Propagation of Ornamental Plants
Vol. 00, № 0, 2000: 00-00

PROPAGATION OF *PAULOWNIA ELONGATA* S. Y. HU BY AXILLARY SHOOTS

Marija Markovic*, Dragica Vilotic, and Marija Popovic

Belgrade University, Faculty of Forestry, 1 Kneza Višeslava str., 11000 Beograd, Serbia
*Fax: + 381 11 2545485, *E-mail marija.markovic@sfb.bg.ac.rs

Abstract

Paulownia elongata is an ornamental, fast-growing species and resistant to urban conditions. It was successfully adapted to the growing conditions in Serbia. Twelve-year-old elite tree, originating from a plantation in Bela Crkva was used as stock plant for the experiments. *In vitro* culture was successfully established using disinfection of nodal segments of annual shoots with 0.1% $HgCl_2$ for 10 min. The most favorable shoot multiplication response (6.7 shoots per explant) was recorded on MS medium supplemented with 6 mg l^{-1} BA and 0.5 mg l^{-1} IBA, and rooting was successful (95%) on MS medium with 0.8 mg l^{-1} of IBA and the same amount of NAA.

Key words: culture establishment, micropropagation, ornamental tree

INTRODUCTION

Paulownia elongata S. Y. Hu (Scrophulariaceae) is a deciduous tree species originating from China. It was purposefully introduced into the territory of Serbia in 1993, when an experimental trial was established near Bela Crkva (Stankovic et al. 2009b). Today, this ornamental species is grown in parks and alleys of Serbian urban areas. The research conducted by Stankovic et al. (2009a,b) showed that *P. elongata* is a tolerant species resistant to urban environmental conditions, and can also be recommended for growing in tree alleys and wind protection zones along urban and regional traffic lines. This fast-growing species has also significant value as a short-rotation woody crop. It produces flowers for honey production and timber for solid wood products (Zhu et al. 1986).

If we assume that a geographic area or the conditions prevailing in a particular region affect the development and growth of plants cultivated there (Li et al. 2010), then the genotypes that proved the best over time should be favored and propagated for ornamental purposes as well as in order to provide planting stock for new orchards. *P. elongata* can be propagated from seed, root cuttings, shoot cuttings and through *in vitro* techniques (Zhu et al. 1986, Khan 1992, Ipekci and Gozukirmizi 2003, Castellanos-Hernandes et al. 2006, Vilotic et al. 2006). Bergmann (1998) showed that vegetatively propagated plants of *P. elongata* (shoot cuttings and micropropagated shoots) have greater survival rate, height, and diameter at breast height in field conditions than *P. elongata* plants propagated from seeds. However, the studies on the micropropagation of *P. elongata*, focused on propagation of young plants, which were no more than one year old (Bergmann 1995, 1998, Ipekci et al. 2001).

The aim of our research was to study the effect of disinfection, different plant growth regulators (PGRs) and their concentrations on the induction, multiplication, and rooting of axillary shoots of *P. elongata*.

MATERIAL AND METHODS

Selected, 12-year-old tree with the best phenotypic characteristics (tree height, diameter at breast height, health) from a plantation near Bela Crkva was used as a stock plant material. In May 2011 the selected *P. elongata* tree was pruned and in early July of 2011 newly-formed shoots (10 - 15 cm in length) were excised from the tree. These shoots were rinsed under running water for 20 min in a laboratory. Single-node cuttings of 1-1.5 cm in length were excised and their leaves were removed. The disinfection was performed in 6 different ways: treatments with 70% ethanol for 30 s followed by 0.1% solution of $HgCl_2$ for 5 min or 2% solution of NaClO (2%) for 5 min; treatments with 0.1% $HgCl_2$ for 10 min or 0.2% $HgCl_2$ for 5 min; treatments with 2% NaClO for both 10 and 15 min. Solutions of $HgCl_2$ and NaClO contained 1-2 drops of Tween-20. After that, the explants were rinsed three times with sterile distilled water, their ends were cut off leaving approximately 5 mm above and below the bud, and

Received: November 10, 2012 *Accepted: May 28, 2013*

Table 1. Disinfection of explants.

