THE EFFECT OF GELLING AGENT ON THE MICROPROPAGATION OF COMMON LILAC (*SYRINGA VULGARIS* L.)

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Abstract. In order to assess the effect of gelling agents upon lilac in vitro proliferation rate, eight types of gelling agents were tested, at concentrations considered to be optimal, based on preliminary tests as follow: 3 g/L Fibrous Agar, 6g/L Daishin Agar, 4 g/L Gelcarin, 2 g/L Gelrite, 15 g/L Isubgol, 4 g/L Micro Agar, 6 g/L Phyto Agar and 4 g/L Plant Agar. The culture media was Murashige & Skoog (MS) supplemented with 4 mg/L 6-benzylaminopurine (BAP). The plant material consisted of shoots picked from in vitro cultures on MS+0.5 mg/L BAP gelled with Plant Agar. The highest multiplication rate was 3.76, obtained in the experimental treatment with the media gelled with 15 g/L Isubgol, while the lowest proliferation rate was 1.4, on the media gelled with Daishin Agar. The shoots obtained in these eight experimental treatments were rooted ex vitro and acclimatized using the floating perlite method, in two experimental treatments: one with hormone-free floating perlite and one supplemented with 1mg/L indole-3-butyric acid (IBA). The highest rooting and acclimatization percentage (90%) was obtained in the hormone-free floating perlite experimental treatment, from shoots obtained in the media gelled with 6 g/L Daishin Agar.

Keywords: acclimatization, Daishin Agar, Isubgol, ‘Sensation’, tissue culture

Abbreviations
BAP 6-Benzylaminopurine
IAA-Indole-3-acetic acid
IBA-Indole-3-butyric acid
MS-Murashige and Skoog medium
NAA-Naphthalene Acetic Acid
TDZ- thidiazuron
Kin- kinetin

INTRODUCTION

Common lilac (*Syringa vulgaris* L.) is an attractive plant with colorful and fragrant flowers, often used in urban areas. Lilac cultivars are mainly propagated by vegetative methods to maintain genetic stability. In practice, plant nurseries reproduce the common lilac only by budding or grafting due to poor rooting results with conventional cuttings (Waldenmaier and Bünemann, 1991). However, the production of large quantities of grafts is limited by the season and the long period needed for rootstock production, and the success of propagation highly depends on the grafting technique (Ilczuk and Jagiełło-Kubiec, 2015).

Syringa vulgaris L. micropropagation was investigated by several researchers, who studied various aspects, like in vitro culture initiation, use of several plant growth regulators at various concentrations, in vitro rooting and acclimatization (Ilczuk and Jagiello-Kubiec, 2015; Parvanova et. al., 2015; Gabryszewska, 2011; Cui et al., 2009, Charlebois and Richer, 2004; Marks and Simpson, 2000). Rooting difficulties were mainly observed in the hybrids of *Syringa vulgaris*, but now, through tissue cultures, *Syringa* can be rooted to a high percentage and grown on their own roots.

Previously published reports indicate that the basal salt medium used for shoot multiplication of various *Syringa cvs*. such as 'Katherine Havemeyer' and 'Sensation' (Ilczuk and Jagiełło-Kubiec, 2015), *Syringa* x *hyacinthiflora* 'Luo Lan Zi' (Cui et al., 2009), 'Katherine Havemeyer' and 'Charles Joly' (Charlebois and Richer, 2004) was Murashige and Skoog, 1962. Tomsone et al. (2007) studied the influence of inorganic salts on 'Liega' and 'Dobeles sapņotājs' shoot multiplication by testing different macro-salt compositions such as Murashige-Skoog (1962) 100%, 150%, Anderson (1984) 100% or Anderson together with Murashige-Skoog (7:3).

