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Production of Seedlings of Fast - Growth Tree of *Paulownia elongata* S. Y. Hu

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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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 the tissue culture method is the most modern biotechnological method. In Bosnia and Herzegovina and regions of former Yugoslawia, more and more Paulownia elongata seedlings are being produced and new plantations of Paulownia elongata areestablished. This paper deals with the methods of propagation and problems in raising Paulownia elongata planting materials. The work aims to produce seedlings of fast-growth Paulownia elongata, Shan Tong hybrid and the possibility of propagation through different methods. Propagation by green cuttings, root cuttings and *in vitro* propagation was tested. After 15 days, the percentage of rooting for the green cuttings was 100%, and there were no dead plants, the average number of roots was 13.86 pcs per plant and roots were of different lengths. The length of the cuttings had an impact on the growth of plants because

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the smallest cuttings was of 1.5 cm (4 pcs from 30 plants or 1.33%), whereas cuttings of 5 cm (26 out of 30 plants or 86.6%), showed the best rooting. 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 provides 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 method because a large number of seedlings can be produced in a short time. It is recommended that *in vitro* propagation should be used to build mother plant stock and that in the coming 2-3 years the green cuttings from super-elite planting material are going to be used.

Keywords: 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 of 3-4 m, and after 5-7 years the growth rate decreases [1]. A ten years, the trees reach 15-18 m in height, with a trunk diameter of 40-50 cm [2]. *Paulownia elongata* is an excellent air cleaner because of the large surface of leaf blades which during 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 is of good quality,and can be used in the wood industry, even for the production of furniture [3].

Paulownia elongata is a very modest plant. It can be grown at an altitude of 2000 m, but best growth can be obtain at an altitude less than 1000 m. As per the soil requirement, it is not picky. Therefore it is used for afforestation of degraded areas on poor soil [4]. It is better to use lighter and sandy soil than heavy and clayey. Also, it does not tolerate a high level of groundwater or waterlogged spots. *Paulownia elongata* can withstand extremes and drought temperature. The root system is deep and well-developed. The root mass is formed at a depth of 1 m or more [5,6].

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 in the nursery is vegetative or asexual [7]. There are two most effective vegetative propagation methods for *Paulownia elongata*. The first, traditional method, is the technique of macro propagation 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 practiced in China more than half a century. Cuttings taken from any part of the root system of *Paulownia elongata* can regenerate new tissues, provided the cuttings are of sufficient size.

The use of *in vitro* propagation techniques provides healthy, good quality planting stock for biomass production of *Paulownia elongata*. Efficient vegetative micropropagation has many advantages over the seedling propagation of *Paulownia ssp*. [8]. This method allows multiplication of basic material without the risk of infection and produces a large number of healthy uniform plants that will be suitable for further multiplication or development besed on root depending on the production goal [9]. In this method, using a tiny area, it is possible to produce a large number of seedlings from only one mother plant [10].

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) which owns a specialised 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).

For the production of the seedlings, two techniques were used: cutting technique (green cutting and root cutting) and *in vitro* technique [11]. The technique of root cuttings enables the

production of cheap seedlings in large quantities [7,12].

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 kept in a plastic tunnel during the winter. An average length of the 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 were 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 were counted for each plant.

For *in vitro* propagation meristems of mother plants were used for establishing the tissue

