CONTRIBUTION TO THE IMPROVEMENT OF *IN VITRO* PROPAGATION BIOTECHNOLOGY OF *SYRINGA VULGARIS* CULTIVARS

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Abstract
The valuable biological, ecological and landscaping features of liliac, the real interest among the plant lovers, the increased requirements for the container culture, the limits imposed by conventional breeding techniques, the reduced quantity of planting material towards the requirements were the premisses to develop a technology for a rapid propagation material production of some cultivars of Syringa vulgaris by in vitro culture. The explants were represented by meristems with 2-3 leaflets and uninodal fragments sampled in December and June. The disinfection time varied with the stage of vegetation. The explants had a good behaviour on the initiation and multiplication culture media. 'Charles Joly' obtained the best result, with a growing percentage after innoculation of 92%. 'Mme. Lemoine' obtained the best multiplication rate of 12.33 microshoots/explant. The rooting phase was done in vivo on perlite substrate. The obtained results, 91% for 'Mme. Lemoine', 90.85% for 'Charles Joly' and 88.1% for 'Sensation' respectively, allowed us to recommend the usage of in vivo rooting on perlite substrate, simultaneously with the acclimatization, shortening the rooting time of microcuttings and production costs. The researches were expanded also on the fortification breeding material. The substrate made of Danmuld peat determined significant shoots' growth for all three studied cultivars. In two years 'Mme. Lemoine' reached 16.4cm in height, 'Charles Jolly' 11.65cm and 'Sensation' 19.5cm in height. The number of suckers was between 3.4-6.1 suckers/plant and wasn't influenced by genotype and culture substrate.

Keywords: lilac, explants, microcuttings, in vivo rooting, fortification

1. INTRODUCERE

Lilac is among the favourite ornamental shrubs used to decorate parks and gardens. It has a small tree habitus of 8-10 m or a shrub-like habitus of 3-5 m (Sonea and Palade, 1969). The port is characterized by its rich foliage, abundance, colour, shape and fragrance of flowers (Neagu et al., 1976). Its ornamental features, drought and pollution resistance recommend its landscape utilisation as isolated bush in the middle of lawns, in groups, massives, hedges, alignments, shapes grafted on the trunk, even in large urban centres. Lilac's good behaviour on containers led to its usage as portable plant during flowering time to decorate public and residential buildings, patios, porches and balconies (Kiselev, 1956).

2. MATERIAL AND METHODS

Researches were initiated in 2007, in the Laboratory of Plant Biotechnologies from Pitesti University, Faculty of Sciences. The biological material was represented by three cultivars of *Syringa* genus brought in containers from Pistoia Nursery, Italy.

- 'Mme. Lemoine': French cultivar, created in 1890, by Victor Lemoine (Fialla, 2002). It has vigorous growth, reaching up to 4-5 m height and 3m wide. It is loved for its beautiful and elegant white coloured inflorescences.
- 'Charles Joly': French cultivar, of the famous breeder Victor Lemoine, introduced in culture in 1896. It may reach up to 3.5-4 m height and 2.5-3 m width. It flowers at the end of spring beginning of summer and it is very appreciated for its colour.
- 'Sensation': was introduced by Dirk E. Maarse, in the year 1938 and it is a mutation of a prior lavender colour variety - 'Hugo de Vries' (Fiala, 2002). It reaches up to 7 m height and 3-4 m width. It flowers during May-June, and the fragrant flowers in shades of purple and with white edges have a unique distinction.

The explants were represented by meristems with 2-3 leaflets, taken from mother plants in December and uninodal fragments, taken in June. The biological material's disinfection was effectuated by water washing and 2 drops of chloride based disinfectant, followed by sterilization in 94% ethyl alcohol and 6% calcium hypochlorite (Table 1), the disinfection time varied with the stage of vegetation.
The culture media used for the initiation and multiplication are presented in table 2 (Murashige and Skoog, 1962; Linsmaier and Skoog, 1965; Lee and De Fossard, 1977; White, 1937c).
During the experiments, in the growth chamber were assured the required conditions: 24°C±2ºC temperature, 14 hours photoperiodism and 3000 lx light intensity.
For the *in vivo* rooting phase simultaneously with the vitroplants acclimatization were used two substrates made of peat and perlite (1:1) and perlite only.
After the *in vivo* rooting phase simultaneously with the vitroplants acclimatization the plants of the three lilac cultivars were trimmed and planted in pots with Ø=10cm, in order to fortificate them in different substrates: peat and clay-sandy soil mixture and Danmuld peat (1:1).
After six months and one year and six months, were effectuated biometrical measurements regarding plants heigh and the number of suckers/plant.

