IN VITRO MICROPROPAGATION BIOTECHNOLOGY IMPROVEMENT OF *SYRINGA VULGARIS* L. SPECIES

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Abstract
The paper's main objectve was to compare the efficienty of in vitro and in vivo rooting of lilac microcuttings belonging to the 'Madame Lemoine', 'Charles Joly' and 'Sensation' cultivars. The results obtained allow the recommendation of in vivo rooting simoultaneously with the acclimatization, shortening the rooting time of microcuttings and production cost, which will lead to an improvement of the in vitro micropropagation biotechnology of lilac.

Keywords: lilac, in vitro, in vivo, rooting %, acclimatizing %

1. INTRODUCTION

The research regarding the establishment of *in vitro* micropropagation biotechnology of lilac were initiated in 2007, in the Laboratory of Plant Biotechnologies from Pitesti University.
From my experience in the field of landscape gardening I observed a high interest for some lilac cultivars, especially for those white and red – purplish inflorescences. The large range of the *Syringa* genus allowed to be chosen into study three genotypes: 'Madame Lemoine', 'Charles Joly' and 'Sensation', their ecological and ornamental particularities being in concordance with the market requirements (elegant shape, attractive colors, different blooming periods, low temperature resistance, soil adaptability, association with other ornamental species in garden landscapeing).

2. MATERIAL AND METHOD

The undertakeing research fallowed the highlightning of the factors implicated in the *in vitro* and *in vivo* rooting capacity, simultaneously with acclimatization for the microcuttings of 'Madame Lemoine', 'Charles Joly' and 'Sensation' cultivars.
I. For *in vitro* rooting. At the end of the multiplication phase, the microcuttings belonging to the three cultivars taken into study, bouquets-like, were individualized and fragmented into two nodes microcuttings. As in the multiplication phase, the foliage area was reduced.
In the *in vitro* rooting phase, the trimmed microcuttings were transfered on specific nutrient media (Hildebrandt V. & Harney P.M., 1983; Tomsone S. et al., 2007), and in each culture vessel were 25 microcuttings.
The work procedure was ended by marking the culture vessels, their covering with poliethilene protective film and their incubation in the growing chamber.
For *in vitro* rooting of lilac microcutting resulted from the multiplication phase, were tested three vatriants of nutrient media (Murashige T. & Skoog F., 1962; Linsmaier E.M. & Skoog F., 1965; Lee E.M.C. & De Fossard R.A. , 1977).
The experience is trifactorial, 3×3×3 type, totalizing 27 variants (Table 1).

Factor A – Genotype (Figure 1)

- A.1 – Cultivar 'Madame Lemoine'
- A.2 – Cultivar 'Charles Joly'
- A.3 – Cultivar 'Sensation'

Factor B - Nutrient medium (Table 2)

- B.1
- B.2
- B.3

Factor C - Photoperiod

- C.1 - photoperiod 12 ore
- C.2 - photoperiod 14 ore
- C.3 - photoperiod 16 ore.

Table 1. Expression of in vitro rooting capacity of lilac, depending on genotype, nutrient medium and photoperiodism

Variant	% rooted plants	Variant	% rooted plants
V.1: A.1, B.1, C.1	93,2 E	V.15: A.2, B.2, C.3	67,3
V.2: A.1, B.1, C.2	92,4	V.16: A.2, B.3, C.1	66,1
V.3: A.1, B.1, C.3	89,3	V.17: A.2, B.3, C.2	65,0
V.4: A.1, B.2, C.1	89,3 E	V.18: A.2, B.3, C.3	57,0
V.5: A.1, B.2, C.2	87,2	V.19: A.3, B.1, C.1	90,2
V.6: A.1, B.2, C.3	82,4	V.20: A.3, B.1, C.2	87,5
V.7: A.1, B.3, C.1	60,0 V	V.21: A.3, B.1, C.3	82,0
V.8: A.1, B.3, C.2	63,4 V	V.22: A.3, B.2, C.1	90,7
V.9: A.1, B.3, C.3	48,2 V	V.23: A.3, B.2, C.2	90,2
V.10: A.2, B.1, C.1	78,4 E	V.24: A.3, B.2, C.3	87,0
V.11: A.2, B.1, C.2	75,1	V.25: A.3, B.3, C.1	52,4
V.12: A.2, B.1, C.3	70,8	V.26: A.3, B.3, C.2	50,0
V.13: A.2, B.2, C.1	74,4	V.27: A.3, B.3, C.3	42,1
V.14: A.2, B.2, C.2	74,1		