The effect of auxin type and cytokinin concentrations were also examined by many authors varying the concentration and the combination of the basal medium as follow: 0.5, 1.0, 5.0 mg/L BAP or zeatin 0.5 mg/L and 1.0 mg/L in combination with 0.1 mg/L IBA (Lyubomirova and Iliev, 2013), BAP (1.00, 0.50, 0.10 mg/L) mixed with Z (0.10, 0.02mg /L) and IAA 0.25 mg/ L (Cui et al., 2009). Ilczuk and Jagiełło-Kubiec (2015) carried out ~~a~~ research about the effect of growth regulators on the proliferation rate in two *Syringa cvs*. ('Katherine Havemeyer' and 'Sensation') using 0.02 mg/L NAA in combination with BA, KIN, 2iP in concentrations of 1.25, 2.50 and 5.0 mg/L, or meta-Topolin (mT) in concentrations of 1.25, 2.5, 5.0 and 10.0 mg/L.

Likewise, chemical and physical characteristics of agars and gelled media have been studied to explain the effect of agars on in vitro cultures by many authors (Parvanova et al., 2015; Ilczuk et al., 2015; Lyubomirova et al., 2013). Scholten and Pierik, (2008) investigated the weight and number of roots on stem explants of the Rosa hybrida cv. 'Motrea' obtained on standard medium with different solidifying agents used from different brands. The results showed the different agars had a clear effect on the total weight and length of adventitious roots on explants of the flower stem of the 'Motrea' rose. Daishin Agar and Becton, Dickinson and Company purified agar performed significantly better than the others and were classified as good ones. Bacto Agar (Becton, Dickinson and Company, Difco™) grade A gave poor results. Moderate classification was given to Agar (Merck 1614), MC 29 and Bacto Agar (Becton, Dickinson and Company, Difco™) granulated.

According to the plant material used for micropropagation, the correct choice of the explant has an important role in the success of tissue culture. Thus, for the in vitro multiplication of *Syringa vulgaris,* Charlebois and Richer (2004) declare that different explant types can be used obtaining a good proliferation rate as follow: nodal segment explants (Einset and Alexander, 1985; Welander, 1987; Pierick et al., 1988), shoot apex (Pierick et al., 1988) as well as axillary buds (Hildebrandt and Harney, 1983; Gabryszewska, 1989; Waldenmair and Bünemann, 1991; Refouvelet et al. 1998).

In the acclimatization~~,~~ stage of the *Syringa vulgaris* plantlets, both rooted and non-rooted plantlets were used from the in vitro culture. In order to induce in vitro rooting of the shoots, different auxin concentrations were tested: MS medium supplemented with NAA or IBA in concentrations of 0.5, 1.0, 2.0 mg/L (Ilczuk and Jagiełło-Kubiec, 2015), MS medium with a ¼ of the quantity of macro- and microelements supplemented with 0.1, 0.5, 1.0, 3.0 and 5.0 mg/L IBA (Nesterowicz et al., 2006), MS supplemented with 1.0, 5.0 and 7.5 mg/L IBA (Lyubomirova and Iliev, 2013).

In contrast to in vitro rooting, ex vitro rooting is a promising method due to its cost effectiveness through labour, materials (auxins) and time reduction of the rooting stage (Martin, 2003). Tomsone (2007) reported a successful rooting of 28 *Syringa vulgaris* cultivars performed (in a peat substrate) in a single step together with ex vitro acclimation that took approximately a month and the survival was 99%.

The aim of our research was to improve the existing micropropagation protocols of *Syringa vulgaris* L. by investigating the influence of different gelling agents (Agar Fibre 3 g/L, Daishin Agar 6 g/L, Gelcarin 4 g/L, Gelrite 2 g/L, Isubgol 15 g/L, Micro Agar 4 g/L, Phyto Agar 6 g/L and Plant Agar 4 g/L) on *Syringa vulgaris* L. 'Sensation' cultivar and to replace the in vitro rooting stage of the plantlets obtained the tested tissue cultures with direct ex vitro rooting in floating perlite as described by Clapa et al. (2013).

MATERIALS AND METHODS

Plant material and culture initiation

Plant material: Two-node stem segments were excised with a length of 1.5-2 cm from a previously established 10-week-old in vitro culture of *Syringa vulgaris* L. 'Sensation' cultured on MS medium supplemented with 0.5 mg/L 6-benzylaminopurine (BAP) and gelled with 5 g/L Plant Agar. Growth induction and shoot multiplication were assessed by inserting the two-node segments into the media prepared with the eight different above-mentioned gelling agents. Five explants/vessel and five vessels/variants were inoculated and prepared to define the experimental design.