Sterilization treatments	Contaminated explants (%)	Necrotic explants (%)	Normally developed explants (%)
30 s ethanol + 5 min 0.1% $HgCl_2$	45.3 ± 5.4 ab	34.7 ± 2.3 a	20.0 ± 3.2 ab
10 min 0.1% $HgCl_2$	29.3 ± 4.9 a	38.7 ± 1.7 a	32.0 ± 4.1 b
5 min 0.2% $HgCl_2$	49.3 ± 3.8 b	36.0 ± 1.2 a	14.7 ± 2.6 a
30 s ethanol + 5 min 2% NaClO	40.0 ± 2.6 ab	37.3 ± 2.0 a	22.7 ± 2.6 ab
10 min 2% NaClO	38.7 ± 2.3 ab	37.3 ± 2.6 a	24.0 ± 3.1 ab
15 min 2% NaClO	30.7 ± 2.7 a	40.0 ± 3.6 a	29.3 ± 2.9 b

Values are means ± SE from three independent experiments. Means followed by the same letters are not significantly different at $p \leq 0.05$ by the least significant difference test.

were placed onto the medium containing 4.0 mg l^{-1} BA (N^6-benzyladenine) and 0.2 mg l^{-1} IBA (indole-3-butyric acid). All explants used in this study were taken from a single tree, which was selected from a group of trees, propagated by rooted cuttings.

After the establishment of *in vitro* culture, axillary shoots 10-15 mm in length were excised and placed on different variants of the medium for multiplication. All variants of the medium used contained MS nutrients and vitamins (Murashige and Skoog 1962), 3% sucrose, 0.8% agar (Sigma-Aldrich), and pH value was adjusted to 5.8 before autoclaving. In the multiplication phase the axillary shoots were placed onto the medium supplemented with 2, 4 or 6 mg l^{-1} BA and 0.1, 0.5 or 1.0 mg l^{-1} IBA, and in the rooting phase onto MS medium supplemented with 0.2, 0.4 or 0.8 mg l^{-1} IBA and 0.4, 0.8 or 1.2 mg l^{-1} NAA (α-naphthaleneacetic acid).

The number and length of axillary shoots in the multiplication phase were measured after 25 days. The percentage of rooted shoots and the number and length of roots in the rooting phase were measured after 15 days of culture. The length of these shoots and roots on a single medium varied. Therefore, the shoots and roots were placed in different length categories (less than 10 mm, 10-20 mm and more than 20 mm) and the length of shoots and roots belonging to a certain category was evaluated as a percentage of the total number of shoots on each variant of the medium. Similarly, according to their length, the roots were also placed in different categories (less than 10 mm, 10-25 mm and more than 25 mm) and the number of roots belonging to a appropriate length category was expressed as a percentage of the total number of roots on each variant of the medium. Twenty five explants were used per treatment with three replications. The data were analyzed statistically using the Version 4.2 STATGRAPHICS software, (STSC, Inc. and Statistical Graphics Corporation, Orem, UT). The significance of differences among the treatments was determined by analysis of variance (ANOVA), and the means were compared using the least significant difference (LSD) multiple range test at a significance level of $p < 0.05$. Before the analysis, arcsine transformation was used to convert the percentage data.

RESULTS

The highest number of survived explants was obtained after disinfection treatments with 0.1% $HgCl_2$ for 10 min (32.0%) or with 2% NaClO for 15 min (29.3%) (Table 1). New axillary shoots were successfully regenerated during the multiplication phase (Table 2). Most shoots (61-83%) on all variants of the medium were up to 10 mm in length, 15-28% of them were 10-20 mm in length and few were longer than 20 mm (0-5%), except the ones on the medium with 2 mg l^{-1} BA and 0.1 mg l^{-1} IBA (13.3%), and on the plant growth regulator-free medium (15.8%) (Table 3). The mean number of shoots ranged from 1.6 to 6.7 (Table 2). The medium supplemented with 6 mg l^{-1} BA and

Table 2. The effects of BA and IBA on multiplication of axillary shoots.

