PRODUCTION OF SEEDLINGS OF FAST - GROWTH TREE OF *Paulownia elongata* S. Y. Hu

ABSTRACT:

The major method of propagation of varieties and hybrids of Paulownia elongata is vegetative (asexual) method. Paulownia elongata can be propagated by macropropagation techniques (root cuttings, green cuttings and by micropropagation technique, tissue culture or in vitro. Today tissue culture method is the most modern biotechnological method. In Bosnia and Herzegovina and region of former Yugoslawia, more and more Paulownia elongata seedlings are being produced and new plantations of Paulownia elongata are established. This paper gives answers to some questions such as the methods of propagation and problems in raising Paulownia elongata planting materials. The aim of this work is to produce seedlings of fast-growth tree of Paulownia elongata, Shan Tong hybrid and the possibility of propagation through different methods. Propagation by green cuttings, root cuttings and in vitro propagation were tested. After 15 days, the percentage of rooting of the green cuttings was 100% and there was no dead plants, the average number of roots was 13.86 pcs per plant and roots were different lengths. The length of the cuttings had an impact on growth of plants because number of living cuttings 1.5 cm long was the smallest (4 pcs from 30 plants or 1.33%) weil in cuttings with a length of 5 cm, there was the best rooting (26 out of 30 plants or 86.6%). For in vitro propagation meristems of mother plants were used for establishing of tissue culture. The plants showed a survival rate of 80-90% .Production of Paulownia elongata seedlings by different methods of vegetative propagation provide a variety of options to producers, depending on what kind of equipment they have. In vitro production is the most expensive but also the fastest because a large number of seedlings can be produced for a short time. It is recommended that in vitro propagation is used to form mother plant stock, and that in the coming 2-3 years the green cuttings from super-elit planting material are going to use.

Key words: fast-growing trees, seedlings, renewable energy sources, introduction, propagation

1. INTRODUCTION

Paulownia elongata is a fast growing species suitable for plantation. In the first few years after planting, the annual growth in height can be 3-4 m, and after 5-7 years growth is slower. Already after ten years the trees reach 15-18 m in height, with a trunk diameter 40-50 cm. *Paulownia elongata* is an excellent air cleaner because of large surface of leaf blades which make photosynthesis, bind carbon dioxide and releases oxygen. In addition, a good substrate is obtained by composting the leaves. At the time of flowering it is extremely decorative, and besides, it is a honey plant. The wood has good quality, it can be used in the wood industry, even for the production of furniture.

Paulownia elongata is a very modest plant. It can be grown at an altitude of 2000 m, but best growth is at an altitude less than 1000 m. According to the soil it is not picky, therefore it is used for afforestation of degraded areas on poor soil. It is better to use lighter and more sandy soil than heavy and clayey. Also, it does not tolerate a high level of groundwater or waterlogged spots. *Paulownia elongata* tolerates temperature extremes and drought. Root system is deep and well-developed. The root mass is formed at a depth of 1 m or more.

Paulownia elongata can be propagated by generative or vegetative methods. The seed germinates slowly and unevenly so this method is rarely used. The primary method of propagation of varieties and hybrids of *Paulownia elongata* in nursery is vegetative or asexual (Drvodelić, D., 2018). There are two most effective vegetative propagation methods for *Paulownia elongata*. The first, traditional method, is technique of macropropagation with root cuttings and the other, more modern, is micropropagation technique by tissue culture '*in vitro*'. Propagation by root cuttings is the most common method for multiplication of *Paulownia elongata* that is being carried out in China already more than half a century. Cuttings taken from any part of the root system of *Paulownia elongata* have the capacity to regenerate new tissues, providing the cuttings of a sufficient size.

The use of in vitro propagation techniques provides healthy, good quality planting stock for biomass production of *Paulownia elongata*. Efficient vegetative micropropagation have many advantages over seedling propagation of *Paulownia ssp*. (Markovic et al., 2013). This method allows multiplication of basic material without risk of infection and production a large number of healthy uniform plants that will be suitable for further multiplication or development on their own root depending on the production goal. In a very small area it is possible to produce a large number of seedlings from only one mother plant.