Culture medium: In this experiment, 8 types of culture media were tested, prepared from the Murashige & Skoog 1962 (in powdered form from Duchefa Biochemie B.V.) basal salts supplemented with 30 g/L sucrose (Cristal from the trade market) and 4 mg/L 6-Benzyladenine (BA) provided by Duchefa Biochemie B.V. and gelled with the following agents:

V1 – Agar Fibre 3 g/L - (Merck)
V2 - Daishin Agar 6 g/L - (Duchefa Biochemie B.V)
V3 - Gelcarin 4 g/L - (Duchefa Biochemie B.V)
V4 - Gelrite 2 g/L - (Duchefa Biochemie B.V)
V5 - Isubgol 15 g/L - (Natural Brand™ Colon Pure™ Unflavored, Code 350971);
V6 – Micro Agar 4 g/l - (Duchefa Biochemie B.V)
V7 – Phyto Agar 6 g/L - (Duchefa Biochemie B.V)
V8 – Plant Agar 4 g/L - (Duchefa Biochemie B.V)

Tap water was used for preparing the culture media (local tap water analysis report: http://www.casomes.ro/wp-content/uploads/2014/12/Buletin-de-calitate-Cluj.pdf). All the components were added to the media before followed by the adjustment of the pH to 5.8±0.1 with HCl or NaOH. The media were then distributed in glass containers (50 ml/container) and autoclaved at 121 °C for 20 minutes.

Culture vessels: The containers used for the in vitro culture were glass jars of 320 ml with a 4-mm filter hole on their lids to prevent the passage of micro-organisms and microbial spores. The holes on the lids were covered with a 2.5 cm width double-layered Leucotape (Duchefa Biochemie B.V).

In vitro culture incubation: The cultures were incubated in a growth chamber at 23 ±2 °C with a 16 h light/8 h dark photoperiod. The light intensity was 36 μmol m^{-2} $s^{-1}$from cool white fluorescent tubes.

Rooting and ex vitro acclimatization of the plantlets. Rooting and ex vitro acclimation of the plantlets were performed using two different methods: *1-Rooting and acclimatization of the shoots and plantlets in floating perlite without hormones added to the medium* and *2-Rooting and acclimatization in floating perlite with growth regulators added to the medium.* According to the first method, the newly developed individual shoots without

roots were taken from all the 8 types of in vitro culture and were introduced in floating perlite at equal distances between them, as suggested by Clapa et al. (2013) at the UASVM/HRS Cluj-Napoca. The cultures were partially covered with plastic lids for 2 weeks to prevent excessive humidity and to ensure suitable air circulation.

The second method used for rooting and hardening of the plantlets was similar to the first one, with the exception that 1 mg/L IBA was added to the floating perlite media to enhance rooting. Both rooting and acclimation methods were carried out under laboratory conditions.

Statistical analysis. All recorded data were subjected to analysis of variance (ANOVA) and then compared applying Tukey's multiple range test (P=0.05). The data shown are mean values± SE. The data were calculated and the scatterplots were generated using SPSS Statistics 19 and MS Excel 2016 based on Pearson's correlation coefficient.

RESULTS AND DISCUSSIONS

The influence of the gelling agent on the in vitro proliferation of *Syringa vulgaris* cv. 'Sensation'

In our experiment the highest proliferation rate (3.79) was observed after 10 weeks of in vitro culture on the medium gelled with Isubgol 15 g/L while the smallest proliferation rate (1.68) was recorded on the medium solidified with Daishin agar 6 g/L (Table 1). These results show that the gelling agent used for the in vitro culture influenced not only the number of shoots developed on the explant but also the length of the proliferated shoots. Thus, the highest mean number of shoots/explant reached 3.91±0.31 on the medium gelled with Isubgol 15 g/L and the longest shoots recorded with a mean value of 9.22±1.09 cm were developed on the media gelled with Gelcarin 4 g/L. Ilczuk and Jagiełło-Kubiec (2015) in one of their research articles recorded a regeneration rate of 91.7% on MS medium supplemented with 5.0 mg/L BAP, 0,02 mg/L NAA and gelled with 8.0 g/L Bacto Agar and a medium shoot length of 4.1 cm.

