Micropropagation of six *Paulownia* genotypes through tissue culture

Микроразмножаване на шест генотипа *Paulownia* чрез тъканни култури

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Abstract

We investigated the effect of genotype and culture medium on the *in vitro* germination and development of plantlets from seeds of 6 different *Paulownia* genotypes (*P. tomentosa*, hybrid lines *P. tomentosa* × *P. fortunei* (Mega, Ganter and Caroline), *P. elongata* and hybrid line *P. elongata* × *P. fortunei*). Nodal and shoot tip explants were used for micropropagation of *Paulownia* genotypes by manipulating plant growth regulators. The highest germination percentage for all genotypes was obtained for seeds inoculated on medium supplemented with 50 mg*L GA_3 (MSG2). On Thidiazuron containing media, the explants of hybrid line *P. elongata* × *P. fortunei* exhibited the highest frequency of axillary shoot proliferation following by *P. tomentosa* × *P. fortunei.* The results are discussed with the perspective of applying an improved protocol for *in vitro* seed germination and plantlet formation in several economically valuable *Paulownia* genotypes.

Keywords: germination, micropropagation, nodal explants, *Paulownia* genotypes, plant growth regulators, shoot tips, tissue culture

Abbreviations: BAP (6-benzylamino purine), GA_3 (Gibberellic acid), IAA (Indole-3-acetic acid), IBA (Indole-3-butyric acid), MS (Murashige and Skoog), PGRs (plant growth regulators), TDZ (Thidiazuron)

Резюме

Проучен е ефектът на генотипа и на хранителната среда върху покълването и развитието на растения-регенеранти от семена *in vitro* при 6 различни генотипа *Paulownia.* Получените резултати са обсъждани с перспективата за приложение на подобрения протокол за *in vitro* покълване на семена и формиране на растения при няколко икономически ценни генотипа *Paulownia*.

Ключови думи: експланти, микроразмножаване, покълване, растежни регулатори, стъблени връхчета, тъканни култури

Разширено резюме

Проучен беше ефектът на генотипа и хранителната среда върху *in vitro* покълването и развитието на растения-регенеранти от семена на 6 различни генотипа *Paulownia* (*P. tomentosa*, хибридните линии *P. tomentosa* × *P. fortunei* (Mega, Ganter и Caroline), *P. elongata* и хибридната линия *P. elongata* × *P. fortunei*). Експланти от стъблени връхчета и пъпки бяха използвани за микроразмножаване на генотиповете *Paulownia* чрез вариране на растителните растежни регулатори. Най-високият процент на покълване за всички генотипи бе получен от семена инокулирани в хранителна среда допълнена с 50 mg*L GA_3 (MSG2). В среда съдържаща Thidiazuron, експлантите на хибридната линия *P. elongata* × *P. fortunei* показаха най-висока честота на формиране на стъблени прораствци, следвани от *P. tomentosa* × *P. fortunei.* Резултатите са обсъждани с перспективата за приложение на подобрения протокол за *in vitro* покълване на семена и формиране на растения при няколко икономически ценни генотипа *Paulownia*.

Introduction

The genus *Paulownia* (*Scrophulariaceae*) includes 9 species of trees indigenous to Chine and East Asia (Zhu et al., 1986). Most species of *Paulownia* are fast growing as the harvesting begins within 8-10 years and can continue yearly for as long as is desired. The tree is extremely hardy and its plantation requires only minimal inputs from the grower. The value of *Paulownia* as a short-rotation woody crop plants (Bergmann and Moon, 1997), afforestation (Zhu et al., 1986) and mine site reclamation (Carpenter, 1977) has been reported. *Paulownia* has remarkable combination of qualities that resulted in the use for a wide range of purposes. The wood of *Paulownia* is soft, lightweight with excellent machining and finishing properties (Akyildiz and Sahin, 2010). Its stem bark has been used in Chinese herbal medicine as a component remedies for some infection diseases. Aside from their timber products, some *Paulownia* species have also ornamental use (Puxeddu et al. 2012; Ben Bahri and Bettaieb, 2013). In the last decade, several *Paulownia* species and hybrids were introduced into Bulgaria since the geographic region is suitable for growth the *Paulownia* tree for ornamental purposes and for planting stock for new plantations. Therefore, the application of biotechnological approaches for *in vitro* regeneration and micropropagation techniques of *Paulownia* ssp. have been encouraging, particularly for supply planting material for forestry (Zagorska et al., 2007; Angelova-Romoval et al., 2011; Ivanova et al. 2012).

