). In a number of experiments cultivars were also used: Madame Florent Stepman (50 year old), Mademoiselle Marie Legray (30 year old), Hugo Koster (15 year old), Herman Eilers (45 year old) and Maréchal Foch (50 year old). Repeated subculture to obtain rejuvenation of this plant material is described in the results. Unless otherwise stated, basic experiments on stem elongation, taking of single-node cuttings, and rooting were carried out with rootstock K8.

In most experiments shoot tips and single nodes were grown on the following basic culture medium: Murashige and Skoog (1962) macro-salts at 1.5 strength, Murashige and Skoog (1962) micro-salts (except Fe) at full strength, NaFeEDTA 50 mg/l, saccharose 3.5%, Difco Bacto-agar 0.8%, pH 6.0 before autoclaving and distilled water. Basic media for stem elongation and single-node culture contained 0.8 mg/l 2iP, whereas media for adventitious rooting contained NAA (see results for concentrations used). Pyrex test tubes (diameter 20 mm) each containing 15 ml of medium were used. All media were autoclaved at 120°C for 20 min. To avoid dehydration during the 6-8 weeks culture period and to reduce the number of infections, the test tubes were 'closed' with cotton plugs, aluminium foil and Vitafilm (Good Year). Shoot tip explants from greenhouse-grown material were initially surface-sterilized as follows: a few seconds dipping in alcohol 70% (v/v), 20 min in 1% NaClO (with a few drops of Tween 20) and then rinsed 3 times (for 3, 5, and 12 min respectively) in sterile tap water.

Cultures were incubated in a growth chamber at 25°C under a schedule of 16 h photoperiod provided by fluorescent tubes (Philips TL

54/38 W, 5 Wm^{-2}) and 8 h darkness. In one experiment cultures were grown at various irradiances.

All experiments had in principle one variable factor (nutritional, hormonal, physical, etc.). Each treatment consisted of 24 test tubes each containing one shoot tip or single node. Experiments on stem elongation (figure 1) were in most cases carried out with single-node explants, whereas in the case of rooting experiments with shoot cuttings all starting material was derived from repeatedly subcultured and rejuvenated plants. A few rooting experiments were carried out in rock wool (Grodan) blocks, saturated with tap water and placed in glass containers. All experiments reported were repeated at least once during the years 1985-1987. Data presented in the figures and tables are the means of at least 2 experiments.

After 8 weeks stem elongation was evaluated by measuring stem length and counting the number of leaves and single nodes. The parameter MR (multiplication rate) represents the number of single nodes produced in 8 weeks, that are suitable for further subculturing. Rooting was evaluated after 6 weeks by determining the percentage rooting, the mean number of roots per explant and the mean root length.

3. Results

General

Numerous preliminary experiments with lilac in vitro were carried out during 1984-1985 to determine the medium and other conditions (light, temperature) as described in the material and methods. The choice of our standard conditions was mainly based on results obtained with rootstock K8, although it cannot be assumed that other rootstocks and cultivars have exactly the same requirements.

Advantages of the single-node method

Despite the fact that Hildebrandt and Harney (1983) choose the axillary-bud method to propagate lilacs in vitro, we selected the single-node method (figure 1), since axillary branching in lilacs (both rootstocks and cultivars) requires extremely high cytokinin levels in the medium (5-10 mg/l), resulting in strong leaf curling, a very compact growth habit, difficult to separate shoots, and rather abnormal plants (Pierik and Steegmans, 1985). Our discovery that the cytokinin 2iP at a concentration of 0.8-1.0 mg/l effectively induced stem elongation was the main reason for favouring the single-node method.

Rejuvenation by subculturing

It soon became clear (after isolation of shoot tips from adult plant material) that rejuvenation would be necessary to obtain rapid growth, stem elongation, and ultimately adventitious root formation in vitro. Rejuvenation was attained by frequently subculturing the shoots in vitro (Pierik et al., 1987). Rootstock K8 was the easiest to rejuvenate because this rootstock was only 2 years old at the moment of isolation. It soon became clear that rather old plants, such as the

the rootstocks A2 and A3, and the cultivars Madame Stepman, Hugo Koster. Herman Eilers, etc. were much more difficult to rejuvenate, i.e. it took many subcultures before they were rejuvenated.

