

**IN VITRO PLANT REGENERATION OF PAULOWNIA TOMENTOSA
(THUNB.) STEUD. FROM SHOOT TIP AND LEAF SEGMENT**

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Abstract

An efficient protocol was developed for *in vitro* mass propagation of *Paulownia tomentosa* (Thunb.) Steud. using shoot tip and leaf segment explants from field grown plant. Different concentrations and combinations of BAP, Kn, zeatin and NAA were used for multiple shoot regeneration. Among two types of explants, leaf segment produced the highest number of shoots per explant (12 ± 0.4) when they were cultured on MS supplemented with 3.0 mg/l Kn and 0.5 mg/l NAA. Addition of 10% CW to above mentioned medium increased the number of shoots (18) per culture. Shoot tip explants also produced multiple shoots in the same medium, but their performance was not good as leaf segment explants. For shoot elongation, 100 mg/l urea was more effective when added with best shoot induction medium. Shoots rooted well in half-strength MS supplemented with 2.0 mg/l NAA, within 12 - 15 days. Regenerated plantlets were successfully acclimatized and established in poly bag containing a mixture of soil and compost in 2:1 ratio. About 90% plantlets survived under open field conditions.

Paulownia tomentosa (Thunb.) Steud. is a very fast growing hard wood tree commonly known as Empress tree belongs to the family Paulowniaceae. It is not only ornamental tree but also a multi-purpose species and source of renewable energy. Paulownia tree is also used for paper pulp, electric poles, construction materials, plywood and furniture (Barton *et al.* 2007).

Now Paulownia is widely planted for the protection of environmental pollution, increase the soil texture, enhance the soil fertility by adding nitrogen in the soil and also reduce the soil erosion. Paulownia could be propagated by seeds and root cuttings (Rao *et al.* 1996, Ozaslan *et al.* 2005). Traditional methods of propagation through seeds are unreliable because of disease and pest problem, poor germination and also slow growth than cuttings (Bergmann and Moon 1997). But on the other hand *in vitro* technique has greatly enhanced the scope and potentiality of mass propagation by exploiting the regeneration behavior in a wide range of selected horticultural plants (Bajaj 1986, Bonga and Durjan 1987).

Many authors already reported the method of Paulownia micropropagation (Marcotrigiano and Stimart 1983, Venkateswarlu *et al.* 2001, Ipekci *et al.* 2001, Litwinezuk and Bochnia 2012, Burger *et al.* 1985, Rajbahak and Sah 2010, Taha *et al.* 2008). But their is no report regarding *in vitro* propagation of *Paulownia tomentosa* (Thunb.) Steud. in our country. Therefore, the importance of this woody plant and to overcome the problem of propagation through conventional method, attempt has been made to develop an efficient and reproducible regeneration protocol for clonal propagation of this plant.

For plant materials, shoot tips and young leaves were collected from field grown plants at Tongi, Gazipur, Dhaka. They were surface sterilized with aqueous solution of 0.1% HgCl₂ for six min under aseptic conditions in laminar air flow cabinet and rinsed four - five times with autoclaved distilled water to wash away the traces of HgCl₂ completely. Before implanting onto the culture media they were cut into small pieces (approximately 1.0 - 1.5 cm in length). Shoot tip and leaf segment were cultured on MS supplemented with different concentrations of cytokinins

(BAP, Kn, zeatin) and auxin (NAA) singly or in combination for shoot regeneration. Half-strength MS supplemented with auxin such as IBA, IAA and NAA were used for root formation. Media supplements such as coconut water (CW) and urea were added to the medium for the determination of their effects on shoot multiplication and elongation, respectively. The cultures were regularly sub-cultured at three weeks intervals on fresh medium. For hardening, the test tubes containing rooted shoots were kept at room temperature and light for five days. Thereafter, the well rooted *in vitro* regenerated plantlets were taken out from the test tubes and gently washed to free the agar. They were then transplanted to poly bag containing a mixture of soil and compost in 2 : 1 ratio. After one month the plantlets were transferred to open field.

