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Rapid Micropropagation of *Paulownia tomentosa*

D.W. Burger, L. Liu, and L. Wu
Department of Environmental Horticulture, University of California, Davis, CA 95616

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Abstract. **Nodes from mature tissue of *Paulownia tomentosa* Steud. were cultured on a modified Murashige-Skoog (MS) medium containing BAP and NAA. Axillary buds elongated most rapidly from nodes of greenhouse-grown trees on a medium containing 1.0 mg BAP/liter and 0.1 mg NAA/liter. After 7 days on this medium, the elongated shoots were transferred to media containing IBA (0.5–1.0 mg/liter) or the shoots were quick-dipped (15 sec) in the K-salt of IBA (KIBA, 500–1000 ppm) and rooted under mist in the greenhouse. The shoots rooted in 7–10 days and were easily transplanted to 6 packs (6-celled plastic flat) for continued development. This propagation scheme provides a method for the rapid multiplication of plants from mature tissue *Paulownia* in a very short period of time (14–17 days). Chemical names used: 1-naphthaleneacetic acid (NAA); benzylaminopurine (BAP); 1*H*-indole-3-butanoic acid (IBA).**

The genus *Paulownia* has been introduced to Europe and North America, but is native to East Asia (5). It has become naturalized in the southern and central hardwood forests of the United States. *Paulownia* is characterized by fast growth, attractive growth habit and flowers and drought tolerance (4). This genus has potential for use as a biomass producer on California marginal lands (1, 2).

Paulownia is propagated by seeds and by seedling root cuttings, but root cutting propagation is used more widely (5). Adventitious shoots arise readily from seedling (juvenile) roots, but as the plants mature, adventitious shoot formation from roots decreases (5). Problems associated with root cutting propagation include physical damage of the root epidermis and cortex, decay due to high, tropical temperatures (> 35°C) and attack by pathogenic organisms (*Pythium* spp.) (5).

In vitro propagation techniques have been attempted using seedling tissues of *Paulownia spp.* (3, 4). However, work with mature tissue has not been reported. The work reported here is an attempt to propagate *Paulownia tomentosa* Steud. vegetatively using tissues (nodes) from mature trees.

Stem segments (6–12 mm diameter, about 1 cm long) containing one node from mature, flowering trees (>15-years-old) growing on the Univ. of California, Davis campus, or from greenhouse-grown trees derived from mature cuttings were used as explants in all experiments (Fig. 1A). Explanted tissues were disinfested by a 20-sec dip in 70% ethanol immediately followed by a 10-min soak in 0.5% sodium hypochlorite (10% commercial bleach). The disinfestants were removed by 3 successive rinses in autoclaved, deionized water. The last rinse consisted of a 30-min soak. Modifications [inorganic salt dilution, combinations of BAP and NAA] of an MS medium were used routinely (see table descriptions for treatment details). The pH of the medium was set at 5.6 prior to autoclaving.

Explants were grown under conditions of 16 hr of light (42 μmol s^{-1} m^{-2}) daily at 24° to 28°C. Elongated shoots (1–2 cm in length, Fig. 1B) were either subcultured onto a medium containing one-half × MS salts and various concentrations of IBA to initiate roots or the elongated shoots were dipped in various KIBA (K-salt of IBA) solutions for 15 sec and rooted under mist in cell-packs containing a 1 sand: 1 redwood sawdust : 1 peat moss (by volume) in a greenhouse with a 26° day temperature and an 18° night temperature and with an interrupted night (4 hr of continuous incandescent light, 10 PM to 2 AM). The mist interval was 2.5 sec of mist every 5 min. After 7–10 days, the rooted shoots (Fig. 2) were transplanted to 10 cm pots containing the same potting mix used for rooting and grown in the greenhouse.

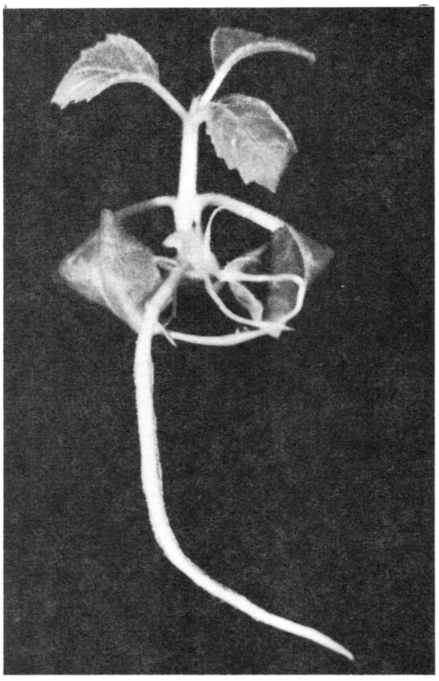

Fig. 2. Axillary shoot after 7 days on a modified Murashige-Skoog medium containing 1.0 mg IBA/liter (3X).

Few axillary buds elongated into shoots from nodal explants taken in early spring (5 or 27 Apr.) from trees which had not yet broken dormancy (Table 1). The highest percentage of elongating shoots from these dormant nodes occurred on a modified MS medium containing 5.0 mg BAP/liter and 0.5 mg NAA/liter. This response was not satisfactory for rapid development of buds, since only 62% of the nodes had elongated buds, and this development took 8 weeks to achieve. After 6 more weeks, however, the trees had begun to leaf out, and axillary buds from nodes explanted at that time (8 June) elongated within 21 days (Table 1).