Temperature

Numerous experiments in our laboratory with 3 rootstocks and several cultivars showed that relatively high temperature (27°C) as used by Hildebrandt and Harney (1983) and Einset and Alexander III (1984) was detrimental for lilac growth in vitro. Even at 25°C growth and development of lilac was abnormal: leaf curling and yellowing occurred. A temperature of approximately 21°C appeared to be optimal for stem elongation (results not shown).

Irradiance

Despite the fact that Hildebrandt and Harney (1983), Einset and Alexander III (1985) and Welander (1987) cultivated their lilac shoots at a relative high irradiance (8-10 Wm^{-2}), experiments with our rootstocks and cultivars clearly demonstrated that the irradiance should be lowered to 4-5 Wm^{-2} to induce effective stem elongation. Figure 2 shows that shoot length and MR were strongly decreased by increasing the irradiance from 2 to 10 Wm^{-2}. Since too low irradiance (1-3 Wm^{-2}) resulted in tiny leaves and very thin stems 4-5 Wm^{-2} was choosen for our experiments.

Concentration of macro-salts

Remarkable effects on stem elongation (figure 3) were obtained by varying the macro-salt strength concentration of Murashige and Skoog (MS, 1962). It is quite clear that shoot length and MR are optimal at an MS strength of 1.0 and 1.25 respectively. However, a more important effect of increasing the MS strength was that a physiological disease (leaf curling and shrinkage of the leaves) strongly declines and completely disappears at an MS strength of 1.5. For this reason most of our rootstocks and cultivars are now grown at MS 1.5 strength.

Iron nutrition

Since in our preliminary experiments yellowing of the leaves often occurred, the concentration of NaFeEDTA was varied. The results are presented in figure 4, which shows that yellowing can be prvented by adding the correct iron concentration (50 mg/l). When the iron concentration is further increased, stem elongation declines and leaf curling and shrinkage occurs.

Sugar

For most rootstocks and cultivars saccharose appeared to be more favourable than glucose as the sugar source. The influence of saccharose at various concentrations is shown in figure 5, and 3.5% saccharose is optimal for shoot length and MR.

pH

Varying the pH of the culture medium in the range 5.5-6.5 had virtually no effect on stem elongation and MR (results not shown).

Cytokinin

Preliminary experiments (Pierik and Steegmans, 1985) showed that cytokinin is one of the most important factors for stem elongation in vitro. Figures 6 and 7 show conclusively that stem length and MR are strongly dependent on the type and concentration of cytokinin used. Kinetin appeared to be rather ineffective, BA only slightly effective, whereas zeatin(riboside) and 2iP induced the best stem elongation. Since zeatin is too expensive for routine use in micropropagation, our experiments were continued with 1.0 mg/l 2iP.

Rootstock and cultivar-specific reactions

Although the various rootstocks and cultivars used in our research reacted in the same way as K8, the following differences were observed:

Irradiance: The cultivar Hugo Koster requires a slightly lower (3-4 Wm^{-2}) irradiance for stem elongation than the other lilacs.

Temperature: The cultivar Madame Florent Stepman is sensitive to higher temperatures.

Macro-salts: Madame Florent Stepman requires a much lower salt content in the medium (1.0 strength MS), whereas Hugo Koster requires 1.25 strength MS.

Sugar: The rootstock A2 and the cultivars Madame Florent Stepman and Hugo Koster require a lower saccharose concentration (3%) than the other lilacs (3.5%).