Explants were cultured on MS supplemented with different concentrations of BAP, Kn, zeatin and NAA alone or in various combinations for multiple shoot induction. In both shoot tip and leaf segment explants, the highest percentage of shoot proliferation was observed on MS supplemented with 3.0 mg/l Kn and 0.5 mg/l NAA. In the case of shoot tips, 90% of cultures were found to regenerate shoots and the number of regenerated shoots per explant was 10 ± 0.4 on the above mentioned medium (Table 1, Fig. 1a). In the same medium, multiple shoots induction from the leaf segment explant was 12 ± 0.4 per culture (Table 1, Fig. 1b). After first subculture in the same medium, both shoot tip and leaf segment explants produced more shoots (Figs 1c, d).

Table 1. Effect of growth regulators in MS basal medium on shoot proliferation from shoot tip and leaf segment explants of *Paulownia tomentosa*. Data were taken after four weeks of culture.

Growth regulators (mg/l)	% of explants produced shoots		Average number of shoots/culture	
	Shoot tip	Leaf segment	Shoot tip \pm SE	Leaf segment \pm SE
BAP				
1.0	78	70	7 ± 0.4	5 ± 0.3
1.5	70	68	6 ± 0.2	4 ± 0.2
2.0	64	60	6 ± 0.2	3 ± 0.2
Kn				
1.0	58	50	4 ± 0.3	5 ± 0.3
1.5	60	52	4 ± 0.2	5 ± 0.2
2.0	64	58	5 ± 0.3	6 ± 0.3
Zeatin				
1.0	44	40	4 ± 0.2	-
1.5	50	44	5 ± 0.3	-
2.0	48	40	3 ± 0.2	-
BAP+NAA				
1.0 + 0.1	84	78	8 ± 0.4	10 ± 0.4
1.5 + 0.1	78	70	5 ± 0.3	6 ± 0.3
2.0 + 0.1	60	58	5 ± 0.2	5 ± 0.3
Kn + NAA				
1.0 + 0.5	60	50	5 ± 0.3	6 ± 0.3
2.0 + 0.5	78	68	7 ± 0.4	8 ± 0.3
3.0 + 0.5	90	80	10 ± 0.4	12 ± 0.4
3.5 + 0.5	80	74	6 ± 0.3	6 ± 0.2
Zeatin + NAA				
1.0 + 0.5	44	40	4 ± 0.2	-
2.0 + 0.5	58	54	5 ± 0.2	2 ± 0.2

Ipekci *et al.* (2001) and Litwinczuk and Bochnia (2012) reported that MS medium supplemented with 1.0 mg/l BAP and 0.1 mg/l NAA was optimum to regenerate multiple shoots in *Paulownia* from shoot tip explant. Superiority of BAP over other cytokinins for multiple shoots formation in *Paulownia* has also been reported (Taha *et al.* 2008).



Figs 1a-j. *In vitro* regeneration of *Paulownia tomentosa*. a-b. Multiple shoot regeneration from shoot tip and leaf segment explant on MS supplemented with 3.0 mg/l Kn and 0.5 mg/l NAA. c - d. More shoots after first subculture in the same medium. e - f. Positive effect of 10% CW on increase the number of shoots in shoot tip explant and leaf segment explant. g. Effect of 100 mg/l urea on shoot elongation. h. *In vitro* root induction on half-strength MS supplemented with 2.0 mg/l NAA. i. Regenerated plantlets in poly bag containing a mixture of soil and compost in 2 : 1 ratio. j. Survived plant in open field conditions.

For further development of the medium and enhanced shoot proliferation, coconut water (CW 5 - 20% v/v) was added to the medium. Addition of 10% CW increased the number of shoots (15 and 18 in case of shoot tip and leaf segment explant, respectively) per culture (Figs 1e, f). Different concentrations of urea (50 - 150 mg/l) were added to the medium to determination of their effects on shoot elongation. Addition of 100 mg/l urea to the medium increased the length of shoots (Figs 1g, 2). Roy (2008) reported that addition of 10% CW increased the number of shoots

in *Boerhaavia diffusa* L. culture. Rahman *et al.* (1999) also observed similar effects of CW (10%) on *in vitro* shoot multiplication of *Emblica officinalis*. Minocha (1981) reported that urea was important media factor in the *in vitro* culture of *Betula papyifera*. Roy (2008) and Ahmed *et al.* (2001) also reported that urea was fruitful media supplement for *in vitro* shoot elongation.

