PAULOWNIA TOMENTOSA L. *IN VITRO* PROPAGATION

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Abstract

The purpose of this study was to obtain in vitro Paulownia tomentosa L. vigorous and less expensive seedlings than the traditional method. Micropropagation was performed starting from the seed germinated made in sterile culture medium Murashihe-Skoog (1962) ½ with Gamborg et al. (1968) ½ vitamin, solid, without growth regulators. At 3 months from the germinated were obtained seedlings with 3-4 cm stem size with a well-developed root system, capable to ex vitro transfer. Plantlets acclimatization survival percentage was 65% in peat: perlite 2: 1 substrate. By comparison with traditional breeding methods we found that the production of in vitro seedlings assumed three times less cost, but it disadvantage is still producing clones devoid of genetic variability.

Key words: *Paulownia*, micropropagation, *ex vitro*, bioeconomy, ecoeconomy

INTRODUCTION

Paulownia tomentosa is a tree species with rapid growth (Swearingen, 2009), originating in China (Radu, 1986; Clinovschi, 2005; Ștefan, Oprea, 2007) belongs to the *Plantae* kingdom, *Spermatophyta* phylum, *Angiospermae* subphylum, Dicotyledonatae class, *Asteridae*, subclass Scrophulariales order, *Scrophulariaceae* family (Săvulescu, 2007), cultivated by Europeans increasingly, both ornamental purposes, but also economically very valuable for timber (Kaymakci et al., 2013). *Paulownia tomentosa* is an herb, leaf, fruit and extracting wood by products of metabolism (Yadav et al., 2013). It has been demonstrated that the species is good accumulators of heavy metals in contaminated soils (Azzarello et al., 2012; Miladinova et al., 2014) and *in vitro* (Bahri et al., 2013, 2014, 2015). *Paulownia* could be cultivated not only for timber but also as a curtain forest. However, it must consider not become invasive (Essl, 2007; McDonald, Urban, 2006; Kuppinger, 2008).

Traditional propagation of the species requires large land areas, numerous human resources, can be achieved under milder climate and sheltered spaces, extendable with caution and its surrounding areas, compliance with temperatures as favourable isotherms. The multiplication of species through tissue culture, prospect of simultaneous large seedlings quantity planting, characterized by maximum biological uniformity, soundness and without heavy expenditure, regardless of season, climate, altitude etc. (Bergmann, 1998).

Micropropagation is an instrument of ecoeconomic and bioeconomic planting material production (Petruș, 2011; Petruș-Vancea, Cachiță, 2013).

Numerous recent studies reported either in terms of *medium* optimization, for example basal medium MS, DKW, QL, McC or N_6 fortified with BAP (0.5 mg/l) and IBA (0.01 mg/l) (Chunchukov, Yancheva, 2014), MS medium supplemented with GA_3 50 mg/l or thidiazuron (Shtereva et al., 2014), nutritive medium plus DMSO (Garelkova, Naydenova, 2015), either by choosing the *explant type*, namely apex, leaf with petiole, nodal stem or roots (Ozaslan et al., 2015), or leaves (proximal half with petiole) from the first node to micropopagated shoots (San-José et al., 2014), axillary buds and seeds (Clapa et al., 2014), or the effects of different *every light* in *in vitro* regenerations (Yang et al., 2013).

MATERIAL AND METHOD

Plant material consists in *Paulownia tomentosa* L. seeds (Table 1) collected from a solitary tree from the University of Oradea court. Research started in June 2015 and finished in May 2016.

Table 1

Research protocol

Step I – *in vitro cultures initiation*

Explant type	*Paulownia tomentosa* seeds
Disinfestations	Alcohol Sodium hypochlorite 5% + Tween 20 for 10 minutes
Culture medium	MB-MS+G ½ , solid, without growth regulators
Culture recipients	Uncoloured glass recipients, with 11 cm height and 2 cm diameter
In vitro growth conditions	1700 lx, 16/24 h light, 24 – 25 ˚C
Culture period	3 months

Step II – *ex vitro acclimatization*

Plant material	Plantlets
Substratum	Peat: perlite 2:1 rate
Culture recipients	Incubators with 5/22/35 cm size
Ex vitro growth conditions	Natural illumination, 22 ˚C
Culture period	1 month

Note: MB-MS – Basal Murashige-Skoog (1962) medium; G – Gamborg et al. (1968) vitamins.