Cytokinin: The most rapid stem elongation using 2iP was obtained with K8, A2 and A3. The cultivar Hugo Koster had a slower stem elongation with 2iP. Stem elongation of Madame Florent Stepman was slow on 2iP-containing media; this cultivar reacting far better on zeatin(riboside) at a concentration of 0.5-1.0 mg/l. After numerous subcultures the cultivars Herman Eilers and Maréchal Foch failed to elongate, not even on media containing zeatin. The optimum 2iP requirement for stem elongation is rootstock/cultivar dependent: Mademoiselle Marie Legray requires 0.4-0.6 mg/l, the rootstocks K8, A3 and A2 require 0.8-1.0 mg/l, and the cultivar Hugo Koster requires 2-5 mg/l.

Rooting

When following stem elongation shoots are subcultured in vitro to induce rooting, 2iP should be ommitted. Most cultivars and rootstocks are capable of regenerating 1-2 adventitious roots per cutting after 6 weeks without auxin, although the rooting percentage does not reach 100%. Addition of auxin promotes rooting, but the auxin requirement is

low. NAA is more effective than IBA; an NAA concentration of 0.01-0.1 mg/l being optimal. When, during rooting, low 2iP (0.1 mg/l) levels are combined with NAA, stem elongation and rooting can be obtained simultaneously; by increasing the 2iP concentration to stimulate stem elongation, rooting decreases. with increasing 2iP concentrations.

Best rooting was obtained in vivo by transferring shoots, dipped in NAA at low concentration, directly into plugs of rock wool, saturated with tap water. During the rooting phase in rock wool, transpiration should be lowered by placing the rock wool plug plates in glass bottles.

4. Discussion

A comparison of the literature on lilac in vitro with the results presented here clearly shows that differences exist (table 1).

Table 1. Comparison of studies on the in vitro culture conditions required for lilac.

	Light (Wm^{-2})	Temp. (°C)	Cytokinin (mg/l)	Sugar (%)	Macro-salts (Strength)	Auxin (mg/l)
Hildebrandt* and Harney (1983)	8	27-28	BA 7.5	3	MS 1.0	IAA 0.25
Einset and Alexander III* (1984)	10	27	2iP 6.0	3	MS 1.0	not added
Pierik et al.(this paper)**	4-5	21	2iP 1.0	3.5	MS 1.5	not added
Welander (1987)**	7	21	BA 3.0	2.0	MS 1.0	0.1 NAA

* Axillary bud method used
** Single-node method used

To use the single-node method efficiently, it is necessary to obtain good stem elongation and a large number of separable nodes from an elongated stem. The ultimate number of separable nodes determines the multiplication rate. From our experiments (see table 1), it is clear that at least 5 factors strongly determine the multiplication rate: low irradiance, relative low temperature, an effective cytokinin, a relatively high saccharose concentration and a high macro-salt concentration. From table 1 it is clear that in all other reports on lilac these requirements were not (fully) fulfilled. Although not investigated here, it can be concluded from the work of Hildebrandt and Harney (1983) and Welander (1987) that auxin can be essential for multiplication.

5. References

Berg, van den, A.J., 1986. Ontwikkeling in trekheesterteelten nog lang niet ten einde. Vakblad Bloemisterij 41(41):16-23.

Einset, J.W., and Alexander III, J.H., 1984. Multiplication of Syringa vulgaris species and cultivars in tissue culture. Comb. Proc. Plant Prop. Soc. 34:628-636.

Hildebrandt. V., and Harney, P.M., 1983. In vitro propagation of Syringa vulgaris 'Vesper'. HortScience 18:432-434.

Murashige, T., and Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Pierik, R.L.M., and Steegmans, H.H.M., 1985. Vegetatieve vermeerdering van sering in vitro. Vakblad Bloemisterij 40(23):44-45

Pierik, R.L.M., Steegmans, H.H.M., and Molenberg, H., 1986. Vegetative propagation of Syringa vulgaris L. in vitro. Abstr. Congress Plant Tissue and Cell Culture 6:434.

Welander, N.T., 1987. Propagation of Syringa chinensis cv Saugeana by in vitro culture of nodal explants. J. Hort. Science 62:89-96.