Other gelling agents, like Phytagel and guar gum, were also used and tested by Dobránszki et al. (2011), who demonstrated that both of these agents can have significant effects on in vitro multiplication of shoots of Galaxy apple and black locust. Therefore, the highest number of healthy shoots per explant for Galaxy apple (7.8) and black locust (3.6) was obtained on the media gelled with agar/guar gum and Phytagel/guar gum blends. The longest shoots for Galaxy apple had a length of 19.8 mm on the medium gelled with agar/Phytagel blend, while the longest shoots (18.8) for black locust were measured on the medium gelled with Phytagel/guar gum. The potential use of guar gum in the micropropagation of both species studied can be justified by the improved multiplication rates and reduced production costs observed for both. The agar brand on which plantlets were grown also influenced water retention capacity and water content of the shoots.

During our experiment, in the multiplication stage, we observed that *Syringa vulgaris* cv. 'Sensation' had a tendency to develop long shoots (Fig. 1. A, B) on all the variants tested reaching a mean length of 6.48 cm (Table 1). Therefore, it can be concluded that the gelling agent used played an important role in shoot development determining variations in shoot length.

Fig. 1. Lilac shoots propagated on MS medium with 4.0 mg/L BAP gelled with Isubgol 15 g/L (A and B); Ex vitro rooted plantlets in floating perlite supplemented with 1 mg/L IBA (C); Hardened plantlets (D)

Table 1.

The data shown are mean values±SE. Different lowercase letters denote the statistically significant differences between the experimental treatments according to Tuckey's HSD test at $P<0.05$

Solidifying agent	Mean number of shoots/inoculum	Mean length of the shoots (cm)	Proliferation rate
Agar Fibre 3 g/L	1.88±0.08 b	4.56±0.26 a	1.88 c
Daishin Agar 6 g/L	1.40±0.06 a	6.75±0.99 d	1.40 a
Gelcarin 4 g/L	2.52±0.19 c	9.22±1.09 f	2.52 d
Gelrite 2 g/L	2.96±0.17 d	5.09±0.57 b	2.96 e
Isubgol 15 g/L	3.91±0.31 e	5.80±1.34 c	3.76 f
Micro Agar 4 g/L	3.04±0.16 d	6.36±0.49 d	3.04 e
Phyto Agar 6 g/L	1.68±0.08 b	8.58±1.11 e	1.68 b
Plant Agar 4 g/L	3.14±0.28 d	5.55±0.91 c	3.04 e

Among the experimental treatments, the longest shoots were measured on the medium solidified with Gelcarin 4 g/L and Isubgol 15 g/L and the shortest on the media gelled with Agar Fibre 3 g/L and Daishin Agar 6 g/L. The development of long shoots in the multiplication stage of *Syringa* can constitute an advantage for micropropagation, because long shoots can increase the multiplication rate by the number of inoculi that can be excised from them and used for further subcultures. Beruto et al. (1999) stated that the type of gelling agent (Oxoid, Merck and Roth) can influence to a large extent clonal propagation of *Ranunculus asiaticus* L. through axillary bud stimulation. On Oxoid and Merck gels, growth was satisfactory, although the former had a more promotive effect on fresh and dry weight production and on multiplication rate. Until now, the proliferation rates reported by other researchers for *Syringa vulgaris* are rather low (Cui et al., 2009; Oprea and Duta, 2012; Dobránszki et al., 2011).