***V – vitrified; *E – etiolated;**

Table 2. The composition of nutrient media tested for in vitro rooting

COMPOSITION	ROOTING		
	B1	B2	B3
MS Macro	1/2n	1/2n	1/2n
MS Micro	1/2n	1/2n	1/2n
MS Vitamine	n	-	-
LS Vitamine	-	n	n
NAA (mg/l)	0,5	-	-
IBA (mg/l)	-	0,6	1
G_3 A (mg/l)	-	0,1	0,1
NaFe EDTA (mg/l)	5	32	32
Dextrose (g/l)	20	30	30
Agar (g/l)	7	7	7
Activated charcoal (g/l)	-	0,3	0,3

Figure 1. Lilac cultivars: 'Charles Joly' (left), 'Madame Lemoine' (center), 'Sensation' (right) (original)

The acclimatization fallows the in vitro rooting phase and contains: the preacclimatization and the acclimatization itself.

II. For *in vivo* rooting-acclimatization. Groups of the three cultivars microcuttings, from the multiplication phase, were removed from culture vessels, cleaned and washed by the nutrient medium, after which it was proceeded to their individualization. To avoid dehydration, microcuttings remained in water throughout the operation. To stimulate microcuttings' rooting, they were treated with root stimulant for woody species.

There were used two rooting substrates: perlite and peat + perlite (1:1). Temperature was maintained at 25 ± 2°C and humidity 80-85%, by covering with polyethylene film. To avoid infections with phytopathogenic agents, were applied fungicide treatments with Topsin 0.1% every three days.

Factor A – Genotype

- A.1 – Cultivar 'Madame Lemoine'
- A.2 – Cultivar 'Sensation'
- A.3 – Cultivar 'Charles Joly'

Factor B - Nutrient medium

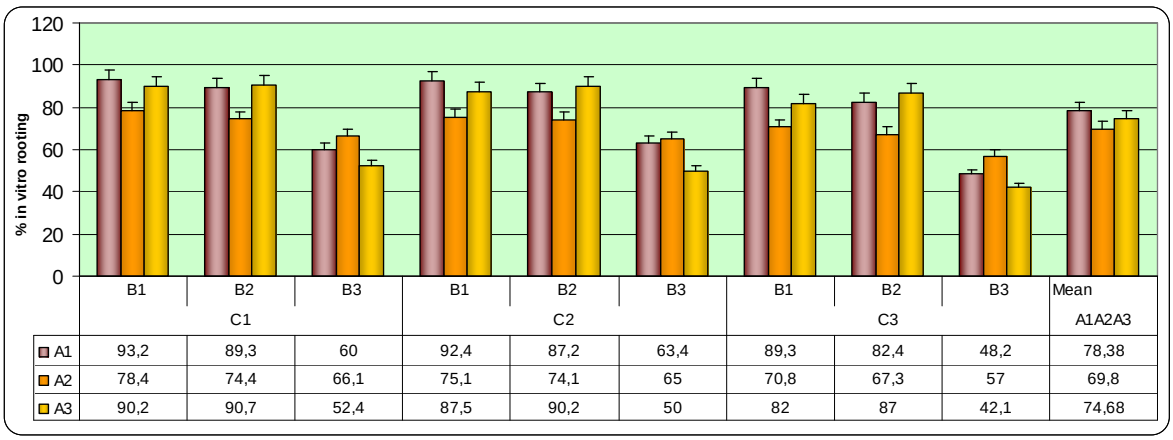
- B.1 – Perlite
- B.2 – Peat+Perlit (1:1).

3. RESULTS AND DISCUSSIONS

In vitro rooting of lilac microcuttings (Figure 2) was influenced by genotype, nutrient medium and photoperiod.