We chose to use seeds in *in vitro Paulownia* culture initiating, because some of vegetative explants, such as stem shows a higher risk of developing

infections. We investigated the possible problem that may hold *Paulownia tomentosa* vegetative organs, in terms of anatomy. At the end of the experiment we showed normal roots, stems and leaves structures of donor plants, except that marrow of stems which was resorbed. It can be a gateway to additional various microorganisms, making it difficult subsequently disinfecting explants, even impossible due to possible endogenous infections (Crișan, Petruș-Vancea, 2016).

RESULTS AND DISCUSSION

At 1 month from *in vitro* initiation, from 443 seeds, 250 germinated which represents 55.1% (Fig. 1).

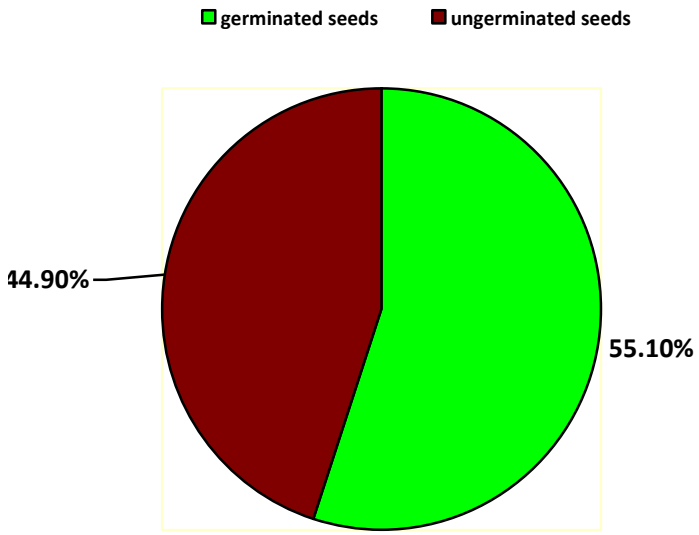

Fig. 1. *Paulownia tomentosa* seeds germination, one month after placing on the aseptic medium

At 3 months after seeds placing on the sterile medium, seedlings generated by them had a well-developed root system (Fig. 2), expressed by the root average length and the roots average number by values of 3.68 cm, respectively 3, 48 (Table 2). Stems lengths were similar to that of roots, were presented 2 nodes/cm and two leaf at one node (Table 2).

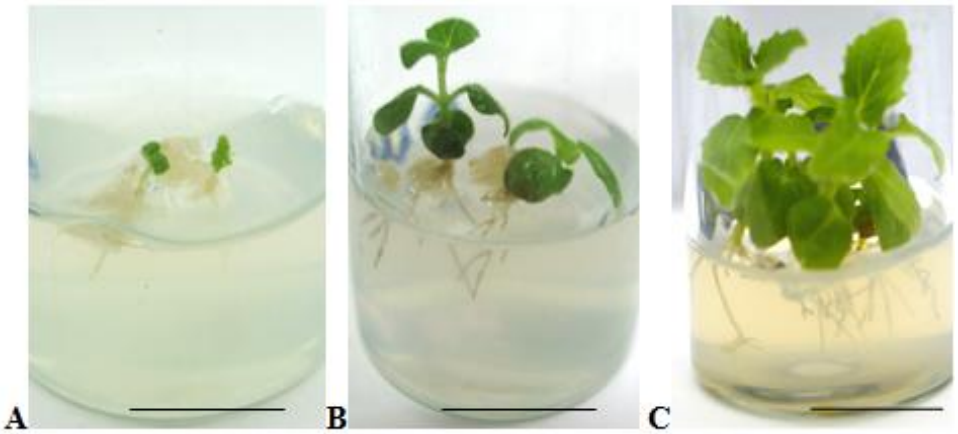

Fig. 2. Aspects of growth and development of *in vitro Paulownia tomentosa* plantlets, at 1 month (A), two months (B) and tree months (C) from culture initiation (bars means 1 cm)

Zayova et al. (2014) reported that the highest rooting percentage (100%) and the maximum roots number per plant were recorded on a ½ MS medium supplemented with 0.5 m / l IBA.

Table 2

Paulownia tomentosa plantlets growth indices at 3 months after the release of germinated

Measurements	Roots length (cm)	Roots no.	Stem length (cm)	Nodes no.	Leaves no.
Average ±standard deviation	3.68±1.57	3.48±0.87	3.52±0.71	5.96±0.79	11.92±1.58

At three months after *in vitro* culture, the plantlets were transferred to *ex vitro* conditions. The survival percentage in the acclimatization period was 65% (Fig. 3).