BA mg l^{-1}	IBA mg l^{-1}	Regeneration of shoots (%)	Mean number of shoots
0	0.0	90.7 ± 3.1 a	1.6 ± 0.18 a
2	0.1	96.0 ± 2.8 a	1.7 ± 0.15 a
2	0.5	94.7 ± 2.9 a	2.0 ± 0.16 b
2	1.0	93.3 ± 2.4 a	2.2 ± 0.16 b
4	0.1	97.3 ± 2.1 a	5.2 ± 0.24 d
4	0.5	100.0 ± 0.0 a	5.7 ± 0.18 de
4	1.0	97.3 ± 2.3 a	5.4 ± 0.32 de
6	0.1	96.0 ± 2.8 a	4.4 ± 0.19 c
6	0.5	98.7 ± 1.2 a	6.7 ± 0.19 f
6	1.0	97.3 ± 2.3 a	4.6 ± 0.17 c

Values are means ± SE from three independent experiments. Means followed by the same letters are not significantly different at $p \leq 0.05$ by the least significant difference test.

Table 3. The effects of BA and IBA on length of axillary shoots.

BA (mg l^{-1})	IBA (mg l^{-1})	Shorter than 10 mm (%)	10-20 mm (%)	Longer than 20 mm (%)
0	0.0	48.5 ± 3.9 a	35.7 ± 3.2 d	15.8 ± 2.9 d
2	0.1	74.8 ± 1.9 cd	25.2 ± 1.9 c	0.0 ± 0.0 a
2	0.5	61.8 ± 5.4 b	24.9 ± 3.0 c	13.3 ± 2.6 d
2	1.0	66.8 ± 4.5 b	28.1 ± 2.7 c	5.1 ± 1.7 c
4	0.1	79.8 ± 2.6 de	15.0 ± 1.4 a	5.2 ± 1.1 c
4	0.5	82.7 ± 4.9 e	15.5 ± 1.3 a	1.8 ± 0.8 ab
4	1.0	79.7 ± 4.9 de	18.6 ± 2.7 a	1.7 ± 1.1 ab
6	0.1	70.1 ± 1.0 bc	25.4 ± 2.8 c	4.5 ± 1.8 c
6	0.5	75.8 ± 2.4 cd	21.3 ± 2.0 bc	2.9 ± 1.0 ab
6	1.0	76.5 ± 2.5 cd	19.9 ± 2.2 b	3.6 ± 1.2 bc

Values are means ± SE from three independent experiments. Means followed by the same letters are not significantly different at $p \leq 0.05$ by the least significant difference test.

Table 4. The effects of IBA and NAA on rooting of axillary shoots.

IBA mg l^{-1}	NAA mg l^{-1}	Rooted explants (%)	Mean number of roots per explant
0.0	0.0	13.3 ± 2.4 a	1.7 ± 0.5 a
0.2	0.4	82.7 ± 3.0 b	5.5 ± 0.5 b
0.4	0.4	86.7 ± 1.1 bc	10.4 ± 0.7 c
0.8	0.4	92.0 ± 1.7 d	13.9 ± 0.8 cd
0.8	0.8	94.7 ± 2.2 d	18.7 ± 1.1 e
0.8	1.2	89.3 ± 1.0 c	16.1 ± 1.2 de

Values are mean ± SE of three independent experiments. Means followed by the same letters are not significantly different at $p \leq 0.05$ by the least significant difference test.

0.5 mg l^{-1} IBA (6.7 shoots) proved to be most favorable (Fig. 1 A, B). Successful rooting was achieved on all variants of the medium studied except on auxin-free medium (Table 4). The highest percentage of rooting was obtained on medium supplemented with 0.8 mg l^{-1} IBA and NAA (94.7%). In most of the rooted shoots the largest number of roots (including primary and secondary roots) was 10-25 mm in length (64.6-75.4%), and only small number of roots were shorter than 10 mm and longer than 25 mm (Fig. 1 C, D). As an exception, 82.1% of the shoots rooted on the medium with 0.2 mg l^{-1} IBA and 0.4 mg l^{-1} NAA formed roots shorter than 10 mm (Table 5). The greatest mean number of formed roots was obtained on medium containing 0.8 mg l^{-1} IBA and 0.8 mg l^{-1} NAA (18.7).

DISCUSSION

Compared to the studies of the species *P. elongata*, *P. tomentosa* and *P. kowakamii* (Ipekci et al. 2001, Ozaslan et al. 2005, Lobna et al. 2008), the plant material used in this research was disinfected for a longer period of time and solutions with lower concentrations were applied. However, Burger (1989) reports use of very low concentration of NaOCl (0.5%) for surface disinfection of stem segments containing axillary buds from greenhouse or field-grown P. tomentosa trees. By contrast, the above studies do not report results referring to the effectiveness of disinfection.