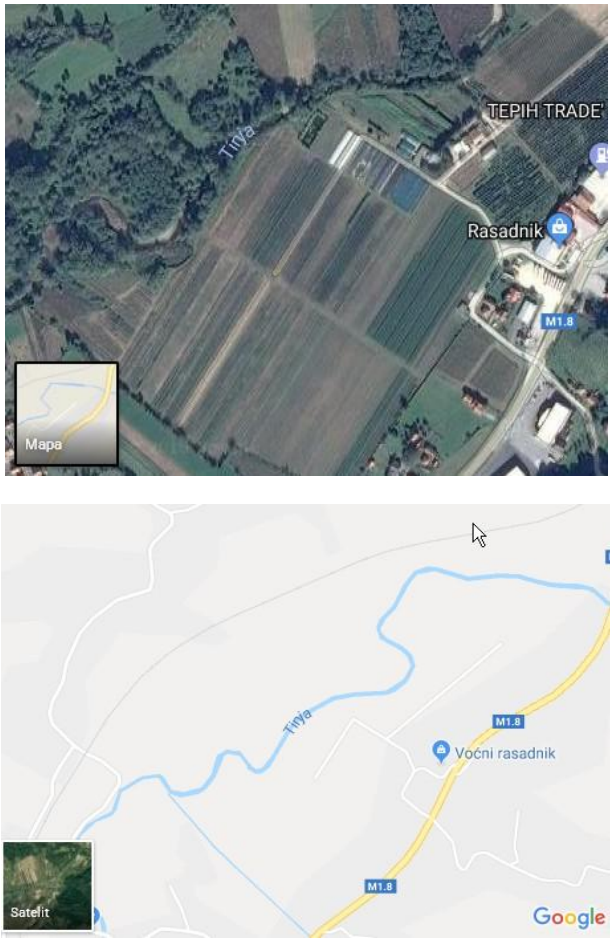

Fig. 1. Map and satelite of 'Vocni rasadnik' company

(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)

culture. *In vitro*, seedlings were grown in the 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 in every 4 weeks. 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 litre).

3. RESULTS AND DISCUSSION

3.1 Green Cuttings

The average number of roots per plant is 13.86 pcs. Data on the number of roots are given in Table 1 and Table 2 gives a moisture and temperature regime.

Table 1. 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

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

In Table 3 data about height (growth increase) of the plants, the number of leaves, length and width of leaves after 21 days of planting green cuttings are given. The growth of the plant is visible as well as the increase in the number of leaves and the increase of the most developed leaf. In Fig. 2 a well-developed root system can be seen.

Fig. 2. Rooted green cuttings

3.2 Root Cuttings

The percentage of plants which started to grow is presented in Table 4.

Based on the data from Table 3. It is concluded that the length of the cuttings had an impact on the growth of the plants because the number of living cuttings of 1.5 cm long was the smallest 4 pcs from 30 plants or 1.33%. In cuttings with a length of 5 cm, best rooting was seen, 26 out of 30 plants or 86.6%.

Table 2. 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

Table 3. Morphometric features

Number	Height cm	Leaf number pcs	Leaf width cm	Leaf lengh 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

Root cuttings

Rooted cuttings

Paulownia seedlings

Fig. 3. Rooting of root cuttings

Table 4. 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

Cuttings 1.5 cm

Fig. 4. Root cuttings-growing

Research implemented in New Zealand in 2007 and 2010 was observed 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 for 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 of 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 direct planting to a permanent site while this 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.

3.3 Production of Seedlings by *In vitro* Technique

After the initialisation and establishment of the 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 acclimatisation 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 substrate and watered in which the fungicide was 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 a 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 grew through the holes at the bottom of the pots (3 weeks), the plants were placed on a container box, covered with a shade net and kept there for 3-4 days for complete acclimatisation. The plants showed a survival rate of 80-90% After that, planting of plants in a permanent place may begin. At that time they reach a height of about 15 cm and have a well-developed root system.

Initilisation **Multiplication** **Growth room** **Acclimatised seedlings**

Fig. 5. *In vitro* production

In *'in vitro'* production there were almost no problems until the moment of acclimatisation. At the time of acclimatising, it is necesessary to standardise temperature and moisture because the *Paulownia elongata* does not allow temperature fluctuations and humidity reduction. If there is no deadlines and no stable heat source acclimatisation should be plan on warmer days (April), and solve the problem of moisture by covering the plants with glass jars (370 mL jars).

Research in Bulgaria on *in vitro* rooting of *Paulownia elongata* showed that plants were successfully transferred from laboratory to a greenhouse. They were characterised 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 acclimatisation. The study did not faced problem because the level of moisture was maintained by covering plants with jars. Many plants can die during this period [13]. Acclimatisation depends on the development of adventitious roots and this is affected by the substrate type and the physical parameters of *ex vitro* conditions. Acclimatisation was evaluated by the percentage of the survived plants, plant height and the 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* acclimatisation. It is suggested that the peat mixture, which contains peat and perlite, improves aeration and reduces water retention leading to root growth [14].

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). Acclimatisation of *Paulownia elongata* plants is demanding, and it reacts negatively on suddenly temperature changes and humidity so in their acclimatisation it is recommended to use glass jars for 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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