In contrast, all axillary buds from nodal explants from greenhouse-grown plants had elongated after 7 days, regardless of the culture medium (Table 2). The greatest elongation came from nodes explanted onto a modified MS medium containing 1.0 mg

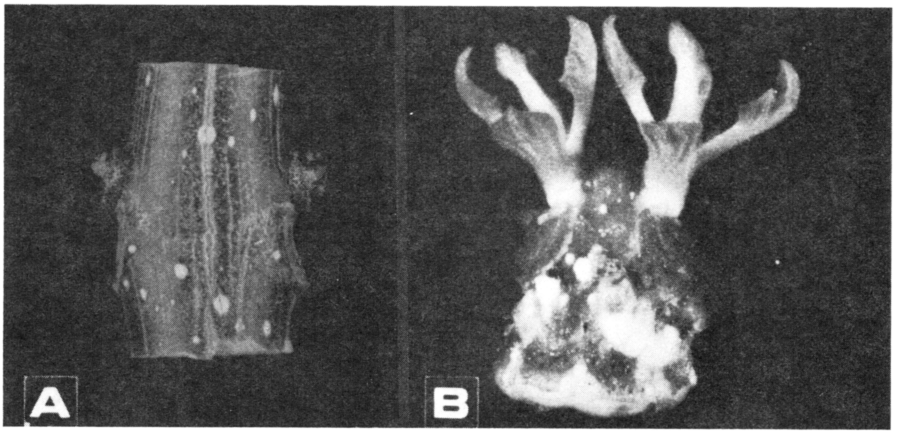

Fig. 1. **A.** Nodal explant of *Paulownia tomentosa* used to initiate all experiments (3X). **B.** Nodal explant after 7 days in culture on a one-half X MS medium containing 1.0 mg BAP/liter and 0.1 mg NAA/liter showing elongating axillary buds (3X).

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Table 1. Percentage of nodal explants from field-grown *Paulownia tomentosa* trees with elongated axillary buds (>1 cm long) over time on a modified Murashige-Skoog medium (half-strength salts) containing varying BAP and NAA concentrations. Twenty-five nodes explanted per treatment.

Treatment		Explant	Nodes (% elongating)		
BAP (mg/liter)	NAA (mg/liter)	date	Day 21	Day 35	Day 56
1.0	0.1	5 Apr.	0	0	0
		27 Apr.	9	9	9
5.0	0.5	5 Apr.	0	18	18
		27 Apr.	27	54	62
		8 June	92	92	92
10.0	1.0	5 Apr.	0	0	0
		27 Apr.	0	0	0

Table 2. Percentage of nodal explants taken 5 Apr. from greenhouse-grown plants of *Paulownia tomentosa* with elongated (>1 cm long) axillary shoots and mean shoot length on a modified Murashige-Skoog medium containing varying concentrations of BAP and NAA. Sixteen nodes explanted per treatment. Data were taken 7 days after explanting.

Treatment			
BAP (mg/liter)	NAA (mg/liter)	Percent	Shoot length (cm)
1.0	0.1	100	1.6[z]
5.0	0.5	100	0.8
10.0	1.0	100	0.7

[z]SE = 0.2

BAP/liter and 0.1 mg NAA/liter, much less BAP and NAA (one fifth X) than required to obtain elongation from field-grown *Paulownia* trees. Concentrations of BAP and NAA greater than 1.0 and 0.1 mg/liter respectively, inhibited the elongation of buds from greenhouse-grown plants (Table 2).

Application of IBA or KIBA was required to obtain rooting of the elongated shoots (Table 3). In general, root formation occurred faster in vitro than under mist. The 2 methods are not easily compared because the 2 environments are very different in temperature, light quality, light duration, light intensity, and relative humidity. Table 3 does show that for the rapid propagation of in vitro derived shoots, however, rooting under mist is possible and may be preferable, even though it takes slightly longer (10 vs. 7 days), since the plantlets are already in soil and there is one less transfer to perform. Transplant problems or acclimatization of in vitro-grown tissue to greenhouse environments did not occur.

Table 3. Percent of elongated axillary shoots derived from in vitro culture with roots. Nine to 16 shoots per treatment.

Treatment	Time period	Percent with roots
In vitro, 1/2 X MS salts, no IBA	7 days	0
In vitro, 1/2 X MS salts + 0.5 mg IBA/L	7 days	100
In vitro, 1/2 X MS salts + 1.0 mg IBA/L	7 days	100
Under mixt, no KIBA treatment	10 days	0
Under mist, 15 sec. dip in 500 ppm KIBA	10 days	17
Under mist, 15 sec. dip in 1000 ppm KIBA	10 days	100

Paulownia tomentosa has proven to be a choice woody plant to work with in vitro. Rapid micropropagation can be achieved from greenhouse or field-grown plants; however, consistent application of this technique requires the use of greenhouse-grown plant material. The total time from explanting nodes to producing rooted plantlets growing in 6 packs is 14 days. This plant has great potential for teaching in vitro propagation techniques because of ease of culture and quick response to culture media.

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