The use of *in vitro* propagation techniques provides healthy, homogeneous planting stock for a forestation and woody biomass production of *Paulownia*. Efficient vegetative micropropagation has been demonstrated to have many advantages over seedling propagation of *Paulownia* ssp. (Bergmann and Moon, 1997; Bergmann, 1998; Rout et al., 2001). *In vitro* propagation of *Paulownia tomentosa* trough shoot bud regeneration (Rao et al., 1996) and mass multiplication of *Paulownia elongata* trough nodal culture and axillary shoots has also been reported (Bergmann and Whetten 1998; Ipekçi et al., 2001; 2003; Lobna and Taha 2008; Imad et al. 2010; Markovic et al., 2013). It is widely accepted, that success of *in vitro* regeneration depends on the control of morphogenesis, which is influenced by several factors

namely genetic background, kinds of tissue and explants, nutritional components, growth regulators and culture environment (Giri et al., 2004; Ozaslan et al. 2005).

The aim of this study was to develop an *in vitro* regeneration effective protocol for micropropagation of six *Paulownia* genotypes and to assess the factors that influence the optimal plant regeneration and propagation.

Materials and methods

Plant material and explant source

This study was carried out at the Department of Applied Genetics and Biotechnology, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia. Seeds from 6 Paulownia genotypes – *P. tomentosa* and hybrid lines *P. tomentosa* × *P. fortunei* (Mega, Ganter and Caroline), *P. elongata* and hybrid line *P. elongata* × *P. fortunei* were kindly provided by Technogen Inc., Turkey.

Establishment of aseptic intact seedlings

The mature seeds were surface-disinfected by 3 different sterilization procedures:

- immersion in 70% ethylene alcohol for 6 min;
- immersion in 70% ethylene alcohol for 1 min followed by swirling in 0.1% (w/v) mercuric chloride ($HgCl_2$) solution for 3 minutes, and
- immersion in 30% solution of sodium hypochlorite for 15 minutes. A drop of Twin-20 was added into the decontamination solution. Following the surface disinfection, seeds were finally rinsed 3 times for 15 min each in sterile distilled water.

Culture media and culture conditions

Three different seed germination media were examined for their effectiveness in promoting germination and subsequent development of *Paulownia* seeds. Basal MS (Murashige and Scoog, 1962) media were supplemented with 30 g*L sucrose, 7 g*L Difco bactoagar and different concentrations of GA_3 (20 mg*L and 50 mg*L). Media were adjusted to 5.6 pH before autoclaving (20 min at 1.1 kg*cm^{-2}, 121 °C). Four replicates of 25 seeds per genotype were sown. Culture tubes were incubated in growth chamber at 25 °C ±1 °C and 60% – 70% humidity in the dark for 4 weeks. Germination responses were scored visually as a percentage at the 10th, 20th and 30th days after sowing.

For shoot regeneration, the segments from nodal sections and shoot tips derived from 30-days-old aseptic seedlings were used as a source material. They were excised and transferred to 4 different shoot induction MS media containing 3% sucrose solidified with 0.6% agar and supplemented with different concentrations of growth regulators BAP, TDZ and IAA (Table 1). The pH of the media was adjusted to 5.6. After 8 weeks of culture, the frequency of explants producing shoots and the average number of shoots per explants were recorded.

For induction of rooting, the *in vitro* raised shoots (1.5 cm – 2 cm in length) were cultured on half-strength MS basal medium supplemented with different concentrations of growth regulator IBA (0.5 mg*L and 1.0 mg*L), 250 mg*L and 500 mg*L casein hydrolyzate and 10 g*L sucrose (Table 1).