2. MATERIALS AND METHODS

The subject of this paper was the *Paulownia elongata, Shan Tong* hybrid and the possibility of propagation using different methods of propagation. The research was carried out in the company "Voćni Rasadnik" ltd Srebrenik (Longitude 44°76' 23.2" N and Latitude 18°49' 71.3" S) who owns a specialized laboratory and trained staff for the production of *Paulownia elongata* seedlings by tissue culture as well as the accompanying objects (greenhouse, plastic tunnel and container field).

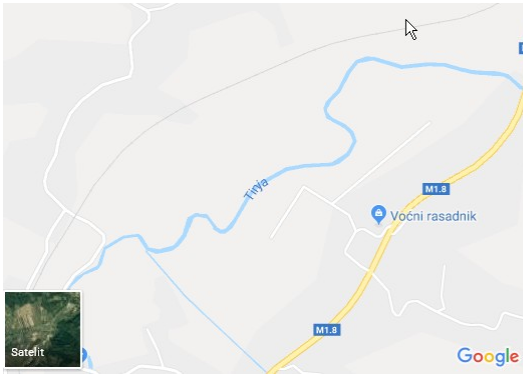

Figure 1: Map and Satelite of 'Vocni rasadnik' Company

(Source:https://www.google.ba/maps/place/Vo%C4%87ni+rasadnik/@44.7622643,18.49742,15z/data=!4m5!3m4!1s0x0:0x9139928ef965e61e!8m2!3d44.7622643!4d18.49742)

For the production of seedlings, two techniques were used: cutting technique (green cutting and root cutting) and in vitro technique. Technique of root cuttings enables production of cheap seedlings in large quantities (Drvodelić, D. 2018)

Root cuttings were taken in the nursery during March. For the root cuttings, the roots of one-year-old plants that were stored in the sand during winter were used.

Before cutting, the roots were taken out from the sand, cleaned and cut after which the root cuttings were planted in plastic pots with 8 cm diameter filled with the substrate ("Vigor plant", based on Irish and Baltic peat). There were 30 root cuttings 1.5, 3 and 5 cm in length and average thickness was 0,57 cm of each size. They were planted at a depth of 2 cm and watered with 0.25% Kaptan solution. During the next 15 days the substrate moisture was maintained by watering with water.

Thirty green cuttings were taken from one-year old container seedlings during March. Containers with plants were during the winter in plastic tunnel. An average length of cutting was 3.7 cm, average thickness was 1.8 mm, and each cutting had two-three leaves. Immediately after taking of green cuttings, they were soaked in a solution of IBA 1% and planted in plastic pots with a diameter of 8 cm filled with a substrate ("Vigor plant", based on Irish and Baltic peat) which was watered with 0.25% Kaptan solution, 30 pots was arranged in a plastic crate and placed in a plastic bag that is folded at the ends. After that, the plants were no longer watered and on the fifteenth day was uncovered, checked for rhizogenesis and roots are counted for each plant.

For *in vitro* propagation meristems of mother plants were used for establishing of tissue culture. *In vitro*, seedlings were grown in medium for multiplication (*Murashige & Scoog*

Medium with $CaCl_2$, Vitamins, Sucrose and Agar) in 375 mL glass jars, in each jar was 20 plants, and multiplication was done every 4 weeks. For rooting plants were grown in special medium (Murashige & Scoog Medium / Van der Salm Modification / with $FeSO_4$, substituted by FeEDDHA and Vitamins Without Sucrose and Agar, 4.46 g of dehydrated medium per liter)

3. RESULTS AND DISCUSSION

Green cuttings

The average number of roots per plant is 13.86 pcs. Data on the number of roots are given in table 2. and table 3. gives a moisture and temperature regime.