The influence of the gelling agent on rooting and ex vitro acclimation of the *Syringa vulgaris* L. "Sensation" plantlets

The highest rooting percentage (90%) and survival rate of the in vitro plantlets were recorded in floating perlite supplemented with 1 mg/L IBA in case of the plantlets developed on the Gelcarin 4g/L medium. Likewise, the highest rooting percentage (60%) and survival rate were observed in plantlets proliferated on the media gelled with Gelcarin 4 g/L even in floating perlite without hormones. Among the eight variants tested, the longest roots emerged with highest mean number of the roots/shoot were detected on the media solidified with Gelcarin 4 g/L (Fig. 2).

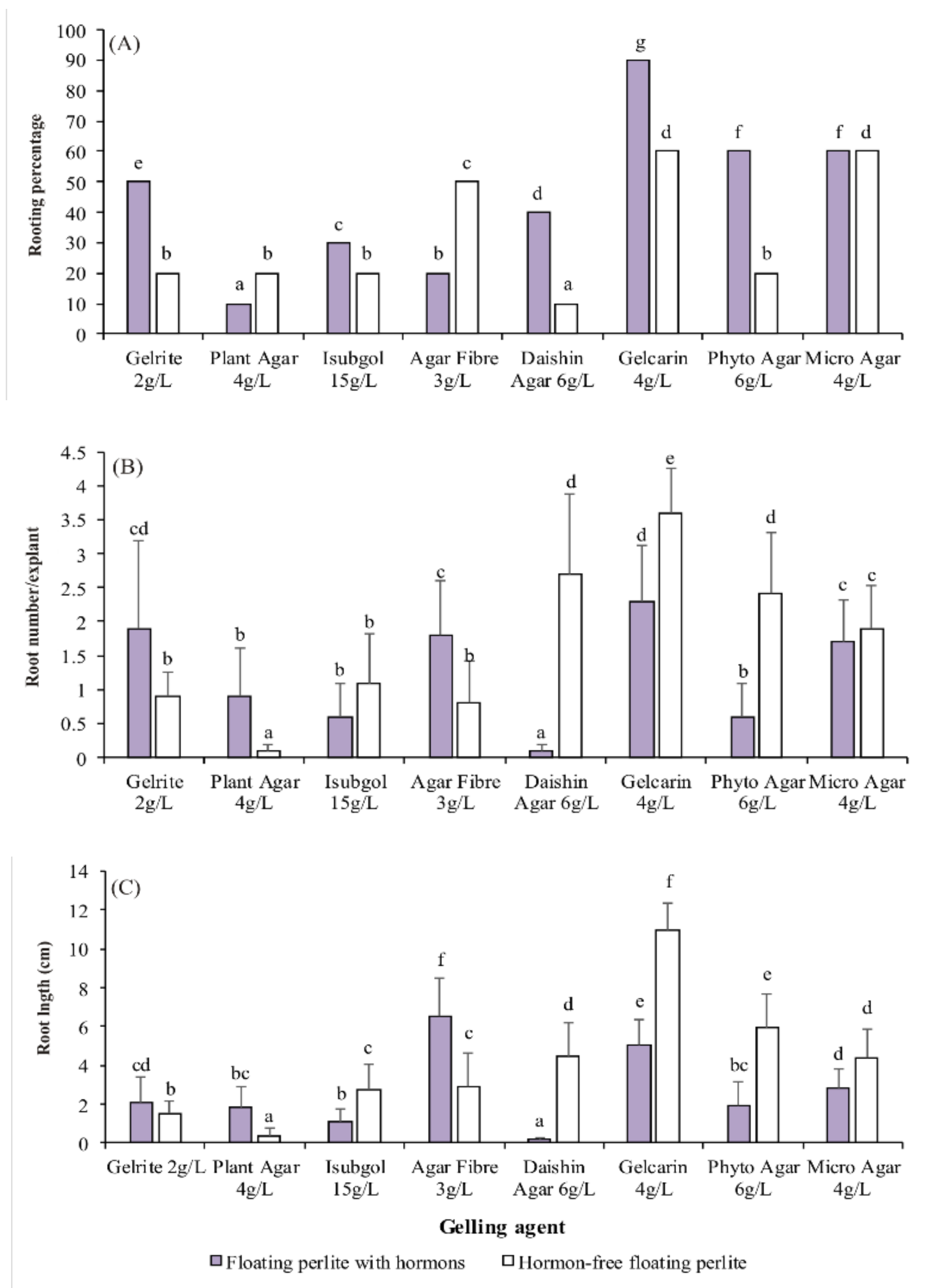

Fig. 2. Ex vitro rooting of the *Syringa vulgaris* L. cv 'Sensation' plantlets obtained on the eight tested media solidified with divers gelling agents: (A) – Rooting percentage (%), (B) – Root number/explant and (C) - Root length (cm). Mean values ± SE are shown. Different lowercase

letters indicate significant differences among the rooting traits recorded in the eight experimental treatments tested