All cultures were incubated under 16/8 h photoperiod in a growth chamber under light intensity 40 $\mu Mm^{-}2s^{-1}$ provided by cool-white fluorescent lamps at 22 °C – 25 °C with 65% – 70% humidity.

Table 1 Composition of culture media used for *in vitro* regeneration and rooting of six *Paulownia* genotypes

Media	Growth regulators, mg*L			
	BAP	TDZ	IAA	IBA
		a/ Organogenesis		
MS0[a]	0	0	0	
MS1[b]	0.5		0.1	
MS2	1		0.1	
MS3		0.5	0.1	
MS4		1	0.1	
		b/ Rooting		
½ MSR0[c]				0
½ MSR1				0.5
½MSR2				1

[a]MS0-control**;** [b]MS1-MS4 – media for organogenesis; [c]MSR –rooting media

Acclimatization of plants under *in vivo* conditions

Well-rooted shoots were removed from the culture medium, and the roots were washed gently with water to remove agar. Plantlets were transferred to small plastic pots (8 cm in diameter) containing a mixture of peat and perlite in the volume ratio 2:1 v/v. They were kept in a plant growth chamber at 22 °C under diffuse light conditions (16/8 h photoperiod, 60 $\mu Mm^{-2}s^{-1}$) and 95% – 98% relative humidity. The humidity was reduced gradually and the plantlets were removed from the growth chamber after 3 weeks. The plantlets were placed in the greenhouse for acclimatization before being transferred outdoors under full sun.

Statistical analysis

Usually, 100 seeds were tested per each treatment and each experiment was conducted at least 3 times. The cultures were examined periodically and the morphological changes were recorded on the basis of visual observations. The effect of different treatments was quantified as the percent of seed germination, mean number of shoots per explant, shoot height, percent of rooting plants and root length. The data were statistically analyzed by Sigma Plot 11.0 test (Systat Software Inc., SSI) (Hill and Lewicki, 2006).

Results and discussion

Contamination of seeds is a constant problem that can compromise development of all *in vitro* techniques (Enjalric et al., 1987; Cassells, 1991; Leifert et al., 1991). In the current study, it was found that the surface sterilization of *Paulownia* seeds by direct treatment with 30% solution of sodium hypochlorite (15 min) removed surface microorganisms resulting in 98% no contaminated sterile seeds. Hence, it could be suggested that preconditioning of the seeds with sterilizing agents such as sodium hypochlorite is the most effective for prevention of surface contamination of *Paulownia* mature seeds.

Table 2 summarizes the germination percentage for controls (MS0) and for seeds inoculated in 2 different culture media MSG1 and MSG2. Germination of *Paulownia* seeds was considered difficult, as specific nutritional and environmental conditions are needed (Bergmann and Moon, 1997; Ipekçi and Gozukirmizi, 2003).

Table 2 Germination response of six *Paulownia* genotypes

Media	Genotypes	Germination, %		
		10^{th} day	20^{th} day	30^{th} day
MSG0[a]	*P. tomentosa*	3.2	12.0	28.2
	P. tomentosa × *P. fortunei*	1.0	3.0	12.6
	P. elongata × *P. fortunei* L Caroline	0.0	3.2	6.5
	P. tomentosa × *P. fortunei* L Mega	0.0	4.5	5.7
	P. tomentosa × *P. fortunei* L Ganter	0.0	0.0	4.0
	P. elongata × *P. fortunei*	2.0	8.5	11.5
MSG1	*P. tomentosa*	32.5	41.0	73.0
	P. tomentosa × *P. fortunei*	12.0	16.2	42.2
	P. elongata × *P. fortunei* L Caroline	5.8	18.0	25.8
	P. tomentosa × *P. fortunei* L Mega	0.0	15.3	28.7
	P. tomentosa × *P. fortunei* L Ganter	0.0	8.9	11.7
	P. elongata × *P. fortunei*	18.6	28.3	40.2
MSG2	*P. tomentosa*	40.0	40.0	40.0
	P. tomentosa × *P. fortunei*	23.3	23.3	23.3
	P. elongata × *P. fortunei* L Caroline	6.7	6.7	6.7
	P. tomentosa × *P. fortunei* L Mega	10.1	10.1	10.1
	P. tomentosa × *P. fortunei* L Ganter	4.2	4.2	4.2
	P. elongata × *P. fortunei*	22.4	22.4	22.4

