

PRODUCTION OF SEEDLINGS OF FAST-GROWING TREES

Paulownia sp. Sieb. & Zucc

Comment [u1]: 1. Delete s

Note: production of seedlings of fast-growing tree of *Paulownia* (Sieb. & Zucc)

2. what is the sp? Specify the species by completing the name(s) of the species.

3. recast the title

ABSTRACT:

The need for renewable energy sources, the consumption of non-polluting energy sources, has caused the production of plant material 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. Introductions should be under control because it is known that uncontrolled introduction of invasive plant species leads to problems that often require enormous efforts and a lot of money.

Comment [u2]: Delete 's'

In our country and region, more and more Paulownia seedlings are being produced and new plantations are founded. This paper gives answers to some questions such as the benefits of growing Paulownia trees, propagation and problems in growing Paulownia planting material.

Comment [u3]: 1. What country and region?
2. specify

Comment [u4]: mention the species

Comment [u5]: mention the species

Comment [u6]: 1. abstract should be in one paragraph
2. Results and recommendations of the research has not been captured in abstract.

Key words: fast growing trees, 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 (Swamy et al., 2006; Mishra et al., 2010). The plantation of fast-growing trees in fields or meadows represents and will represent 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 absorption 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 this is dependent on the purpose (1 year for silage, 7 to 8 years for the wood mass). According to the soil it 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 (Börjesson et al., 1997; Dubuisson and Sintzoff, 1998; Cannell, 2003). The energy value of the paulownia is the same as the best coal. One kilo of dry weight of paulownia wood gives 4.700 kcal, and oak 2.600 kcal.

Comment [u7]: Did not italicize throughout the work, please, italicize et al., as *et al*

Comment [u8]: Do the Same (D.S)

Comment [u9]: Specify by mentioning the species

Comment [u10]: D.S to all

Germany put paulownia 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 has also proved to be a world record holder in carbon dioxide absorption and in the production of oxygen. One tree per hour absorbs 2.25 kg CO₂, and at the same time produces 1.75 kg of O₂. 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 (Woods, 2008; Škvorc et al., 2014).

Comment [u11]: Too long sentence. Break the sentence, using fullstop.

Comment [u12]: CO₂

Comment [u13]: Make it a paragraph

When burning paulownia emits in the atmosphere lot less harmful gases, data on gas emissions are given in table no. 1

Table No.1 Emission of harmful gases

Harmful substances	Paulownia	Coal	Oil
Sulfur oxide (SO)	0	1750	277
Carbon monoxide (CO)	0	7	0
Methane (CH ₄)	0	8	0
Carbon dioxide (CO ₂)	187	550	775
Other gases	0	140	2800

Source: USDOE NREL

Comment [u14]: Year?

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

Based on the facts and experiences in the cultivation of paulownia, 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 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 uncontrolledly in the south, east, and midwest of the United States. It was imported from China in 1840, as an ornamental plant, but from gardens and parks it spreads 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 the paulownia, 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 considered the most modern method of biotechnology. The paper presents the experience in the production of paulownia seedlings with wood cuttings, root cuttings and "in vitro" by tissue culture.

2. MATERIAL AND METHODS OF WORK

The subject of the study was the *Pulownia Shan Tong* hybrid and the possibilities of different ways of propagation. The research was carried out in the company "Voćni Rasadnik" Ltd. Srebrenik who owns a specialized laboratory and trained staff for the production of Paulownia seedlings by tissue culture as well as the accompanying objects (greenhouse, plastic tunnel and container field). For the production of seedlings, two techniques were used: cutting technique (green cutting and root cutting) and in vitro technique. For root cuttings were used one year old plants. Roots are cut to 1.5, 3 and 5 cm in length cuttings and average thickness 5.7 mm. Green cuttings were taken from one-year old container seedlings, an average length of 3.7 cm, an average thickness of 1.8 mm, and two-three leaves.

For 'in vitro' propagation meristems of mother plants are used for establishing of tissue culture. All cuttings were planted in plastic pots with a diameter of 8 cm filled with substrate - "Vigor plant", based on Irish and Baltic peat. Immediately after taking 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, which was watered with 0.25% Kaptan, 30 pots was arranged in, 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 the fifteenth day was uncovered, checked for rhizogenesis and roots are counted for each plant. For the root cuttings, the root of one-year-old plants that were stored in the sand during winter was used.

Immediately before cutting, the roots were taken out from the sand and slaughtered, after which the root cuttings were planted in plastic pots with 8 cm diameter and filled with the substrate. They were planted at a depth of 2 cm and watered with 0.25% Kaptan. During the next 15 days the substrate moisture was maintained by watering with water. In vitro, seedlings were grown in medium for multiplication (Murashige & Scoog Medium with CaCl₂, Vitamins, Sucrose and Agar) in 375 ml glass jars, in each was planted 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 FeSO₄, substituted by FeEDDHA and Vitamins Without Sucrose and Agar, 4.46 g of dehydrated medium per liter)

3. RESULTS AND DISCUSSION

Comment [u15]: 1. Break into paragraphs
2. Referencing the source (s) of these information is very important, especially in academics/research papers.

Comment [u16]: The author should be able to understand the difference between genus and species. If you are referring to a specific species of Paulownia, pls state the species; and if you are referring to all the species of the genus paulownia, then mention all the species.
Note: scientific name(s) should be italicized such as *paulownia sp*

Comment [u17]: What are the materials used?

Comment [u18]: Do you mean *Paulownia* or '*Pulownia*'?

Comment [u19]: 1. Details of the study area should be mentioned with coordinates, longitude and latitude.
2. If possible, the map of the study area should be included.