Figure 2. In vitro rooting of lilac microcuttings (original)

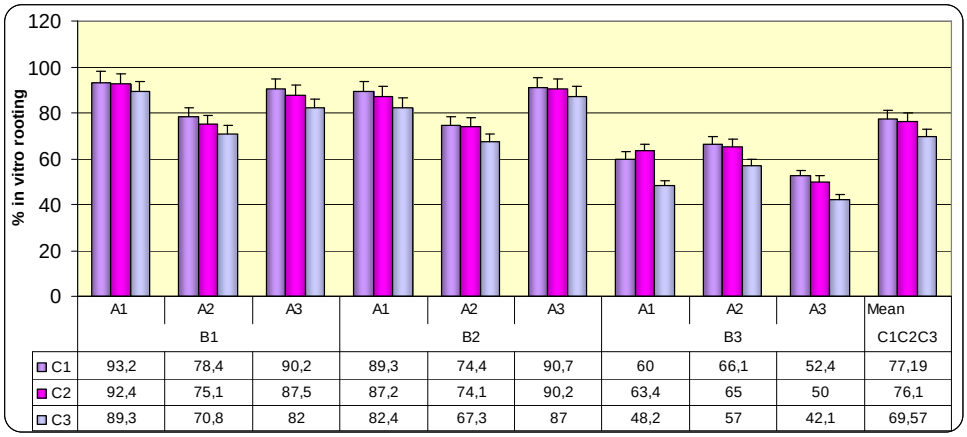
Regarding the influence of nutrient media, the best results were obtained by cultivars 'Madame Lemoine', which recorded a 92.4% of rooting in V2 variant and 89,3% in the V3 variant, on B1 nutrient medium (Figure 3).



	B1	B2	B3	B1	B2	B3	B1	B2	B3	Mean
	C1			C2			C3			A1A2A3
A1	93,2	89,3	60	92,4	87,2	63,4	89,3	82,4	48,2	78,38
A2	78,4	74,4	66,1	75,1	74,1	65	70,8	67,3	57	69,8
A3	90,2	90,7	52,4	87,5	90,2	50	82	87	42,1	74,68

Figure 3. In vitro rooting percent depending on cultivar, for different nutrient media

Both variants of nutriet media had in their composition naphthyl-acetic acid, in a concentration of 0,5 mg /l. Note that, in the version V2 the rooting process was developed in 14 hours photoperiod, and in the V3 variant in 16 hours photoperiod. For a fotoperiodism under 12 hours, the same cultivar showed a rooting percentage of 93,2%, but microcuttings showed the etiolation phenomenon (Figure 4).



	A1	A2	A3	A1	A2	A3	A1	A2	A3	Mean
	B1			B2			B3			C1C2C3
C1	93,2	78,4	90,2	89,3	74,4	90,7	60	66,1	52,4	77,19
C2	92,4	75,1	87,5	87,2	74,1	90,2	63,4	65	50	76,1
C3	89,3	70,8	82	82,4	67,3	87	48,2	57	42,1	69,57

Figure 4. In vitro rooting percent depending on photoperiod, for different cultivars

On B2 nutrient medium, 'Sensation' was noted with a rooting procent between 87,0% and 90,7%, in the presence of indole-butyric acid, at a concentration of 0.6%. Shortening the lighting to 12 hours influenced favorably the microcuttings' rooting of this cultivar (Figure 5).
Increasing the concentration of indole-butyric acid from 0.6 mg/l on B2 medium at 1.0 mg/l on B3 medium, did not stimulate the microcuttings rooting, as it was evidenced by the results. In the process of *in vivo* rooting (Figure 6.) the rooting substrate of perlite favorably influenced 'Madame Lemoine' and 'Sensation' cultivars rooting, the values obtained being of 93% for 'Madame Lemoine' and 91,7% for 'Sensation' (Table 3, Figure 7).

