Nepal Journal of Biotechnology. Dec. 2018 Vol. 6, No. 1: 11-15 ISSN 2091-1130 (Print)/ISSN 2467-9319 (online)

ORIGINAL RESEARCH ARTICLE

Comparative Study of Growth Statistics of Two Species of *Paulownia* and Optimization of Rooting Methods

Hari Krishna Saiju*, Abhishesh Bajracharya, Brishav Rajbahak, Stuti Ghimire
Department of Biotechnology, Asian Institute of Technology and Management (AITM) Lalitpur, Nepal

Abstract

Paulownia is a fast-growing woody tree, native to the forests of China. It belongs to the family Scrophulariaceae and is mainly used as a source of wood for furniture and musical instruments. Due to its fast-growing nature and high-quality of wood, there has been growing interest in cultivation and research of *Paulownia* in Nepal. Growth comparison was performed by measuring shoot length in *in vitro* condition. Among two species of *Paulownia* - *Paulownia tomentosa* (Thunb.) Steud and *Paulownia fortuneii* (Seem.) Hemsl., the growth rate of *P. tomentosa* was found to be 0.355 cm/week while that of *P. fortuneii* was found to be 0.637 cm/week in *in-vitro* conditions in MS medium supplemented with 0.1 mg/l NAA and 1mg/l BAP. Optimization of rooting methods was also performed, in which, sand rooting was found to be easier and more effective than *in-vitro* rooting. Dipping the plantlets in 1 mg/l of NAA was found to produce longer and denser roots than lower or higher concentrations during sand rooting.

Keywords: *Paulownia tomentosa, Paulownia fortuneii,* growth comparison, *in-vitro* rooting, sand-rooting, nodal culture

***Corresponding Author**
Email: hk.saiju@aitm.edu.np

Introduction

Paulownia is a fast-growing, woody tree native to the forests of China [1]. It is a deciduous tree but becomes evergreen in the tropics [2]. It belongs to the family Scrophulariaceae and is majorly used as a source of wood for furniture and musical instruments [3]. It has a very low thermal conductivity making it ideal for construction of insulative structures [4]. Each tree can produce 44 cubic feet of wood in average and can be harvested after 8-10 years of plantation. It is also tolerant to pollutants and can grow in many types of soils. Its leaves and flowers show medicinal properties and can also be used as fodder and fertilizers due to their high nitrogen concentration [5]. Its tolerance to drought and soil extremes makes it commercially important for use in the reclamation of surface-mined land [6]. It is also a suitable raw material for pyrolysis conversion into liquid and gaseous products [7]. The generic name, *Paulownia*, honors Anna Pavlovna of Russia [8].

Paulownia tomentosa was first introduced in Nepal in 1988 AD by International Centre for Integrated Mountain Development (ICIMOD) in Godavari. It was normally propagated through seeds but due to seed dormancy and slow seedling growth, tissue culture products have been used. Among the several species of Paulownia, *Paulownia tomentosa* (Thunberg) Steudel and *Paulownia fortuneii* (Seemann) Hemsley are the most widely used species in the context of Nepal. The former is usually found below an altitude of 1800m while the latter is usually found below an altitude of 2000m [1, 9].

Due to the booming market for Paulownia plants in Nepal, the plantlets have to be manufactured in huge amounts which can be performed by tissue culture. But there have been no publications of research about the *in-vitro* growth statistics and very few data regarding lab-to-land techniques like acclimatization and rooting methods. In this study, we aim to compare the *in-vitro* growth statistics of the two species to shed light on their growth patterns. Furthermore, we have extrapolated the type and concentrations of hormones and the method of rooting required for optimal root initiation.

Materials and Methods

Sample and Material Collection

Two different species of Paulownia, namely, *Paulownia tomentosa* and *Paulownia fortuneii* were used in this experiment. *Paulownia fortuneii* samples were brought in sterile culture jars from the Department of Plant Resources (DPR), Thapathali.

Paulownia tomentosa samples available in the Plant Tissue Culture laboratory, Himalayan White House International College, Khumaltar were used for the project.