Green cuttings

The average number of roots per plant is 13.86 pcs. Data on the number of roots are given in table no. 2 and in table no. 3 gives a moisture and temperature regime.

Table No. 2 Number of roots per plant Table No. 3 Temperature and moisture regime

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
	$\bar{X} 11,17$	$\bar{X} 15,47$	$\bar{X} 14,93$

Day	Night temperature	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
	$\bar{X} 15,10$	$\bar{X} 30,43$	$\bar{X} 93,05$

Comment [u20]: Scientific method of presenting results on tables should be use, i.e. all tables should have only 3 horizon lines only without vertical lines

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 No. 4 Morfometric features

Number	Height Cm	Leaf number pcs	Leaf width cm	Leaf lenght cm
1	5,2	6	4	5
2	5	7	4,2	4,5
3	7	7	4,3	5,3
4	5	5	4,2	5
5	4	5	3,6	4,2
6	3,5	6	4,2	4,5
7	4	4	4,1	4,8
8	3,5	6	4,1	4
9	3	4	3,2	4
10	5,6	4	3,3	4,5
11	4,3	5	4,3	4
12	5,1	6	3,6	5
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	6	5	3,5
18	3,6	4	3,5	4,2
19	4,2	4	4	4
20	4,1	4	6,2	4,5
21	4	3	7	5
22	3,3	4	6,3	4
23	6,2	4	5,5	6
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	4	5,2	5,6
28	7,4	6	5,3	7,7
29	5,4	7	6	5,4
30	7,1	4	5,9	7
	X 4,80	X 4,77	X 4,68	X 4,94

Comment [u21]: D.S



Figure 1. Rooted green cuttings

In table no. 4 are given data about height (growth increase) of the plant, 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 developed leaf. In Fig. 1 you see a well-developed root system.

Root cuttings

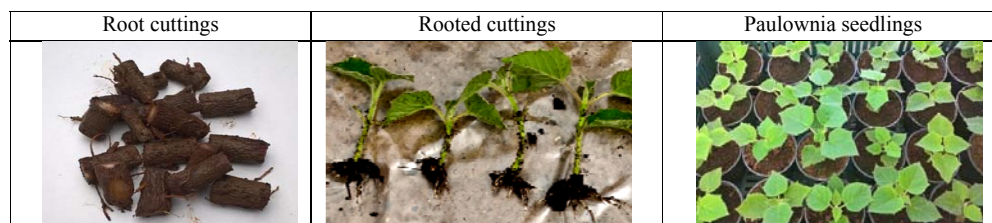


Figure 2. Rooting of root cuttings

The percentage of plants which started to grow is presented in table no.5

Table No.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 conclude 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 the cuttings with a length of 5 cm, there was the best rooting , 26 out of 30 plants or 86.6%.



Figure. 3 Root cuttings-growing

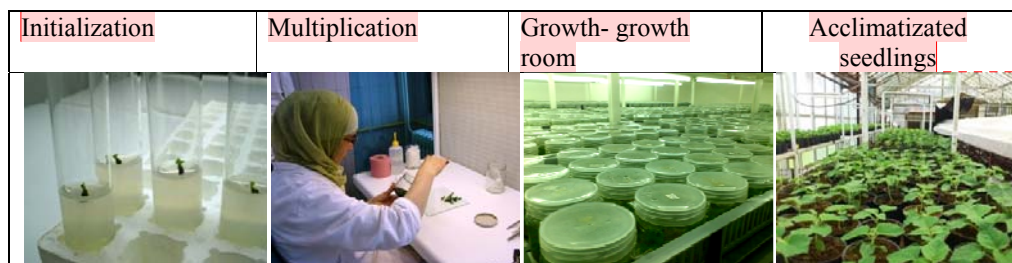
Research implemented in New Zealand in 2007 and 2010 was observed on root cuttings of 0.75 - 2.0 cm thick and 10-20 cm in length. Desinfection with Kaptan, drying for 3 days and keeping in refrigerator 15-21 days after which they were planted in the ground in the open. Similar investigations were carried out in the United States (Donald H. Graves, Jeffrey W. Stringer, 1989) 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 "*in vitro*" technique

Comment [u22]: Bold all titles and subtitles

After the initialization and establishment of tissue culture, the material 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 substrate and watered with water in which the fungicide is dissolved. Planting is done in a greenhouse. Young plants are 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 is removed and the plants are 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 are placed on a container box, covered with a shadenet and stay there for 3-4 days for complete acclimatization. After that, the 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.

Comment [u23]: Break it into 2 paragraphs



Comment [u24]: Titles/subtitles of figures, plates or pictures should be under not above.

Figure no. 4 "In vitro" production

In 'in vitro' production there are almost no problems until the moment of acclimatization. When acclimatizing, it is necessary to standardize temperature and moisture because the paulownia does not allow temperature fluctuations and humidity reduction. If you are not bound by deadlines and do not have a stable heat source, plan acclimatization on warmer days (April), and solve the problem of moisture by covering the plants with glass jars (370 ml jars).

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.

Green cuttings successfully reproduce paulownia plants in the greenhouse. "In vitro" multiplication of paulownia yields good results with a multiplication rate of 10

Acclimatization of the paulownia is demanding and it reacts negatively on sudden temperature changes and humidity.

Comment [u25]: 1. Results and discussion should be separated.
2. to add value to your work, buttress your result with other findings/reports while discussing.

In paulownia acclimatization use glass jars in the first 5 days (covering the plants).

Comment [u26]: Make it a paragraph

5. LITERATURE

Comment [u27]: Change to reference(s)

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