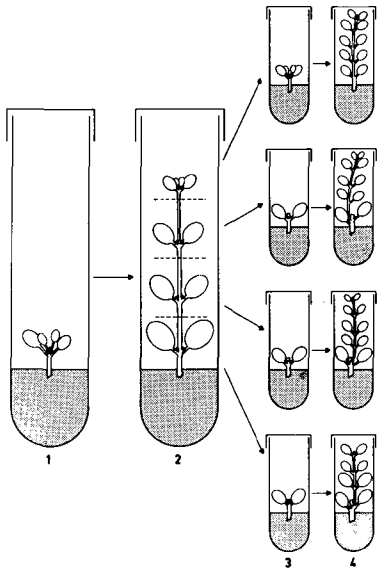

Figure 1 - Schematical representation of the single-node method.

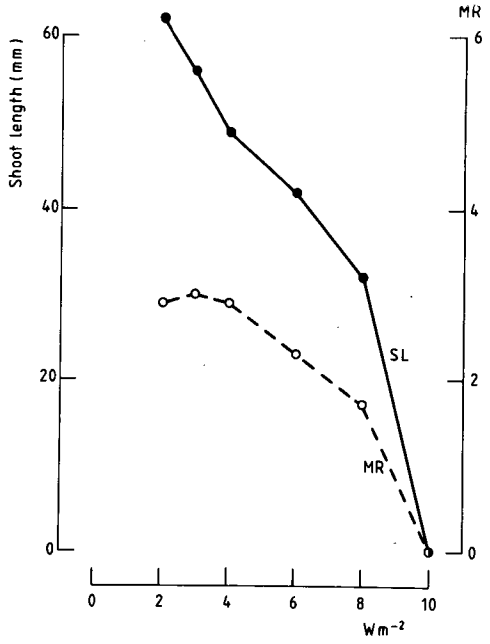

Figure 2 - The influence of the irradiance on shoot length and multiplication rate (MR) of rootstock K8.

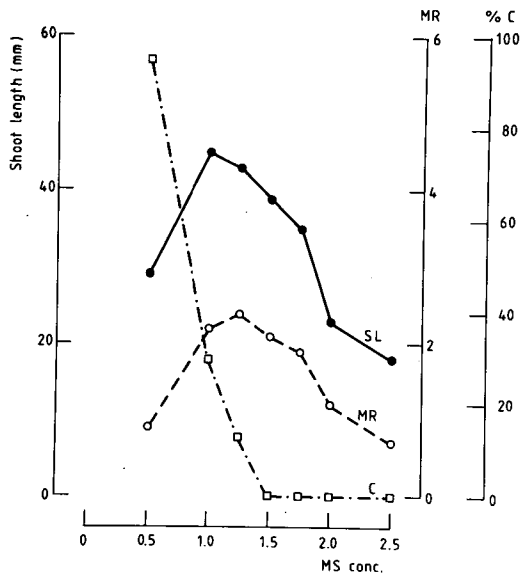

Figure 3 - The influence of the MS concentration on shoot length (SL), multiplication rate (MR), and the percentage of leaf curling (C) of rootstock K8.