3. RESULTS AND DISCUSSIONS

The recorded data are expressed in: growth percent for the initiation phase, ratio of multiplication (microshoots/explant) for the multiplication phase, rooting percent for the *in vivo* rooting simultaneously with the acclimatization.
For the initiation phase, the nutrient media contained macro MS and micro MS, White vitamins, growth regulators - 0.5mg/l BAP, 32 mg/l NaFeEDTA, 40g/l dextrose and 7g/l agar. The addition of 0.5mg/l BAP influenced the growth of 'Charles Joly' cultivar's explants, triggering a percentage of 92%.
For the multiplication phase, the nutrient media contained: macro MS, micro MS, LF vitamins, 0.004% NAA, 2mg/l BAP, 32 mg/l NaFeEDTA, 40g/l dextrose and 7g/l agar. The cultivar 'Mme. Lemoine' had the best multiplication rate, of 12.33 microshoots/explant.
The culture substrates, made of peat and perlite 1:1 and perlite only, led to high *in vivo* rooting percentages, 91% for 'Mme. Lemoine' cultivar, 90.85% for 'Charles Joly' cultivar and 88.1% for 'Sensation' respectively (table 3).
The Danmuld peat determined shoots growth for the three lilac cultivars, while the mixture of peat and clay-sandy soil determined lower values, but the shoots were lignified. The number of suckers wasn't influenced by genotype or substrate nature. The fortification capacity of lilacs cultivars depending on genotype and substrate is given in table 4.
Thus, the phases of *in vitro* culture biotechnology of the three cultivars of *Syringa vulgaris* L. species are: culture initiation and establishment, microshoots individualisation and multiplication *in vivo* rooting simultaneously with acclimatization, fortification of plants. (fig. 1).

4. CONCLUSIONS

Summarizing, we recommend:

- Utilisation of 0.5mg/l BAP for the explants' initiation phase;
- 0.004 mg/l NAA/1.2 mg/l BAP auxin/cytokinin for the multiplication phase;
- Reduction of rooting time shortening by the replacement of in vitro rooting with in vivo rooting simultaneously with acclimatization, shortening the rooting time and thus the production costs;
- The Danmuld peat determined shoots growth for the three lilac cultivars, thus lilac can be used for container culture with success, while the mixture of peat and clay-sandy soil determined lower values, but the lignified shoots useful for nurseries and landscaping designs;

- The number of suckers wasn□t influenced by genotype or substrate nature.

Table 1. Biological material disinfection

Vegetation phase	Presterilization	Sterilization	Disinfection time (minutes)
Dormant phase	Tap water + 2 – 3 drops of chloride based disinfectant	94% Ethyl alcohol	10
			15
		6% Calcium hypochlorite	20
			10
			15
			20
Active growth	Tap water + 2 – 3 drops of chloride based disinfectant	94% Ethyl alcohol	5
			8
			10
		6% Calcium hypochlorite	5
			8
			10

Table 2. Nutrient media composition

Composition	Initiation	Multiplication
Macro MS	N	-
Micro MS	N	-
Vitamins W	N	-
Dextrose (g/l)	40	-
Agar (g/l)	7	-
BAP (mg/l)	0.5	-
NaFeEDTA (mg/l)	32	-
Macro MS	-	N
Micro MS	-	N
Vitamins LF	-	N
Dextrose (g/l)	-	40
Agar (g/l)	-	7
BAP (mg/l)	-	1.2
NAA (mg/l)	-	0.004
NaFeEDTA (mg/l)	-	32

Table 3. Expression of in vivo rooting capacity of lilac microcuttings

Culture substrate	% of in vivo rooted plants		
	Mme. Lemoine	Charles Joly	Sensation
Peat and perlite 1:1	93	91.7	88.2
Perlite only	89	90	88

Table 4. Expression of fortification capacity of lilac cultivars depending on different substrate after one year and month