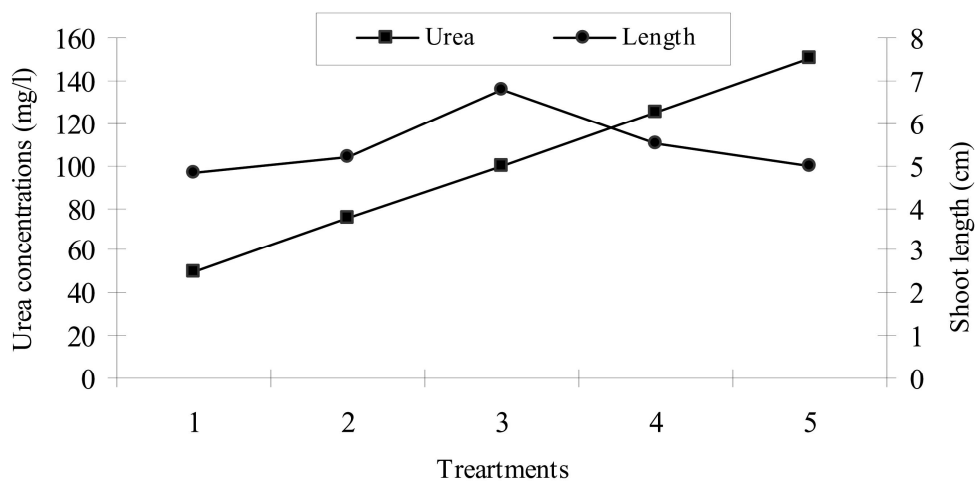


Fig. 2. Effect of different concentrations of urea (50 - 150 mg/l) along with MS + 3.0 mg/l Kn + 0.5 mg/l NAA on shoot elongation.

Table 2. Effect of auxins in half-strength MS medium on root formation from regenerated shoots of *Paulownia tomentosa*. Data were taken after four weeks of culture.

Auxins (mg/l)	% of shoots rooted	Days required for rooting	No. of roots per shoots	Average root length (cm.)
IBA				
1.0	84	15 - 18	6 - 8	3 ± 0.2
1.5	80	15 - 18	5 - 7	3 ± 0.2
2.0	80	18 - 20	6 - 8	4 ± 0.3
IAA				
1.0	48	18 - 20	4 - 6	2 ± 0.3
1.5	60	18 - 20	6 - 8	3 ± 0.2
2.0	54	20 - 22	6 - 8	3 ± 0.2
NAA				
1.0	90	14 - 16	8 - 10	4 ± 0.2
1.5	88	16 - 18	5 - 7	4 ± 0.3
2.0	98	12 - 15	10 - 12	5 ± 0.3
2.5	80	15 - 17	6 - 8	4 ± 0.2
IBA + IAA				
1.0 + 0.5	68	18 - 20	6 - 8	3 ± 0.3
1.5 + 0.5	50	20 - 22	3 - 5	2 ± 0.2
NAA + IBA + IAA				
1.5 + 0.5 + 0.5	68	15 - 17	5 - 7	3 ± 0.2
2.0 + 0.5 + 0.5	74	16 - 18	6 - 8	4 ± 0.3

For root induction, *in vitro* regenerated well-developed and elongated shoots were excised and cultured on rooting medium containing half-strength MS with different concentrations and combinations of IBA, IAA and NAA. The best result was obtained in half-strength MS supplemented with 2.0 mg/l NAA (Table 2). In this combination, it was observed that 98% shoots rooted well within four weeks of culture and each microcutting produced 10-12 roots (Fig. 1h). Taha *et al.* (2008) and Burger *et al.* (1985) reported that NAA was more effective for *in vitro* rooting of Paulownia. They observed that half-strength MS with 1.0 mg/l NAA was suitable combination for best rooting, which is similar with my second highest result. For acclimatization and plant establishment under the natural conditions, the well rooted plantlets were transferred to poly bag containing a mixture of soil and compost in 2:1 ratio (Fig. 1i). After acclimatization, 90% plantlets were survived in the open field. The results of the present investigation demonstrate a reproducible and efficient regeneration protocol of *Paulownia tomentosa* (Thunb.) Steud. through *in vitro* culture. The technique described here appears to be readily adaptable for large scale clonal propagation and plantation in the forest floor for sustainable uses.

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