Multiplication

Due to the requirement of a high number of plants, the samples were mass propagated in MS medium supplemented with hormone concentrations of 0.1 mg/l Naphthalene Acetic Acid (NAA - manufactured by Sigma Chemical Co.) and 1 mg/l Benzyl Amino Purine (BAP - manufactured by S.D. Fine-Chem Limited) in 150 ml culture vessels. The growth medium and culture equipment were autoclaved (manufactured by Life Steriware) at 121°C temperature and 15 lb./sq. inch pressure and nodal culture of the samples was performed in a Laminar Air Flow Hood (manufactured by Amar Chand & Company (ACCO). As a result, we performed subcultures twice and prepared a total of 30 vessels for each sample, with an average of 5 explants per vessel, which were stored in an incubation room, under fluorescent tube-lights (2000 lux) at a constant temperature of 25°C [1].

Shoot Growth Comparison

After two subsequent subcultures, we met the required explant number which was estimated to be 250 explants. Following this, nodal cultures were performed using MS medium with 0.1 mg/l NAA and 1 mg/l BAP, with only one explant per vessel, for ease of measurement. 20 vessels were produced for each plant species, which were also stored in the incubation room, under 2000 lux fluorescent tube lights at 25°C temperature. Culture vessels were recorded alongside a scale every week for 7 consecutive weeks [1].

Rooting Optimization

Sand Rooting

In this experiment, a total of 30 culture vessels were removed from the incubation room and exposed to indirect sunlight at room temperature for 10 days for acclimatization process. Nodal cuttings of the *in-vitro* plants, including at least one leaf, were prepared and placed into solutions of differing concentrations of NAA (0.5 mg/l, 1 mg/l and 1.5 mg/l) for about 10 minutes. The cuttings were then transplanted into rooting trays packed with autoclave-sterilized wet sand and placed into a polythene chamber. After a week of incubation (with water spraying twice a day) at room temperature, liquid MS media was added to the sand twice a week. The plantlets were removed from the sand after 4 weeks of incubation, for the measurement of root density and length [10-12]

In-vitro Rooting

In this experiment, a total of 30 plantlets from culture vessels were transferred into MS media with differing NAA concentrations (0.5 mg/l, 1 mg/l, and 1.5 mg/l). These vessels were stored in the incubation room under 2000 lux fluorescent lights at 25°C temperatures for two months. Plants were carefully extracted from the media using forceps for measurement of root density and length [13, 15].

Results

In-vitro Growth Statistics

According to the data in **Table 1**, *P. fortuneii* was found to have a higher average growth rate of 0.637 cm (SD=0.22) per week in comparison to 0.355 cm (SD=0.12) per week growth rate of *P. tomentosa.* There was a significant difference in the growth rates of *P. tomentosa* and *P. fortuneii*; t (6)=5.150, p = 0.002.

Both the line graphs represented peaks followed by a gradual decline in growth rate. *In-vitro* growth was found to peak during the 3rd week for *P. tomentosa* (0.543 cm) and during the 4th week for *P. fortuneii* (0.727 cm). In the following weeks, the growth receded slowly for the rest of the culture period. Within the 7th week, plant height reached to an average of 5.188 cm (n=16; SD=0.98) for *P. tomentosa* and 7.197 cm (n=16; SD=1.68) for *P. fortuneii.*

Rooting Optimization

Sand rooting: Comparative study of root development between two species

Out of 64 explants, 35% survived after 4 weeks of incubation in the polythene chamber. Among those, 55% were *P. fortuneii* samples and 45% were *P. tomentosa* samples.

As shown in **Table 2**, the average root length of *P. tomentosa* samples was estimated to be 3.72 cm (n=11; SD=1.32) and the average root number was calculated to be 10.22 (n=11; SD=5.64). For *P.*

Table 1: Average height growth rate (in cm/week) of both species of plants for 7 weeks (n=16). The average in-vitro growth rate of *P. fortuneii* was found to be higher than that of *P. tomentosa*.

Species\Time	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Avg. Net Growth Rate
P. fortuneii	0.186	0.547	0.789	0.908	0.72	0.65	0.662	0.637
P. tomentosa	0.142	0.331	0.543	0.397	0.359	0.301	0.411	0.355

fortuneii samples, the average root length was estimated to be 3.04 cm (n=12; SD=1.72) and the average root number was calculated to be 7.5 (n=12; SD= 7.38).