At one month from transferring in incubators (Fig. 4), the seedlings had a stem average size of 8.8 cm, 6-7 nodes and 12-13 leaves (Table 3). Now, the seedlings were transferred individually in pots (Fig. 4).

Clapa et al. (2014) managed rooting and *Paulownia ex vitro* acclimatization using Jiffy7 pills and perlite, with percentages above 80% to shoots of 3-5 cm in length and over 60% to 2-2.5 cm long shoots.

Comparing costs about the making of traditional seedlings obtained from a local manufacturer with those made in our laboratory we synthesized the following conditions: for the *in vivo* production of 10 000 seedlings were spending about 30 860 euros, or 3.86 EUR / sapling, and by *in vitro* methods only 9.410 euros, which is 0.94 euro / sapling (Table 4). We note

that if are used LED lighting systems, the costs is significantly reduced (Pop et al., 2014).

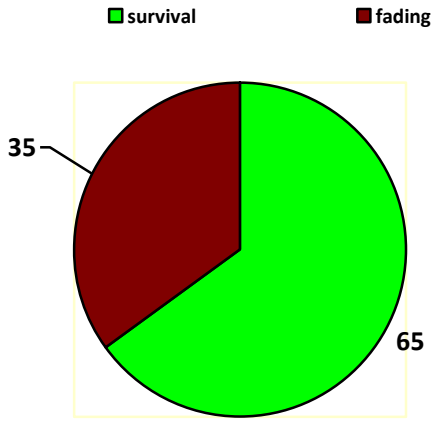

Fig. 3. *Paulownia tomentosa* acclimatization survival percent, at 1 month from *ex vitro* transferring

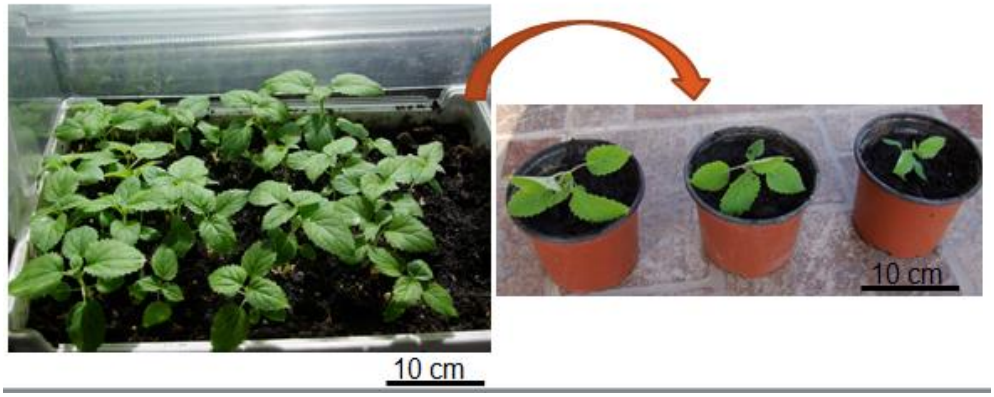

Fig. 4. Aspects of *ex vitro Paulownia tomentosa* growth, at one month from *ex vitro* transferring

Table 3

Paulownia tomentosa plantlets growth indices at 1 month after *ex vitro* transferring

Measurements	Stem length (cm)	Nodes no.	Leaves no.
Average ±standard deviation	8.8±0.58	6.24±0.52	12.56±0.92

Table 4

Comparative estimate of expenses, related *to in vivo* and *in vitro* cultures

Costs (for 10 000 saplings)	***In vivo (euro)***	***In vitro (euro)***
Human resource	6 people X 400 euro/month = 2 400 X 4 months = 9 600 euro	1 people x 400 euro = 400 x 4 months = 1600 euro
Electricity	8 000	800
Culture medium	-	10
Other materials (fertilizer etc)	13 260	7 000
Total costs	*30 860*	*9 410*
TOTAL costs/sapling	**3.86 euro**	**0.941 euro**

CONCLUSIONS

1. Initiating the *Paulownia tomentosa in vitro* culture from seeds was successful on MB-MS + G ½, solid, medium without growth regulators, after three months seedlings being able to acclimatization to septic medium of life in peat: perlite 2: 1 substratum.
2. *In vitro Paulownia tomentosa* seedlings production is three times cheaper than the traditional method, *in vitro* cultures disadvantage is clones obtain without genetic variability, sensitive to abiotic stress factors with the greatest risk of not survive on the environmental conditions. If not properly taken explants (genetic determinations in advance) before the *in vitro* culture initiation, the clones are genetically poor, unsustainable.

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