Table 2. Number of Roots per Plant in the Study Area

Plant number	I sample	II sample	III sample
1	8	16	10
2	10	11	13
3	8	21	9
4	9	15	19
5	14	11	15
6	11	14	24
7	9	13	10
8	5	12	7
9	5	15	12
10	10	13	8
11	12	11	7
12	11	10	24
13	9	12	30
14	10	12	10
15	15	16	29
16	6	15	8
17	6	15	12
18	3	31	14
19	13	23	23
20	8	29	9
21	22	16	10
22	12	14	38
23	6	16	10
24	9	19	12
25	20	20	7
26	11	17	8
27	18	15	14
28	17	13	23
29	23	11	18
30	15	8	15
	$\bar{x}$ 11.17	**$\bar{x}$ 15.47**	**$\bar{x}$ 14.93**

Table 3. Temperature and Moisture Regime

Day	Night temperature °C	Daily temperature	Moisture %
1	12	25	93
2	14	27	94
3	15	30	90
4	17	32	93
5	16	28	92
6	17	31	95
7	13	29	90
8	18	30	92
9	17	32	93
10	13	28	91
11	15	29	95
12	17	35	90
13	12	29	92
14	18	36	91
15	13	30	93
16	12	30	94
17	16	32	95
18	17	34	97
19	14	30	94
20	13	29	95
21	18	33	95
	$\bar{x}$ 15.10	**$\bar{x}$ 30.43**	**$\bar{x}$ 93.05**

After 15 days, the percentage of rooting of the green cuttings was 100% and there was no dead plants, the average number of roots was 13.86 pcs per plant and different lengths.

Table 4. Morfometric features

Number	Height Cm	Leaf number pcs	Leaf width cm	Leaf lenght cm
1	5.2	6	4.0	5.0
2	5.0	7	4.2	4.5

3	7.0	7	4,3	5.3
4	5.0	5	4.3	5.0
5	4.0	5	3.6	4.2
6	3.5	6	4.2	4.5
7	4.0	4	4.1	4.8
8	3.5	6	4.1	4.0
9	3.0	4	3.2	4.0
10	5.6	4	3.3	4.5
11	4.3	5	4.3	4.0
12	5.1	6	3.6	5.0
13	3.6	4	3.7	3.5
14	4.1	4	4.1	4.7
15	6.2	4	4.2	5.5
16	4,3	4	5.1	4.2
17	3.0	6	5.0	3.5
18	3.6	4	3.5	4.2
19	4.2	4	4.0	4.0
20	4.1	4	6.2	4.5
21	4.0	3	7.0	5.0
22	3.3	4	6.3	4.0
23	6.2	4	5.5	6.0
24	5.2	4	6.4	5.5
25	6.1	4	5.1	5.5
26	5.1	4	4.8	7.5
27	6.0	4	5.2	5.6
28	7.4	6	5.3	7.7
29	5.4	7	6.0	5.4
30	7.1	4	5.9	7.0
	$\bar{x}$ 4.80	$\bar{x}$ 4.77	$\bar{x}$ 4.68	$\bar{x}$ 4.94

Figure 2: Rooted green cuttings

In table no. 4 are given data about height (growth increase) of the plants, the number of leaves, length and width of leaves after 21 days of planting green cuttings. The growth of the plant is visible as well as the increase in the number of leaves and the increase of the most developd leaf. In Fig. 2 we see a well-developed root system.

Root cuttings


Root cuttings


Rooted cuttings


Paulownia seedlings

Figure 3. Rooting of root cuttings

The percentage of plants which started to grow is presented in table 5.

Table 5. Number and Percentage of Living Plants from Root Cuttings

Lenght of cutting	Number of plants	Number of living plants	% of living plants
1.5 cm	30	4	13.3
3 cm	30	23	76.6
5 cm	30	26	86.6

Based on the data from Table 4. we concluded that the length of the cuttings had an impact on growth of plants because number of living cuttings 1.5 cm long was the smallest 4 pcs from 30 plants or 1.33%. In cuttings with a length of 5 cm, there was the best rooting, 26 out of 30 plants or 86.6%.