Pearson's correlation analysis based on the mean values of rooting traits for both methods (floating perlite with and without hormones) were used to estimate the correlation coefficients among rooting percentage, number of roots/explant and root length.

The results show that there were significant interactions ($P<0.05$) between the number of roots-rooting percentage and root length-rooting percentage respectively (Fig.3 and 4).

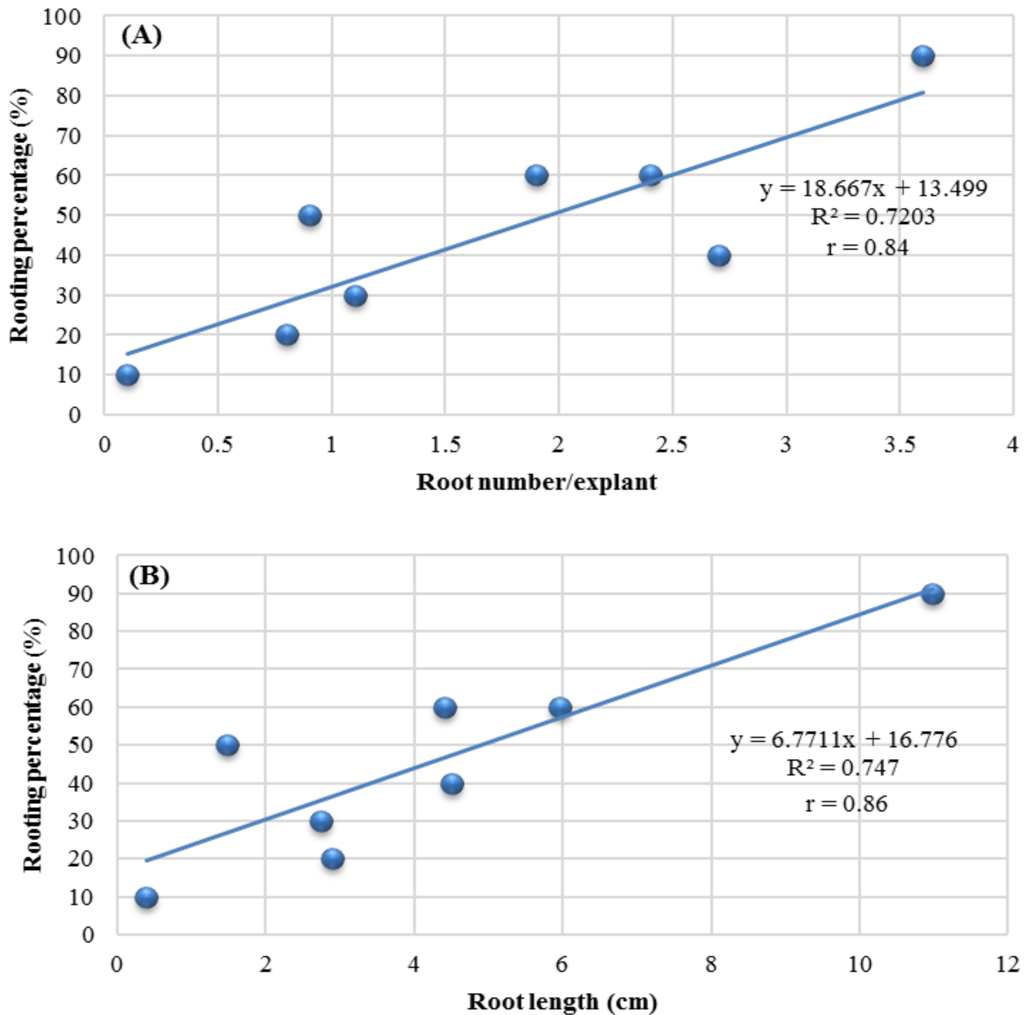

Fig. 3. Scatterplots showing the strong positive linear relationships in hormone-free floating perlite between: (A) - the number of roots and rooting percentage and (B) - root length and rooting percentage