[a]MSG – media for germination;

However, with exception of lines Mega and Ganter of hybrid *P. tomentosa* × *P. fortunei*, seeds of investigated genotypes sown on different media germinated within 10 days of culture. Seed germination percentage varied among the genotypes. The highest germination percentage for all genotypes was obtained for seeds inoculated on medium supplemented with 50 mg/L GA_3 (MSG2). Germination percentages in these variants varied from 4.2% (line Ganter of hybrid *P. tomentosa* × *P. fortunei*) to 40.0% (*P. tomentosa*). In contrast, seeds germinated on medium supplemented with

20 mg*L GA_3 revealed reduction of the germination percentage (11.7% – 73%). Successful germination of seeds is directly related to the plant development and survival. Therefore, the development of a protocol for seed germination of different *Paulownia* species and their hybrids was an essential aspect of the current study. Seeds of *P. tomentosa* revealed the highest germination ability than the all other genotypes following by hybrid line *P. tomentosa* × *P. fortunei* (Table 2). In this study, seed germination of the rest other genotypes assessed at 30th days was comparable and similar and ranged from 11.7% (*P. tomentosa* × *P. fortunei* L Ganter) to 40.2% (*P. elongata* × *P. fortunei)* (MSG1 medium) and from 4.2% *(P. tomentosa* × *P. fortunei* L Ganter) to 22..4% (*P. elongata* × *P. fortunei)* (MSG2 medium). Besides the beneficial effect of culture media, genotype differences may also have contributed for the differences in germination percentages observed among different genotypes. Our results indicated that the seed germination response to media composition is species-specific, although in this study genotypic effects were not directly evaluated.

The morphogenic response of the explants to various growth regulators is summarized in Table 3. On TDZ-containing media (MS3 and MS4), the explants of hybrid line *P. elongata* × *P. fortunei* exhibited the highest frequency of axillary shoot proliferation - 99.6% (MS4) and 97% (MS3) following by *P. tomentosa* × *P. fortunei* – 91.8% (MS4) and 90% (MS3). Also, the number of shoots per explant on these media was higher in comparison with that of MSR1 and MSR2, especially for *P. elongata* × *P. fortunei* hybrid genotype. Thidiazuron has been reported to induce axillary as well adventitious shoots in many tree species (Huetteman and Preece, 1993; Murthy et al., 1998; Ipekçi and Gozukirmizi, 2003). TDZ at 0.5 mg*L^{-1} and 1 mg*L^{-1} was used in the present study to induce axillary shoots in the cotyledonary nodes of *Paulownia* genotypes. The results indicated that TDZ in combination with other growth-regulating substances (auxin IAA) is most effective of all the cytokinins tested for inducing shoot proliferation. These data corroborate those reported by Murthy et al. (1998) and Corredoira et al. (2008) indicating the morphoregulatory potential of TDZ and its application in plant tissue culture for the development of micropropagation systems. The reasons for the high activity of TDZ in woody species have not been investigated at a physiological level but it was suggested that TDZ helps to establish the optimal internal balance growth regulators (Saxena, 1992; Guo et al. 2011).

All 3 media induced root formation in the regenerated shoots (Table 4) as especially effective is MSR2 containing 1 mg*L IBA thus, indicating that the presence of that auxin in higher concentration favoured root induction. MSR2 medium induced about 99.4% shoot rooting in *P. elongata* × *P. fortunei* and 100% in *Paulownia tomentosa* within 4 weeks and roots averaged 4.9 cm and 4.7 cm in length. Each of the shoot formed 2 – 3 roots in the culture. The observations about the effect of some auxins on root induction in other 3 species such as *Cinnamomum zeylanicum* and *Morus* have been reported earlier by Ravishankar Rai and Jagadishch (1987), and Pattnaik and Chand (1997). The rooted plantlets were successfully acclimatized in a plant growth chamber and eventually established at outdoors under full sun. The regenerated plants did not show any detectable phenotypic variations.