Cuttings 1.5 cm

Figure 4: Root cuttings-growing

Research implemented in New Zealand in 2007 and 2010 was oberved on root cuttings of 0.75 - 2.0 cm thick and 10-20 cm in length. Disinfection with Kaptan, drying for 3 days and keeping in refrigerator 15-21 days after which they were planted in the ground in the open field. Similar investigations were carried out in the United States with root cuttings 4-5 inches long (10.16 - 12.7 cm) and 1 inch thick (2.54 cm). The mentioned research with root cuttings refers to their planting directly to a permanent site while our research focused on the production of container seedlings and their subsequent planting in a permanent place after the rhizogenesis and the development of the above-ground system.

Production of seedlings by *in vitro* technique

After the initialization and establishment of tissue culture, the multiplication continued every four weeks. The plants grew first in the tubes for safety from the infection, and later they were placed in glass jars of 370 mL with 50-70 mL of MS media. Preparation for acclimatization implied the transfer of plants to the rooting medium where they spend 12-15 days to form the root system. After rooting, the plants are removed from the medium and washed in lukewarm water to remove the remains of the media. Before planting, pots were filled with substrat and watered with water in which the fungicide is dissolved. Planting was done in a greenhouse.

Thirty young plants were planted in individual plastic pots, sprayed and covered with a glass jar, to create microclimate and for easier moisture maintenance. After 4-5 days, the jar was removed and the plants were covered with lutrasil foil and regularly irrigated, as well as the surrounding area. When the root grows through the holes at the bottom of the pots (3 weeks), the plants were placed on a container box, covered with a shadenet and stay there for 3-4 days for complete acclimatization. The plants showed a survival rate of 80-90% After that, planting of plants in a permanent place can begin. At that time they reach a height of about 15 cm and have a well-developed root system.


Initilization


Multiplication


Growth room


Acclimatized seedlings

Figure 5. *In vitro* production

In *'in vitro'* production there were almost no problems until the moment of acclimatization. When acclimatizing, it is necesessery to standardize temperature and moisture because the *Paulownia elongata* does not allow temperature fluctuations and humidity reduction. If there were not bound by deadlines and there is not have a stable heat source acclimatization should

plan on warmer days (April), and solve the problem of moisture by covering the plants with glass jars (370 mL jars).

Reaearch in Bulgaria on in vitro rooting of *Paulownia elongata* showed that plants were successfully transfered from laboratory to a greenhouse. They were characterized by rapid growth and normal development. The adapted plants did not exhibit any morphological variations when compared with the initial plants. Plant growth and development can easily be disturbed by a change in the environmental conditions after the *ex vitro* transfer and so, plants need a period of acclimatization. We did not have problem because we we maintained the level of moisturesolve moisture level by covering plants with jars. Many plants can die during this period (Clapa, D., et al., 2013). Acclimatization depends on the development of adventitious roots and this is affected by the substrate type and the physical parameters of *ex vitro* conditions. Acclimatization was evaluated by the percentage of the survived plants, plant height and number of leaves and it was seriously affected by the quality of the substrates. The aeration in the root substrate is very important for *ex vitro* acclimatization. It is suggested that the peat mixture, which contains peat and perlite, improves aeration and reduces water retention leading to root growth (Zayova E. *et al*., 2014).

4. CONCLUSION

For root cuttings it is better to use 5 cm long cuttings (container seedlings production) because they give a higher percentage of rooted plants than smaller root cuttings. Green cuttings successfully reproduce *Paulownia elongata* plants in the greenhouse with 100% rooting. *In vitro* multiplication of *Paulownia* elongata yields good results with a multiplication rate of 10 (proliferation rates, number of shoots/explant). Acclimatization of *Paulownia elongata* plants is demanding and it reacts negatively on suddenly temperature changes and humidity so in their acclimatization it is recommended to use glass jars in the first 5 days for plants covering to avoid the death of plants. Production of *Paulownia elongata* seedlings by different methods of vegetative propagation provide a variety of options to producers, depending on what kind of equipment they have. *In vitro* production is the most expensive but also the fastest because a large number of seedlings can be produced for a short time. It is recommended that *in vitro* propagation is used to form mother plant stock, and that in the coming 2-3 years the green cuttings will be taken from super-elite planting material.

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