The results of our study revealed that the type of gelling agent and its concentration applied as a solidifier of the culture medium used for micropropagation, can considerably

influence not only the proliferation rate and rooting traits of the newly regenerated shoots but also the shoot length.

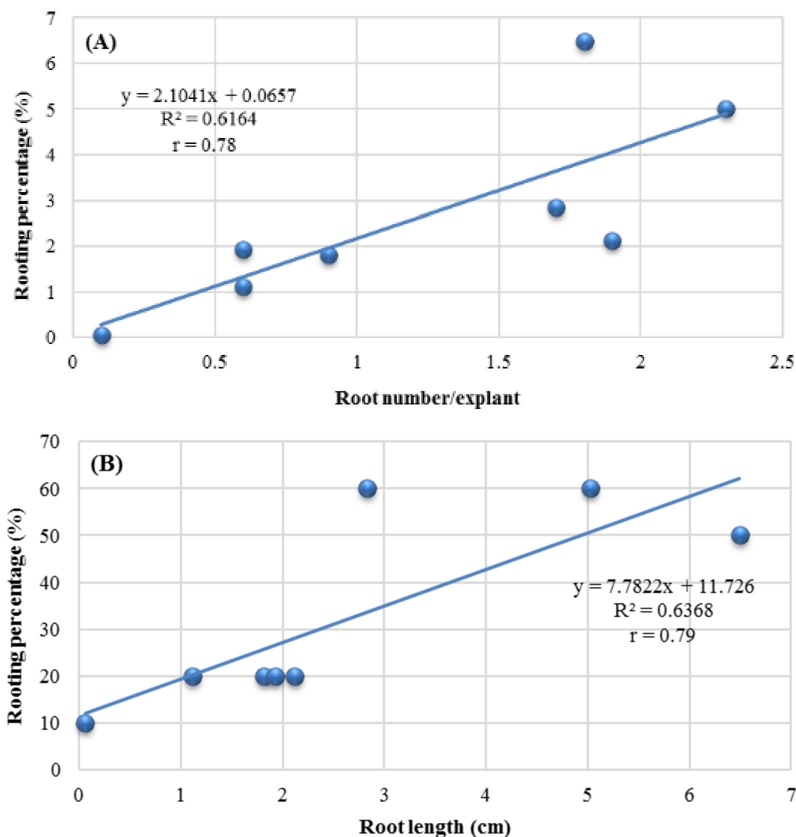

Fig. 4. Scatterplots showing the strong positive linear relationships in floating perlite supplemented with IBA between: (A) - the number of roots and rooting percentage and (B) - root length and rooting percentage

Acknowledgments. This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI-UEFISCDI, project number PN-III-P2-2.1.-BG-2016-0046, within PNCDI III.

REFERENCES

1. Beruto M., Curir P., and Debergh P. (1999). Influence of agar on in vitro cultures: II. Biological performance of ranunculus on media solidified with three different agar brands, In Vitro Cell. Dev. Biol. Plant, 35:94-101.
2. Charlebois D. and Richer C. (2004). In vitro propagation of Syringa vulgaris ‘Katherine Havemeyer’ and ‘Charles Joly’. Can. J. Plant Sci., 84:279–289.
3. Clapa D., Fira A., Joshee N. (2013). An efficient ex vitro rooting and acclimatization method for horticultural plants using float hydroculture, Hort. Sci., 48(9):1159-1167.
4. Cui H., Gu X., Shi L. (2009). In vitro proliferation from axillary buds and ex vitro protocol for effective propagation of Syringa x hyacinthiflora ‘Luo Lan Zi’, Sci. Hortic., 121: 186-191.