Table 2: Rooting Optimization with different concentrations of phytohormone NAA, performed in sand and in-vitro medium

Type of Rooting	Species	Hormone Concentration (mg/l)	Sample Size (n)	Avg. Root Length (cm)	Avg. Root Number
SR	**PT**	0.5	3	3.33±1.44	7.50±5.00
		1	8	3.87±1.35	11.25±5.82
		1.5	0	NA	NA
		Total	**11**	**3.72±1.32**	**10.22±5.64**
	PF	0.5	3	4±1.32	5.83±2.88
		1	5	3.4±2.16	11.50±10.24
		1.5	4	1.87±0.75	3.75±2.50
		Total	**12**	**3.04±1.72**	**7.50±7.38**
	Sum Total		**23**	**3.36±1.55**	**8.80±6.60**
IVR	**PT**	0.5	1	2.60±0	3.30±0
		1	2	1.61±0.43	1.37±0.53
		1.5	2	2.13±0.38	2.35±1.20
		Total	**5**	**1.96±0.44**	**2.15±1.04**
	PF	0.5	4	3.91±1.03	2.92±1.40
		1	3	2.36±0.11	2.51±0.72
		1.5	1	4.40±0	4.80±0
		Total	**8**	**3.39±1.09**	**3.00±1.25**
	Sum Total		**13**	**2.84±1.13**	**2.67±1.20**

Abbreviations: SR - Sand Rooting, IVR - *In-Vitro* Rooting, PT - *Paulownia tomentosa*, PF - *Paulownia fortuneii*, NA – Not Applicable

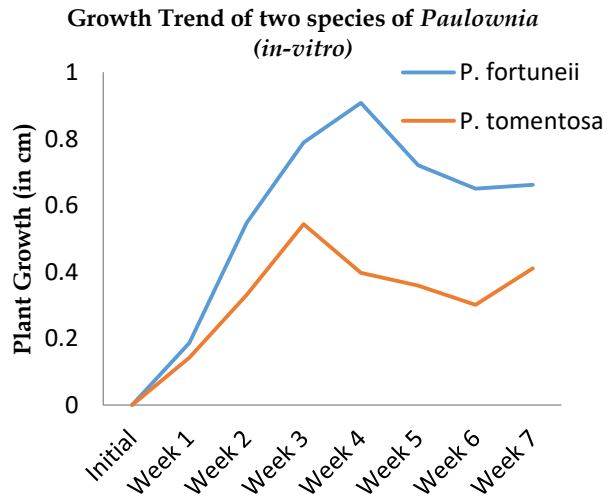

Figure 1: **Line graph showing the growth trend of the two species in in-vitro conditions (n=16).** Plants grown in MS medium with supplementation of 0.1 mg/l NAA and 1 mg/l BAP. Peaks in growth rate can be seen in Week 3 for *P. tomentosa* (0.543 cm/week) and in Week 4 for *P. fortuneii* (0.908 cm/week)

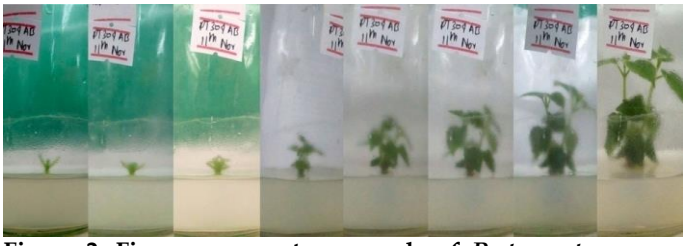

Figure 2: Figure represents a sample of *P. tomentosa* undergoing *in-vitro* growth for 7 weeks. The gradual in-vitro growth in plant height as well as biomass can be clearly visualized from this figure.

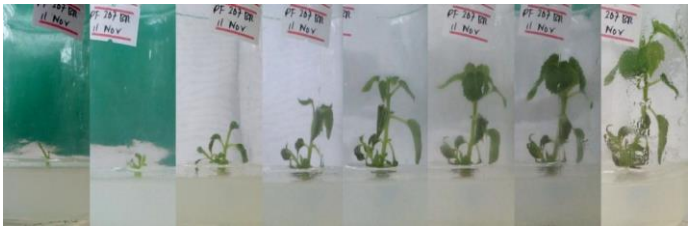

Figure 3: Figure represents a sample of *P. fortuneii* undergoing *in-vitro* growth for 7 weeks. The gradual in-vitro growth in plant height as well as biomass can be clearly visualized from this figure.

Sand rooting: Comparative study of effect of varying concentrations of auxin (NAA) treatment in root development

As shown in **Table 2**, among the surviving samples, 26% had been treated with 0.5 mg/l NAA, 56% had been treated with 1mg/l NAA and 17% had been treated with 1.5 mg/l NAA.