Culture substrate	Height (cm)			Suckers/plant		
	Mme. Lemoine	Charles Joly	Sensation	Mme. Lemoine	Charles Joly	Sensation
Danmuld Peat	16.4	14.7	19.15	4	4.5	4.4
Peat + clay-sandy soil	13.2	11.65	13.5	5.1	3.4	5.1

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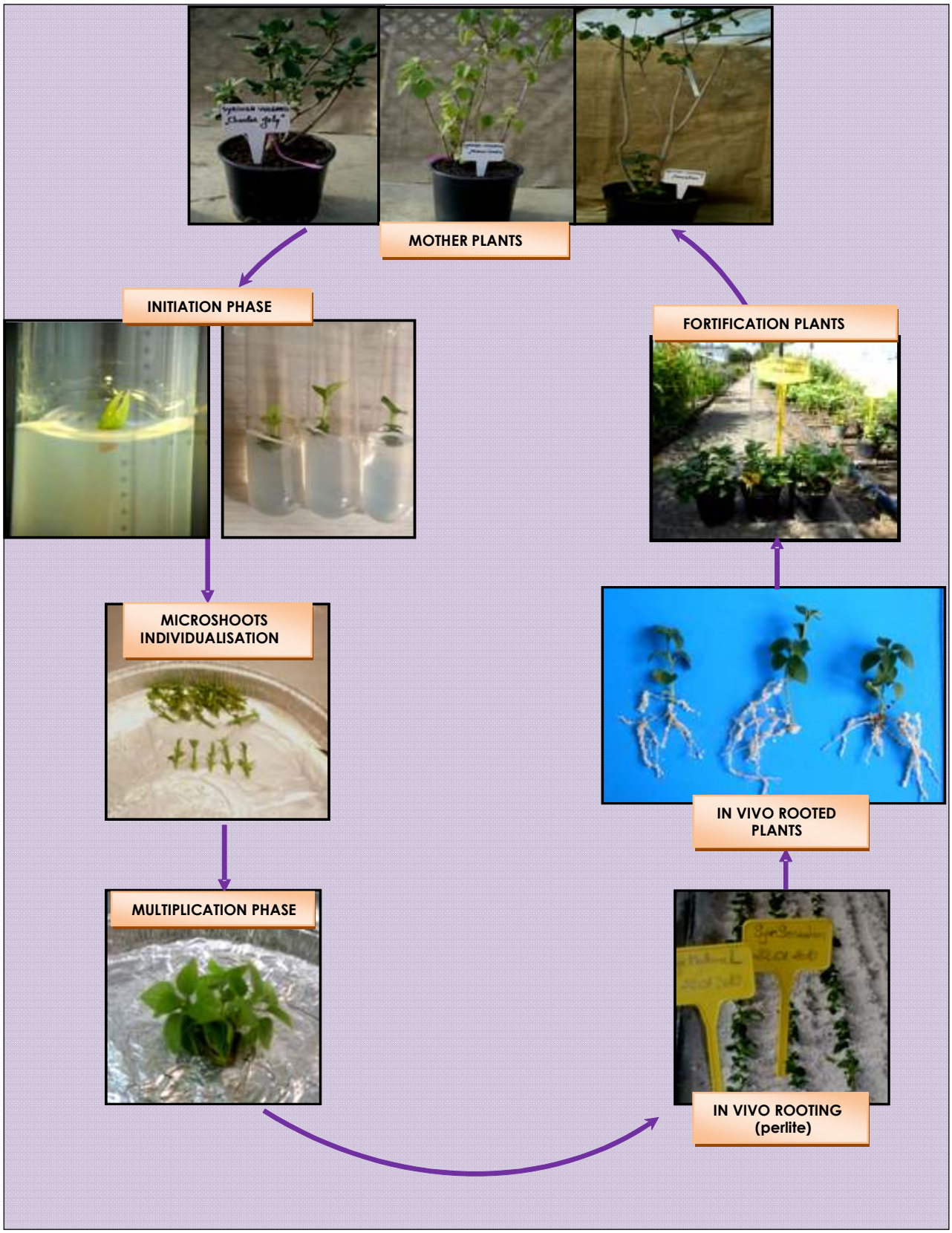

Figure 1. In vitro phases of Syringa vulgaris **L.**