The mean number of axillary shoots per explant (6.7) obtained in this research was significantly higher than the one obtained by Ipekci et al. (2001), who found that the total number of axillary shoots per explant ranged from 1 to 5, using nodal segments as explants. Besides, our research showed that the most favorable variants of the medium contained relatively high con-

Table 5. The effects of IBA and NAA on length of roots.

IBA (mg l^{-1})	NAA (mg l^{-1})	Shorter than 10 mm (%)	10-25 mm (%)	Longer than 25 mm (%)
0.0	0.0	29.4 ± 6.5 ab	29.4 ± 4.4 b	41.2 ± 7.8 d
0.2	0.4	82.1 ± 1.3 c	16.7 ± 0.7 a	1.2 ± 0.8 a
0.4	0.4	14.2 ± 1.4 a	68.4 ± 2.7 c	17.4 ± 1.7 c
0.8	0.4	21.4 ± 3.7 b	64.6 ± 3.2 c	14.1 ± 0.7 c
0.8	0.8	16.9 ± 2.0 a	75.4 ± 3.4 c	7.7 ± 1.5 b
0.8	1.2	19.2 ± 2.8 a	74.3 ± 5.6 c	6.5 ± 2.6 b

Values are mean ± SE of three independent experiments. Means followed by the same letters are not significantly different at $p \leq 0.05$ by the least significant difference test.