Samples treated with 1mg/l of NAA showed highest root density in both *P. tomentosa* and *P. fortuneii*. None of the *P. tomentosa* plants treated with 1.5mg/l NAA survived the sand rooting, but both the average root length and root number from *P. fortuneii* plants treated with 1.5 mg/l were found to be low. *P. fortuneii* plants dipped in 0.5 mg/l NAA showed the highest average root length of 4cm (n=3; SD=1.32).

In-vitro rooting: Comparative study of root development between two species

Callus formation was observed in all the *in-vitro* rooting samples. Root initiation was observed after 3 weeks of sub-culture. Out of 13 viable samples, 38% were *P. tomentosa* samples and 61% were *P. fortuneii* samples.

As shown in **Table 2,** the average root length of *P. tomentosa* samples were observed to be 1.96 cm (n=5; SD=0.44) and the average root number was calculated to be 2.15 (n=5; SD=1.04). For *P. fortuneii* samples, the average root length was observed to be 3.39 cm (n=8; SD=1.09) and the average root number was calculated to be 3 (n=8; SD= 1.25).

In-vitro rooting: Comparative study of effect of varying concentrations of auxin (NAA) treatment in root development

As shown in **Table 2**, among the viable samples, 38% had been treated with 0.5 mg/l NAA, 38% had been treated with 1 mg/l NAA and 23% had been treated with 1.5 mg/l NAA.

Samples treated with 0.5mg/l NAA showed the highest average root length and density for *P. tomentosa*, while samples treated with 1.5mg/l showed the highest average root length and density for *P. fortuneii*.

Comparison of root length and root number between *in-vitro* rooting samples and sand rooting samples

As shown in **Table 2**, while comparing average root length and root number between different rooting techniques, sand rooting was clearly better suited for root development than *in-vitro* rooting technique.

In case of sand rooting samples, their average root length was estimated to be 3.36 cm (n=23; SD=1.55) and the average root number was calculated to be 8.80 (n=23; SD=6.60). For *in-vitro* rooting samples, the average root length was estimated to be 2.84 cm (n=13; SD=1.13) and the average root number was calculated to be 2.67 (n=15; SD= 1.20).

Discussion

Mass propagation of *Paulownia* plants by tissue culture is gaining popularity in Nepal. Due to this, many research works are being carried out on different species of *Paulownia* both in and outside Nepal.

P. fortuneii was found to have higher average growth rate among the two species. This result could be interpreted as *P. fortuneii* having a greater metabolic ability to utilize the energy and nutrients from MS medium than that of *P. tomentosa.*

Despite the lower average growth rate in *in-vitro* condition, *P. tomentosa* overcame its flaws by demonstrating superiority during sand rooting. *P. tomentosa* samples were found to have higher average root length as well as higher root density than that of *P. fortuneii.* Optimization test of hormone pre-treatment showed that the samples treated with 0.5 and 1 mg/l NAA had greater average root length than those treated with 1.5mg/l. Out of 10 *P. tomentosa* samples dipped in 1.5mg/l NAA, none of the samples survived the sand rooting process. *P. fortuneii* samples dipped in 1.5mg/l NAA also resulted in shorter and sparser roots. It was also observed that samples treated with 1mg/l NAA had higher average root density. These results suggest that concentrations of 0.5 and 1mg/l are optimum for treatment before transfer to sand.

Callus formation was observed in *in-vitro* rooting samples which are probably induced by mechanical damage to the nodal cutting and presence of auxin (NAA) during the culture process. *P. fortuneii* demonstrated higher densities and lengths of roots in *in-vitro* rooting. This result also supports our interpretation that *P. fortuneii* may have a greater ability to utilize energy and nutrients from MS medium. Conversely, root length and density were found to be higher in *in-vitro* media having

hormone concentration of 0.5 mg/l and 1.5 mg/l. This inconsistency in the result may have stemmed from inadequate sample size, so further studies must be conducted.

While comparing the results from sand and *in-vitro* rooting techniques, we found sand rooting technique resulted in longer and denser roots. Due to the high availability of water and nutrients during *in-vitro* conditions, smaller and sparse roots may have been enough to sustain the plant whereas the scarcity of water and nutrients during sand rooting may have promoted higher root growth. A study performed by Rodrigues et al. in 1995 demonstrated a significant increase in root density during water deficit, which is similar to our findings [14-16].