Table 3 Effect of different nutrient media on the micropropagation of six *Paulownia* genotypes

Media	Genotypes	Formed shoots, %	No shoots/explant *x* ±SE	Shoot length, cm *x* ± SE
MS0	G1*	38.6	1.3 ± 0.12c	1.8 ± 0.11b
	G2	42.4	1.7 ± 0.14bc	1.5 ± 0.11c
	G3	22.7	1.1 ± 0.11d	0.9 ± 0.07d
	G4	31.2	1.5 ± 0.15c	1.3 ± 0.07c
	G5	34.0	1.6 ± 0.07b	1.6 ± 0.15bc
	G6	45.8	2.6 ± 0.23a	2.4 ± 0.23a
MS1	G1	70.2	2.7 ± 0.21c	2.6 ± 0.28d
	G2	80.6	3.4 ± 0.29b	3.0 ± 0.15c
	G3	51.1	2.4 ± 0.28d	3.1 ± 0.11c
	G4	63.2	2.8 ± 0.11c	3.8 ± 0.08b
	G5	55.4	3.2 ± 0.19b	3.3 ± 0.23c
	G6	90.8	4.6 ± 0.23a	5.2 ± 0.23a
MS2	G1	74.3	3.1 ± 0.30b	2.8 ± 0.27c
	G2	89.1	3.8 ± 0.32b	3.6 ± 0.12b
	G3	60.1	2.6 ± 0.18c	3.1 ± 0.16b
	G4	65.0	2.8 ± 0.20c	3.2 ± 0.11b
	G5	59.0	3.6 ± 0.31b	2.9 ± 0.18c
	G6	95.1	4.8 ± 0.33a	5.6 ± 0.23a
MS3	G1	81.1	6.0 ± 0.41cd	5.2 ± 0.35c
	G2	90.0	6.5 ± 0.32bc	6.0 ± 0.43b
	G3	68.1	5.6 ± 0.27de	5.8 ± 0.39bc
	G4	66.8	5.0 ± 0.60ef	5.7 ± 0.44bc
	G5	60.1	4.7 ± 0.29f	5.6 ± 0.32bc
	G6	97.0	8.4 ± 0.51a	7.6 ± 0.43a
MS4	G1	85.2	6.8 ± 0.53bc	5.7 ± 0.48e
	G2	91.8	7.2 ± 0.50b	6.4 ± 0.08de
	G3	72.8	6.8 ± 0.51bc	6.9 ± 0.46c
	G4	70.0	6.3 ± 0.42c	7.5 ± 0.32b
	G5	69.6	5.2 ± 0.47d	6.7 ± 0.36cd
	G6	99.6	8.7 ± 0.36a	8.9 ± 0.46a

*G1- *Paulownia tomentosa*; G2 **-** *P. tomentosa* × *P. fortunei*; G3 - *P. elongata* × *P. fortunei* L Caroline; G4 **-** *P. tomentosa* × *P. fortunei* L Mega; G5 **-** *P. tomentosa* × *P. fortunei* L Ganter, G6 - *P. elongata* × *P. fortunei*

Table 4. Effect of different nutrient media on *in vitro* rooting of six *Paulownia* genotypes

Media	Genotypes	Rooting, %	Root length, cm *x* ±SE
MSR0[a]	G1*	75.0	1.2 ± 0.24c
	G2	79.0	1.3 ± 0.19c
	G3	83.2	1.9 ± 0.15b
	G4	78.5	1.8 ± 0.25b
	G5	91.0	2.0 ± 0.23b
	G6	92.5	2.7 ± 0.37a
MSR1	G1	99.2	3.9 ± 0.36a
	G2	90.0	3.7 ± 0.39ab
	G3	85.8	3.0 ± 0.33c
	G4	85.0	2.8 ± 0.40c
	G5	86.0	3.2 ± 0.37bc
	G6	94.0	3.8 ± 0.46ab
MSR2	G1	100.0	5.2 ± 0.41a
	G2	99.0	4.4 ± 0.31bc
	G3	94.6	3.8 ± 0.46c
	G4	97.0	4.7 ± 0.56ab
	G5	98.0	4.7 ± 0.54ab
	G6	99.4	5.5 ± 0.47a