5. Dobránszki J., Magyar-Tábori K., Tombácz E. (2011). Comparison of the rheological and diffusion properties of some gelling agents and blends and their effects on shoot multiplication, Plant Biotechnol. Rep., 5:345–352.
6. Einset J. W. and Alexander J. H. (1985). Multiplication of Syringa species and cultivars in tissue culture. Int. Plant Prop. Soc., 34: 628–636.
7. Gabryszewska, E. (1989). A preliminary study on in vitro propagation Syringa vulgaris L. Acta Hortic., 251:205–208.
8. Gabryszewska, E. (2011). Effect of various levels of sucrose, nitrogen salts and temperature on the growth and development of Syringa vulgaris L. shoots in vitro. J. Fruit Ornam. Plant Res., 19(2): 133-148.
9. Hildebrandt V. and Harney P. M. (1983). In vitro propagation of Syringa vulgaris 'Vesper'., Hort Sci., 18:43-434.
10. Ilczuk A. and Jagiełło-Kubiec K. (2015). The effect of plant growth regulators and sucrose on the micropropagation of common lilac (Syringa vulgaris L.). Ann. Warsaw Univ. Life Sci. – SGGW, Horticult. Landsc. Architect. 36:3-12.
11. Lyubomirova T. and Iliev I. (2013). In vitro propagation of Syringa vulgaris L. Forestry Ideas, Vol. 9, No 2 (46):173–185.
12. Marks T. R., Simpson S. E. (2000). Rhizogenesis in Forsythia x intermedia and Syringa vulgaris; application of a simple internode experimental system. Plant Cell Rep., 19:1171-1176.
13. Martin K.P. (2003). Rapid in vitro multiplication and ex vitro rooting of Rotula aquatica Lour., a rare rhoeophytic woody medicinal plant, Plant Cell Rep., 21:415–420.
14. Murashige T., Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15: 473–497.
15. Nesterowicz S., Kulpa D., Moder K., Kurek J. (2006). Micropropagation of an old specimen of common lilac (Syringa vulgaris L.) from the dendrological garden at Przelewice. Acta Sci. Pol., Acta. Sci. Pol-Hortoru Hortoru., 5(1):27–35.
16. Oprea M. I., Duţă M., Concioiu M. E. (2012). In vitro micropropagation biotechnology improvement of Syringa vulgaris L. species. Current Trends in Natural Sciences, 1:1.
17. Parvanova P., T. Lyubomirova, N. Tzvetkova, V. Ilinkin. (2015). Effect of auxin on protein content during in vitro rhizogenesis of Syringa Vulgaris L., Annuaire de l'Université de Sofia "St. Kliment Ohridski" Faculte de Biologie, volume 100, livre 4, pp. 208-215.
18. Pierik, R. L. M., Steegmans, H. H. M., Elias, A. A., Stiekema, O. T. J. Van der Velde, A. J. (1988). Vegetative propagation. of Syringa vulgaris L. in vitro. Acta Hortic. 226: 195–204. DOI: 10.17660/ActaHortic.1988.226.22.
19. Refouvelet E., Le Nours S., Tallon C. and Daguin, F. (1998). A new method for in vitro propagation of lilac (Syringa vulgaris L.): regrowth and storage conditions for axillary buds encapsulated in alginate beads, development of a pre-acclimatisation stage. Sci. Hortic. 74: 233–241.
20. Scholten H. J. and R. L. M. Pierik. (2008). Agar as a gelling agent: chemical and physical analysis, Plant Cell Rep., 17:230–235.
21. Tomsone S., Galeniece A., Akere A., Priede G., Zīra L. (2007). In vitro propagation of Syringa vulgaris L. cultivars. Biologija 53:28–31.
22. Waldenmaier S. and Bünemann G. (1991). Ex vitro effects in micropagation of Syringa L. Acta Hortic. 300:201-209.
23. Welander N. T. (1987). Propagation of Syringa chinensis cv 'Saugeana' by in vitro culture of nodal explants. J. Hortic. Sci., 62: 89–96.