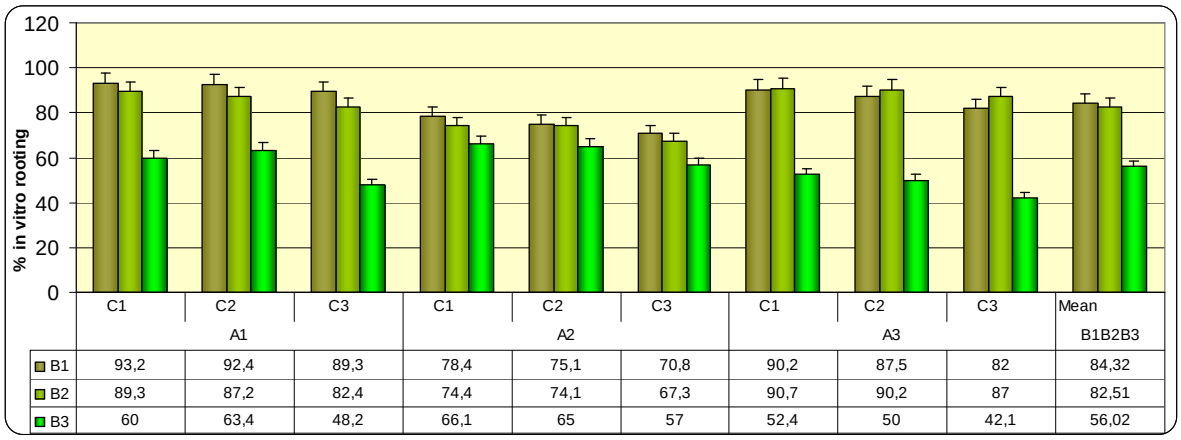


Figure 5. In vitro rooting percent depending on nutrient medium, for different photoperiods

Figure 6. In vitro rooting of lilac on perlite (original)

Table 3. Expression of in vivo rooting capacity oflilac microcuttings

VARIANT	% ROOTED PLANTS
V1: A.1, B.1	93
V1: A.1, B.2	89
V1: A.2, B.1	91,7
V1: A.2, B.2	90
V1: A.3, B.1	88,2
V1: A.3, B.2	88

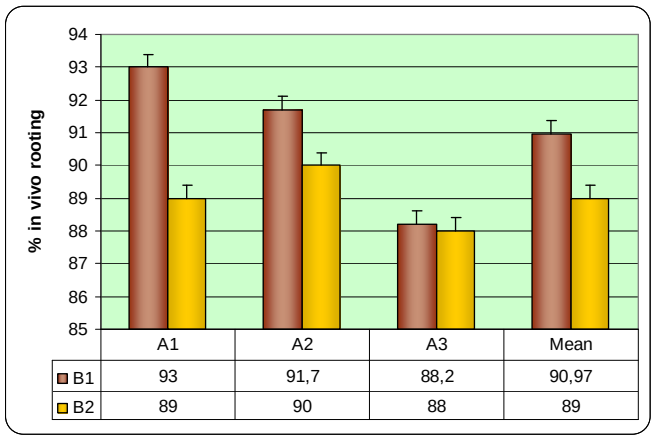


Figure 7. In vivo rooting percent depending on substrate, for different cultivars

In vivo rooted plants were transferred to pots (Φ = 8cm) of mixed peat and perlite for forcing (Figure 8).

Figure 8. Vitroplants in vivo rooted on substrate of peat and perlite (original)

4. CONCLUSIONS

Summarizing the reported findings, the conclusions were:

- The best *in vitro* rooting results were obtained by the cultivars 'Madame Lemoine' and 'Sensation', with the values of 92,4% and 90,7%.
- Auxins NAA (0,5 mg/l) and IBA (0,6 mg/l) had a determinant role in triggering and sustaining the rhizogenesis process, within certain levels.
- Reduced production costs by using a photoperiod of 12 hours, with high efficiency, than a photoperiod of 14 and 16 hours for the rooting of 'Sensation's microcuttings.
- Shortening the rooting time by replacing the *in vitro* rooting phase with the *in vivo* one simultaneously with acclimatization, for the microcuttings harvested from multiplication phase, on perlite substrate.

5. REFERENCES

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