[a]MSR –rooting medium;
*G1 - *Paulownia tomentosa*; G2 **-** *P. tomentosa* × *P. fortunei*; G3 - *P. elongata* × *P. fortunei* L Caroline; G4 **-** *P. tomentosa* × *P. fortunei* L Mega; G5 **-** *P. tomentosa* × *P. fortunei* L Ganter, G6 - *P. elongata* × *P. fortunei*

Conclusions

In conclusion, an attempt was made in this investigation in order to develop an effective micropropagation of several *Paulownia* genotypes. This work shows that organogenic capacity *in vitro* varied among genotypes when grown on identical medium. The *in vitro* protocol described herein for regenerating a large number of plantlets of *Paulownia* using cotyledonary nodes and shoot tips is an improved method as compared to that reported earlier. It could be useful for a large scale plantation programme of economically important *Paulownia* trees. With the method presented here it is possible to produce healthy plants, which could then be released to different habitats.

References

Akyildiz, M.H., Sahin, H., (2010) Some technological properties and uses of *Paulownia* (*Paulownia tomentosa* Steud.) wood. Journal of Environmental Biology, 31(3), 351–355.

Angelova-Romoval, M., Koleva, A., Antova, G., Zlatanov, M., Stoyanova, M., Dobreva, K., Denev, P., Damianova, S., Angelov, B., Stojanova, A., (2011) Lipid composition of *Paulownia* seeds grown in Bulgaria. Trakya University Journal of Science, 13(2), 101–111.

Ben Bahri, N., Bettaieb, T., (2013) *In vitro* propagation of a forest tree *Paulownia tomentosa* (Thunb.) Steud. - A valuable medicinal tree species. Albanian Journal of Agricultural Sciences, 12(1), 37–42.

Bergmann, B. A., Whetten, R., (1998) *In vitro* rooting and early greenhouse growth of micropropagated *Paulownia elongata* shoots. New Forests, 15(2), 127–138.

Bergmann, B.A., (1998) Propagation method influences first year field survival and growth of *Paulownia*. New Forests, 16(3), 251–264.

Bergmann, B.A., Moon, H.K., (1997) *In vitro* adventitious shoot production in *Paulownia*. Plant Cell Reports, 16(5), 315–319.

Carpenter, S.B., (1997) This “princess” heals disturbed land. American Forest, 83, 22–23.

Cassells, A.C., (1991) Problems in tissue culture: culture contamination. In P.,C., Debergh, R.H., Zimmerman, eds. Micropropagation. New York: Kluwer Academic Publisher, pp. 31–44.

Corredoira, E., Ballester, A., Vieitez, M., (2008) Thidiazuron-induced high-frequency plant regeneration from leaf explants of *Paulownia tomentosa* mature trees. Plant Cell, Tissue and Organ Culture, 95(2), 197–208.

Enjalric, F., Carron, M.P., Lardet, L., (1988) Contamination of primary cultures in tropical areas & the case of *Hevea brasiliensis*. ISHS Acta Horticulture, 225, 57–66.

Giri, C.C., Shyamkumar, B., Anjaneyulu, C., (2004) Progress in tissue culture, genetic transformation and applications of biotechnology to trees: an overview. Trees, 18(2), 115–135.

Guo, B., Abbasi, B.H., Zeb, A., Xu, L.L., Wei, Y.H., (2011) Thidiazuron: A multi-dimensional plant growth regulator. Review. African Journal of Biotechnology, 10(45), 8984–9000. DOI: 10.5897/AJB11.636.

Hill, T., Lewicki, P., (2006) Statistics: methods and applications: a comprehensive reference for science, industry, and data mining. StatSoft, Inc.

Huetteman, C.A., Preece, J.E., (1993) Thidiazuron: A potent cytokinin for woody plant tissue culture. Plant Cell, Tissue and Organ Culture, 33(2), 105–119.

