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

ABSTRACT:

The need for renewable energy sources, consumption of non-polluting energy sources, has necessitaded the production of plant materials and planting of fast growing trees such as Paulownia. In addition to the need to preserve indigenous species, there is also a need for introduction of new species. Introduction should be under control because uncontrolled introduction of invasive plant species leads to problems that often require enormous efforts and a lot of money. 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 benefits of growing Paulownia elongata trees, 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

The lack of fossil resources and the increasing demand for wood requires the need for greater use of fast-growing species for the production of wood and biomass (Mishra *et al*., 2010). The plantation of fast-growing trees in fields or meadows represents a valuable and necessary alternative for the supply of biogenic raw materials. It also contributes to the diversity in the cultivation of crops on agricultural land, and can be used for the treatment of wastewater and soil, as well as for the absorbtion of increased amounts of atmospheric carbon. Planned, plantation growing of fast-growing species of trees such as willow (*Salix* L*.),* poplars (*Populus* L.), achilles (*Alnus glutinosa* L.), birch (*Betula pendula* Roth.), black locust (*Robinia pseudoacacia* L.) paulownia (*Paulownia* spp .) etc. is called SRC (Short Rotation Coppice / Crop / Culture) or SRF (Short Rotation Forestry), based on the time from planting till harvest. In *Paulownia* species this is dependent on the purpose (1 year for silage, 7 to 8 years for the wood mass). According to the soil requirements *Paulownia* is not picky, therefore it is used for afforestation of degraded areas, on the poor soil, it is especially interesting for the recultivation of minings. The development of the industry leads to intensified exploitation and use of fossil fuels, which results in negligible negative effects on the environment (Veselinović *et al*., 2012) and interest in wood as a fuel and raw material is again being actualized (Nicholls D.L.; Zerbe J. 2012). The energy value of the *Paulownia elongata* is the same as the best coal. One kilo of dry weight of paulownia wood gives 4.700 kcal, and oak 2.600 kcal.

Germany put *Paulownia* species on the first place in solving the energy problem. On a global level, in the last decades, a number of declarations, conclusions and statements have been accepted. On the basis of which the signatory states have committed to numerous obligations, the most important is the Kyoto Protocol, which obliges the signatory countries to reach a 20% in the use of renewable energy sources (Dražić *et al*., 2010). In addition to its beautiful decorative look, cost-effectiveness and healing properties, *Paulownia elongata* has also proved to be a world record holder in carbon dioxide absorption and in the production of oxygen.

One tree absorbs 2.25 kg CO_2 per hour , and at the same time produces 1.75 kg of O_2. It annually absorbs 19.5 tons of carbon dioxide and produces 15 tons of oxygen. It represents a real small air spa, because it emits oxygen enough to breathe 50 people. So it improves the level of polluted air. All forest plants in the process of photosynthesis produce oxygen and are a good protection for human life. The branched root system (length up to 2 m) with the possibility of penetration into the deeper layers allows soil binding and erosion control, and certainly contributes to the better utilization of the soil potential, as it increases the ability of the plant to absorb water and nutrients from it.

When burning *Paulownia* wood emits in the atmosphere lot less harmful gases, data on gas emissions are given in table 1.

Harmful substances	**Paulownia**	**Coal**	**Oil**
Sulfur oxide (SO)	0	1750	277
Carbon monoxide (CO)	0	7	0
Methane (CH4)	0	8	0
Carbon dioxide (CO2)	187	550	775
Other gases	0	140	2800

Table 1: Emission of harmful gases

Source: US Department of Energy The National Renewable Energy Laboratory

After combustion, the coal leaves up to 35% of the slag, and *Paulownia elongata* up to 5% of the ash.

Based on the facts and experiences in the cultivation of *Paulownia elongata*, it is indisputable to give recommendation for the distribution of this species on our fields, especially on degraded and weak soils. The species and varieties that need to be distributed must be keep in mind . It would be ideal to perform a controlled introduction, but this is not always possible for many reasons. Of course, there is a danger of invasiveness of this kind. For beginning, the experiences of others should be used.

The US Department of Agriculture (USDA) lists the species *Paulownia tomentosa* as invasive because it is expanding uncontrollably in the south, east, and midwest of the United States. It was imported from China in 1840, as an ornamental plant, but it spreads from gardens and parks to fields and forests, where the Americans are trying to eradicate it now, causing great costs. In New England, for now, the spread is limited to the coastal area, according to the Atlas of Invasive Species of New England.

In Croatia, a marketing authorization has been granted and / or introduced into the wild for the purpose of cultivating a foreign species known under the commercial name *Paulownia Bellissima*, as it has been established that there is no ecological risk of its placing on the market and introduction into the wild. There are other, herbaceous, fast-growing species, for example, black locust which is invasive by many parameters, even more adaptive to our conditions than *Paulownia elongata*, and nobody has nothing against the black locust, although it is not an autochthonous species.

The poplar is multiplied in several ways: from seeds, root cuttings, stem cuttings and by tissue culture. Today, *in vitro* method of propagation is the most modern method of vegetative propagation. The paper presents the experience in the production of *Paulownia elongata* seedlings with wood cuttings, root cuttings and *in vitro* by tissue culture.

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).

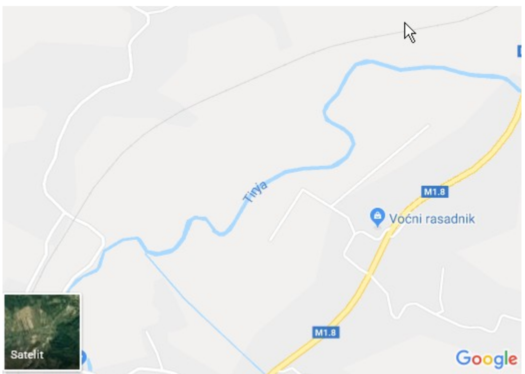

Figure 1: Map and Satelite of 'Vocni rasadnik' Company

(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
	x̄ 11.17	**x̄ 15.47**	**x̄ 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
	x̄ 15.10	**x̄ 30.43**	**x̄ 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.

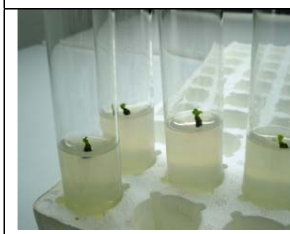			
Initilization	Multiplication	Growth room	Acclimatized seedlings

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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