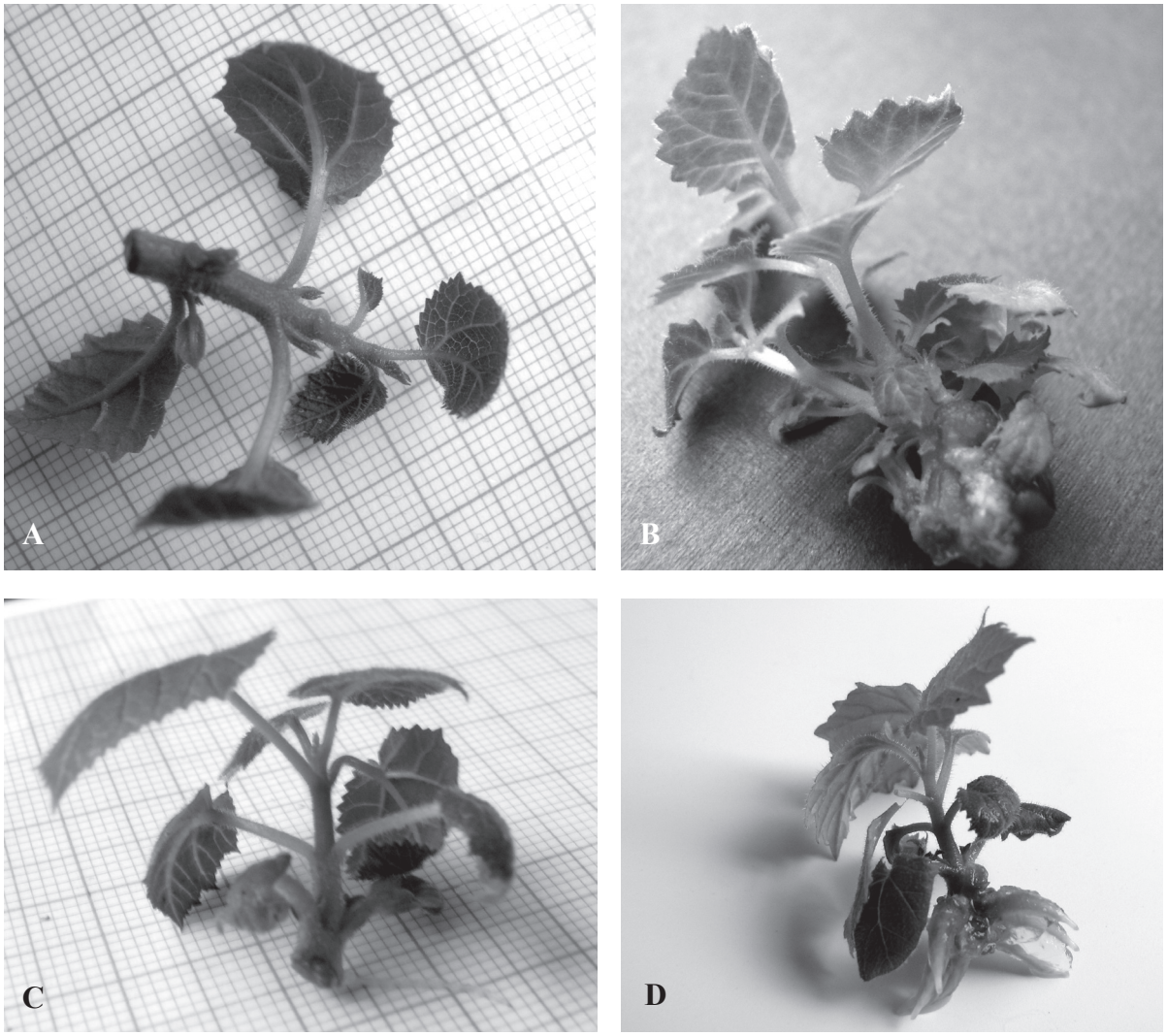

Fig. 1. A) Shoots developed on hormone-free medium supplemented, B) Shoots developed on medium supplemented with 6 mg l^{-1} BA and 0.5 mg l^{-1} IBA, C) Rooted shoots on hormone-free medium, D) Rooted shoot on medium containing 0.8 8 mg l^{-1} IBA and 0.8 8 mg l^{-1} NAA.

centrations of cytokinin (4 and 6 mg l^{-1} BA), whereas Ipekci et al. (2001) obtained the best results by using nodal segments of *P. elongata* on the medium with much lower concentrations of BA (1 mg l^{-1}). Similarly, Burger (1989) reports that the medium containing 1 mg l^{-1} BA and 0.1 mg l^{-1} IBA was the most favourable for micropropagation of *P. tomentosa* using axillary shoot tips (2 cm in length) and that on media with higher auxin concentrations and media lacking BA the lower rates of shoot multiplication were obtained. Likewise, Chang and Donald (1992) the optimal results got on medium containing 1 mg l^{-1} BA by growing the shoot tips obtained from aseptically produced seedlings of *P. elongata*. The medium containing 1 mg l^{-1} BA was also the most favourable medium during the multiplication phase, when the nodal segments were used as explants, after shoot induction from the explants derived from three-month-old plants of *P. elongata* × *fortunei* (Gyuleva 2010). When mature nodes of field-grown *P. tomentosa* were used as explants, the best results were achieved on medium with much higher concentrations of PGRS (5 mg l^{-1} BA and 0.5 mg l^{-1} NAA) than in the case when mature nodes from greenhouse grown plants were used (1 mg l^{-1} BA and 0.1 mg l^{-1} NAA) (Burger 1989). However, Ozaslan et al. (2005) used shoot tips from *in vitro* derived seedlings of *P. tomentosa*, and the number of regenerated shoots was the highest on the medium with a high BA concentration (7 mg l^{-1}). Moreover, high regeneration frequency of meristems was obtained on the medium containing 15 mg l^{-1} BA during propagation of 8-year-old tree of *P. taiwaniana* (Yang et al. 1989). In addition to that, the survival rate of *in vitro* grown meristems derived from adult trees of seven *Paulownia* species differed depending on species (Song et al. 1989). Thus, the survival rate of meristems of *P. tomentosa* was 36.7%, *P. taiwaniana* 21.4%, and

none of meristems of *P. elongata* and *P. kawakammi* survived. Furthermore, Song et al. (1989) indicated that the differences between different individuals of the same species were observed, for example survival rates of meristems derived from two trees of *P. glabrata* were 29.6% and 72.7%. These results indicate that optimal PGR level for shoots multiplication depends on growing conditions of stack plant, on the type of explants as well as on plant genotype. In that way, the positive effect of relatively high concentrations of PGRs in our research could be explained.