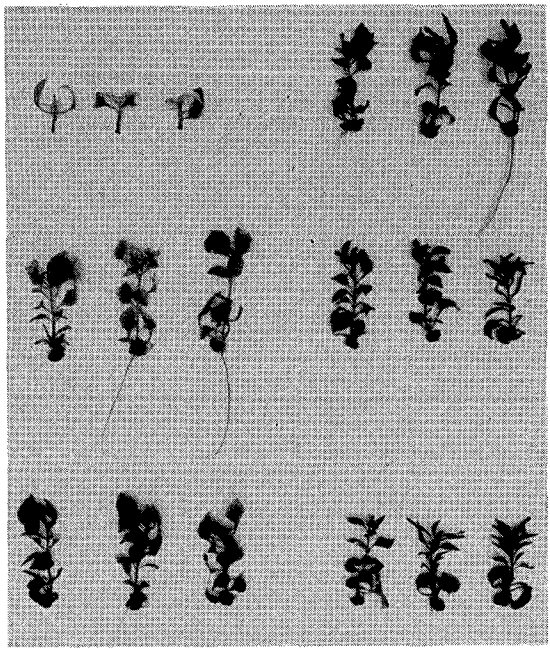

Figure 4 - The influence of the NaFeEDTA concentration on shoot length of the cultivar Mademoiselle Marie Legray. Left, from top to bottom: 0, 25, and 37.5 mg/l NaFeEDTA. Right, from top to bottom: 50, 75, and 100 mg/l NaFeEDTA.

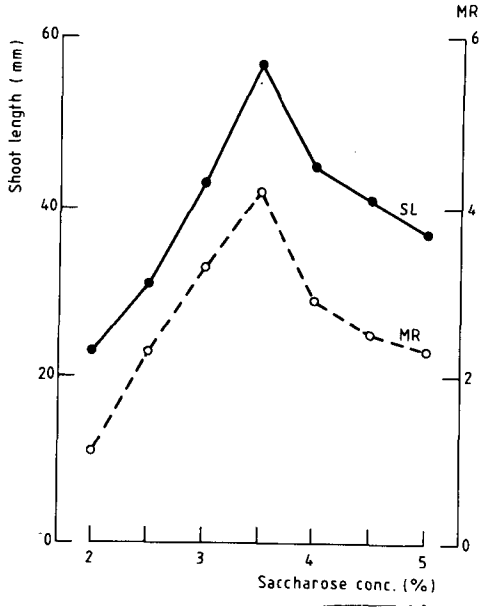

Figure 5 - The influence of the saccharose concentration on shoot length (SL) and multiplication rate (MR) of rootstock K8.

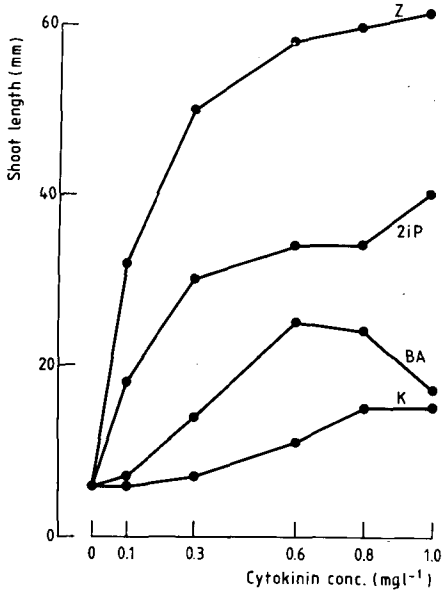

Figure 6 - The influence of the cytokinin concentration on shoot length of rootstock K8.

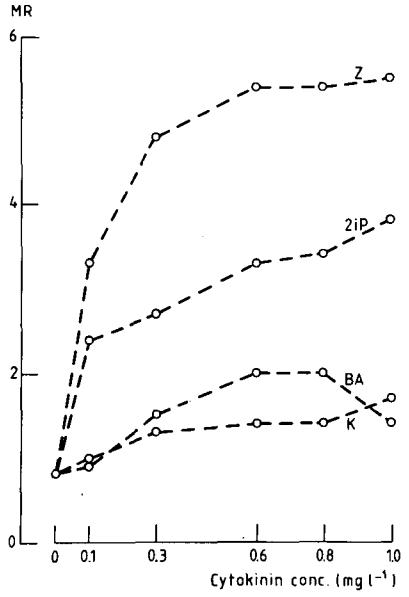

Figure 7 - The influence of the cytokinin concentration on multiplication rate (MR) of rootstock K8. K=kinetin. BA=6-benzylaminopurine. 2iP=6γ,γ-dimethylallylamino)purine. Z=zeatin.