Imad, A. Al-Tinawi, Khuzama, A.Q., Wasseem, I.M., Nabil, A.B., Ahmad, M. Abdul-Kader, (2010) Development of *in vitro* propagation system for *Paulownia tomentosa* L. using tissue culture techniques. Jordan Journal of Agricultural Sciences, 6(4), 617–628.

Ipekçi, Z., Altinkut, A., Kazan, K., Bajrovic, K., Gozukirmizi, N., (2001) High frequency plant regeneration from nodal explants of *Paulownia elongata*. Plant Biology, 3(2), 113–115.

Ipekçi, Z., Gozukirmizi, N., (2003) Direct somatic embryogenesis and synthetic seed production from *Paulownia elongata*. Plant Cell Reports, 22(1), 16–24.

Ivanova, B., Yancheva, S., Bojinov, B., (2012) Molecular differentiation of *Paulownia* species and hybrids. Journal of Central European Agriculture, 13(1), 73–84.

Leifert, C., Ritchie, J.Y., Waites, W.M., (1991) Contaminants of plant-tissue and cell cultures. World Journal of Microbiology and Biotechnology, 7(4), 452–469.

Lobna, S. Taha, Soad, Ibrahim M.M., Farahat, M.M., (2008) A Micropropagation protocol of *Paulownia kowakamii* through *in vitro* culture technique. Australian Journal Basic Applied Sciences, 2(3), 594–600.

Markovic, M., Vilotic, D., Popovic, M., (2013) Propagation of *Paulownia elongata* S. Y. Hu by axillary shoots. Propagation of Ornamental Plants, 13(2), 73–77.

Murthy, B.N.S., Murch, J., Saxena, P.K., (1998) Thidiazuron: A potent regulator of *in vitro* plant morphogenesis. In vitro Cellular and Developmental Biology Plant, 34(4), 267-275.

Ozaslan, M., Can, C., Aytekin, T., (2005) Effect of explant source on *in vitro* propagation of *Paulownia tomentosa* Steud. Biotechnology and Biotechnological Equipment, 19(3), 20–26.

Pattnaik, S.K., Chand, P.K., (1997) Rapid clonal propagation of three mulberries *Morus cathayana* and *M. serrata* Roxb. through *in vitro* culture of apical shoot buds and nodal explants from mature trees. Plant Cell Reports, 16(7), 503–508.

Puxeddu, M., Marras, G., Murino, G., (2012) *Paulownia* tree planting in Sardinia (Italy) and its evaluation for agroforestry systems and sustainable land use. Journal of Environmental Science and Engineering, B 1, 1192–1195.

Rao, D.C., Goh, C.J., Kumar, P.P., (1996) High frequency adventitious shoot regeneration of *Paulownia* ssp. cultured *in vitro*. Plant Cell Reports, 16(3-4), 204–209.

Ravishankar Rai, V., Jagadishch, K.S., (1987) Clonal propagation of *Cinnamomum zeylanicum* Breyn by tissue culture. Plant Cell, Tissue and Organ Culture, 9(1), 81–88.

Rout, G.R., Reddy, G.M., Das, P., (2001) Studies on *in vitro* clonal propagation of *Paulownia tomentosa* Steud. and evaluation of genetic fidelity through RAPD marker. Silvae Genetica, 50(5-6), 208–211.

Saxena, P.K., Malik, K.A., Gill, L.G., (1992) Induction by Thidiazuron of somatic embryogenesis in intact seedlings of peanut. Planta, 187(3), 421–424.

Zagorska, N., Karceva, T., Shtereva, L., Petrova, M., Gjuleva, V., (2007) *In vitro* propagation of certain species and hybrids of genus *Paulownia*. In Propagation of Ornamental Plants : Book of Abstracts, Sofia.

Zhu, Z.H., Chao, C.J., Lu, X.Y., Xiong, Y.G., (1986) *Paulownia* in China: Cultivation and utilization. Singapore: Asian Network for Biological Science and International Development Research Centre of Canada.