Bergmann and Whetten (1998) thoroughly explored the possibilities of *in vitro* rooting of *P. elongata* shoots. These authors successfully rooted shoots (94-100%) on medium with 0.2 mg l^{-1} NAA and 0.4 mg l^{-1} IBA, whereas in our study the lowest percentage of rooted shoots (83%) was obtained on the medium with low levels of auxin (0.2 mg l^{-1} IBA and 0.4 mg l^{-1} NAA). The research conducted by Bergmann and Whetten (1998) also showed that response to auxin treatment differed considerably depending on the used clone of *P. elongata*. This could explain the differences between our and their results, indicating that it is important to determine auxin level for the specific genotype in order to obtain the best results.

Acknowledgments: This paper was supported by the project TR 31041 (Establishment of Wood plantations intender for afforestation of Serbia) of the Ministry of Science and Technological Development of Serbia

REFERENCES

Bergmann B. A. (1995). Micropropagation of *Paulownia elongata*. *In*: Wier R. J., Hatcher A. V. (Eds). Proceedings 23rd Southern Forest Tree Improvement Conference. Asheville, North Carolina, 266.

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

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

Burger D. W. (1989). Empress tree *(Paulownia tomentosa* Steud.), *In*: Bajaj Y. P. S. (Ed.). Biotechnology in Agriculture and Forestry, Vol. 5 Trees II, Springer, Berlin, Heidelberg: 359-369.

Castellanos-Hernandez O. A., Rodriguez-Sahagun A., Rodríguez-Dominguez J. M., Rodríguez-Garay B. (2006). Organogénesis indirecta y enraizamiento *in vitro* de *Paulownia elongata*. e-Gnosis (on-line). 4: 1-12.

Chang S. H., Donald D. G. M. (1992). *In vitro* organogenesis and plantlet formation of *Paulownia elongata*. South African Forestry Journal, 163: 27-29.

Gyuleva V. (2010). Micropropagation of hybrid *Paulownia* from long-term preserved seeds. Silva Balcanica, 11: 45-58.

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

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

Khan M. (1992). Selection of size of root cuttings for vegetative propagation of *Paulownia elongata*. Pakistan Journal of Forestry, 42: 144-147.

Li B., Xie Z., Zhang A., Xu W., Zhang C., Liu Q., Liu C., Wang S. (2010). Tree growth characteristics and flower bud differentiation of sweet cherry (*Prunus avium* L.) under different climate conditions in China. HortScience, 37: 6-13.

Lobna S., Taha M. M., Soad I., Farahat M. M. (2008). A micropropagation protocol of *Paulownia kowakamii* through *in vitro* culture technique. Australian Journal of Basic and Applied Sciences, 2: 594-600.

Murashige T., Skoog F. (1962). A revised medium for growth and bioassays with tobacco tissue culture. Physiologia Plantarum, 15: 473-497.

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

Song S. L., Sato T., Saito A., Ohba K. (1989). Meristematic culture of seven *Paulownia* species, Journal of the Japanese Forest Society, 71: 456-459.

Stankovic D., Igic R., Šijačić-Nikolić M., Vilotić D., Pajevic S. (2009a). Contents of the heavy metals nickel and lead in leaves of *Paulownia elongata* S. Y. Hu and *Paulownia fortunei* Hems. in Serbia. Archives of Biological Sciences Belgrade, 61: 827-834.

Stankovic D., Nikolic M. S., Krstic B., Vilotic D. (2009b). Heavy metals in the leaves of tree species *Paulownia elongata* S. Y. Hu in the region of the city of Belgrade. Biotechnology & Biotechnological Equipment, 23: 1330-1336.

Vilotić D., Vukovojac S., Šijačić-Nikolić M. (2006). Effect of the super absorbent on development of *Paulownia elongata* seedlings. *In*: Isik F. (Ed.). Low Input Breeding and Genetic Conservation of Forest Tree Species. Proceedings of the IUFRO Division 2 Joint Conference: 9-13 October, Antalya, Turkey: 35.

Yang J. C., Chang S. H., Ho C. K. (*1989*). Micropropagation of *Paulownia taiwaniana* from mature tissues. Annals of Forest Science, suppl., 46: 165-167.

Zhu Z. H., Chao C. J., Lu X. Y., Xiong Y. G. (1986). Paulownia in China: Cultivation and utilization. Asian network for biological sciences, Republic of Singapore and International Development Research Centre, Canada, 28-45.