Conclusion

In the comparison of *in-vitro* growth statistics of *P. tomentosa* and *P. fortuneii*, the latter was found to have higher average growth rate. Growth curves were found to peak at 3rd and 4th weeks respectively for the two species.

In the study to optimize rooting, *P. tomentosa* was found to have higher root length as well as root density during sand rooting, but *P. fortuneii* showed better root development in *in-vitro* conditions. Among the different concentrations of NAA, 0.5 and 1 mg/l were found to bear the best results during sand rooting. In case of *in-vitro* rooting, 0.5 and 1.5 mg/l concentrations of NAA were found to give better roots.

Comparison between the two rooting techniques showed that sand-rooting is the better method for root induction in case of *Paulownia* plants as both *P. tomentosa* and *P. fortuneii* demonstrated better results during sand rooting.

References

1. Rajbahak S, et al: **Clonal Propagation of *Paulownia tomentosa* Steud. for Commercial Production.** *Bull. Dept. Pl. Res.* 2014, **36**:56-60.
2. Siebold and Zuccarini Fl: ***Paulownia*.** *Flora of China* 1998, **18**:8-10.
3. Melhuish JH, Gentry CE and Beckjord, P R: **Paulownia tomentosa Seedling growth at Differing Levels of pH, Nitrogen, and Phosphorous.** *J Environ Hort.* 1990, **8**:205-207.
4. Akyildiz MH and Kol HS: **Some Technological Properties and Uses of *Paulownia tomentosa Steud.* wood.** *J Environmental Biology* 2010, **31**:351-355.
5. Zhu ZH, Chao CJ, Lu XY, Xiong YG: ***Paulownia* in China: Cultivation and Utilization.** *Asian Network of Biological Sciences.* Edi- A.N. Rao et al. 1986, 1-65.
6. Tang, RC, et al: ***Paulownia* - A crop tree for wood products and reclamation of surface-mined land.** *South J Appl For.* 1980, **4**:19-24.
7. Yorgun S and Yildiz D: **Slow pyrolysis of *Paulownia* wood: Effects of Pyrolysis Parameters on Product Yields and Bio-oil Characterization.** *J. Anal. Appl. Pyrolysis.* 2015, **114**:68-78.
8. Coombes AJ: **The A to Z of Plant Names.** *USA Timber Press* 2012, 312.
9. Zima A, Hosek J, Treml J, Muselík J, Suchý, P et al: **Antiradical and Cytoprotective Activities of Several C-Geranyl-substututed Flavonones from *Paulownia tomentosa* Fruit.** *Molecules.* 2010 **15**:6035-6049.
10. Rajkarnikar KM and Rajbahak S: **In-vitro Multiplication of *Paulownia fortuneii (Seem.) Hemsl.* through Seed Culture.** *Bull Dept Pl Res.* 2015 **37**:63-65.
11. Kumar PP, Rao CD, Rajaseger G, Rao AN: **Seed Surface Architecture and Random Amplified Polymorphic DNA Profiles of *Paulownia fortunei*, *Paulownia tomentosa* and their Hybrid.** *Annals of Botany* 1999, **83**:103-107.
12. Rajbhandary SB and Bajaj YPS: **Rooting of in vitro produced shoots in nonsterile sand - An inexpensive and efficient technique for enmasse micropropagation.** *Biotechnology in Agriculture and Forestry* 1991, **17**:262-268.
13. Venkateswarlu B, Mukhopadhyay J, Sreenivasan E, Kumar VM: **Micropropagation of *Paulownia fortuneii* through in vitro axillary shoot proliferation.** *Indian J Exp Biol* 2001, **39**:594-599.
14. Magar LB, Shrestha N, Khadka S, Joshi, J., Acharya, J., Gyanwali, G., Marasini, B., Rajbahak, S., & Parajuli, N: **Challenges and Opportunity of in vitro propagation of *Paulownia tomentosa Steud* for Commercial Production in Nepal.** *Int J Appl Sci Biotechnol* 2016, **4**:155-160.
15. Bergmann BA and Whetten R: **In vitro rooting and early greenhouse growth of micropropagated *Paulownia elongata* shoots.** *New Forests* 1997, **15**:127-138.
16. Rodrigues ML, Pacheco CMA and Chaves MM: **Soil-plant water relations, root distribution and biomass partitioning in *Lupinus albus* L. under drought conditions.** *J Exp Bot* 1995, **46**:947-956