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A tissue culture technique for propagation of *Paulownia elongata* tree

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Abstract

Stem tips and nodal explants of *Paulownia elongata* plants grown at controlled conditions were cultured on Murashige and Skoog medium (MS) supplemented with NAA and BAP. Medium containing 2 mg/L BAP and 0.05 mg/L NAA gave the best shoot production and maximum number of shoots from nodal explant (8.2). The results indicated that *P. elongata* has a great potential for vegetative buds formation as the answer strongly related to the composition of medium and culture conditions. The described protocol allows the establishment of numerous cloned elite plants from selected genotypes of *P. elongata*..

Key words: *P. elongata,* Shoot tips, Nodal explants, Micropropagation, Plant growth regulators

1. Introduction

Paulownia (*Paulownia elongata* S. Y. Hu) (princess tree) is an economically important wood in the family Scrophulariaceae. It is native to China and has been introduced into Japan, Brazil, Europe and USA. Paulownia wood has been put to many uses because it is light weight, rot resistant and free of warping (Zhu et al., 1986; Olson et al., 1989; Kays et al., 1997). The fast growth rate of Paulownia may be capitalized upon for agroforestry (Wang and Shogren, 1992; Jiang et al., 1994), biomass production for fodder (Zhu et al., 1986; Song 1988), land reclamation (Carpenter, 1977) and animal waste remediation systems (Bergmann et al., 1997). In the last decade, *Paulownia* received an increasing attention in Bulgaria as a highly drought tolerant, valuable short-rotation woody crop plant.

Paulownia ssp. can be propagated by seeds and stem or root cuttings, with the latter being used more often (Burger et al., 1985; Ipekci and Gozukirmizi, 2004). It was established that propagation of Paulownia by seeds or seedlings production is time-consuming and difficult. The existence of efficient vegetative propagation systems should allow the producing clonal planting stock. Methods for successful tissue culture propagation have been published for seven species and two hybrids of *Paulownia* (Yang et al., 1996; Rao et al., 1996; Bergmann, 1998; Ipekci et al., 2001). The plants derived through tissue culture from various explant sources of *Paulownia* species develop better than these derived from seeds (Bergmann and Moon, 1997). *In vitro* plant regeneration system for woody plants is regulated by an interaction of phytohormones, mainly auxins and cytokinins (Komamine et al., 1992; Bergmann, 1995).

We conducted experiments with *Paulownia elongata* with the aim of developing an efficient protocol for micropropagation and establishing the optimal cultural conditions of plants growth from rooted shoots transferred to the greenhouse.

2. Materials and methods

Young *P. elongata* plants were provided by company "Sortoizpitvane", Elena town, Bulgaria. They were grown in pots (26 x 24 cm) in growth chamber at ambient temperature 24 ± 1ºC and relative humidity 70 %. Shoot tips and nodal segments of *P. elongata* that served as explants were harvested from young developing plants. Disinfection was accomplished by swirling explants in 0.04 % mercuric chloride for 30 minutes (Shaker, SK-600, Jeio Tech Co., Ltd, Korea). Explants were rinsed 15 - min, three-times with sterile, distilled water and placed on Murashige and Skoog (1962) medium (MS). Medium was adjusted to pH 5.6 and contained 0.7 % agar-agar, 3 % sucrose, 0.2 mg/L BAP, 0.2

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mg/L GA and 0.05 mg/L NAA. Each explant was then placed with the basis inserted to a depth to about 0.2 cm into cultural tube (150 x 20 mm). After 2 weeks of culture, the frequency of aseptically explants was scored.

Shoot-buds obtained from nodal sections and from stem tips were transferred to a shoot multiplication medium MS with 1.0 or 2.0 mg/L BAP as the only growth regulator or MS with combination with NAA or IAA (Table 1a, b). Shoot response was recorded four weeks after inoculation. The percent of formed shoots, mean number of shoots per explant and shoots length were measured. Experiment was repeated twice. Developing new axillary shoots were detached and transferred onto each fresh medium for further micropropagation. The data were subjected to statistical analysis (Sigma Stat 3.1 Systat Software, San Jose, California, USA). All cultures were maintained at 22 ± 2 ºC in a growth chamber with 40 µmol $m^{-2}s^{-1}$ illumination from cool-white luminescent lamps, 70 % relative humidity and a 16/8h (light/dark) photoperiod..

3. Results

Stem tip and nodal explants from young *P. elongata* plants were no bacterially contaminated, which indicates that treatment with mercuric chloride in appropriate solution following by several rinses in sterile water excludes the surface contaminants. Thus, explants produced 95-100 % aseptically culture after treatment with 0.04% mercuric chloride. Similar approach for surface sterilization was reported in sugar beet and fodder beet (Slavova, 1988; Slavova and Kaschieva, 2004). Explants were initially cultured on MS medium supplemented with 0.2 mg/L BAP, 0.2 mg/L GA and 0.05 mg/L NAA; the shoot tips developed rapidly (Figure 1a) and each nodal axils produces single vegetative buds after 10-12 days (Fig. 1b).

Propagation of *P. elongata* plants derived from stem tip and nodal explants in culture were studied (Table 1a, b). Numerous factors influenced *in vitro* propagation such as physiological status of the donor plants, plant growth regulators (BAP, NAA and IAA) and its interactions etc. Cytokinins levels were shown to be the most critical for multiplication of many plant species (Abou Dahab et al., 2005; Sayed and Gabr, 2007, Zayova et al., 2010). In our case, BAP at concentrations 1.0 and 2.0 mg/L resulted in significant variation on the development of axillary buds in both explant types. BAP stimulated the development of buds after two weeks of culture. On MS1 medium, stem tips produced 1-2 buds. On the same medium, nodal explants produced higher number of vegetative buds (3.5 buds/ explant) with mean length 1.9 cm.

a b

Figure 1. Establishment of *P. elongata in vitro* cultures a) stem tips; b) nodal explants

On MS2 medium, the level of produced vegetative buds was higher but they are smaller and shorter than these on MS1. Hence, BAP stimulated the rate of multiplication as the influence depends on concentrations of the growth regulator. BAP at 1.0-2.0 mg/L and NAA at 0.05 mg/L had a promising effect on shoot proliferation and buds formation. On MS3, 60 % of nodal explants produced buds (1.7/explant) with mean length 2.4 cm (Table 1b). This combination yielded a number of vegetative buds (90 %) in the basal part of the plants derived from nodal segments (Table 1b). Higher rate of vegetative buds induction was observed at higher concentration of BAP (2 mg/L) in a medium containing NAA. In this case, the vegetative buds were well differentiated with intensive growth and development. Figure 2a and 2b demonstrated the results obtained on MS4 medium. The micropropagation frequency of the formed buds reached 70 % for stem tips and 95 % for nodal explants and maximum number of shoots/explant was 2.5 and 8.2, respectively.

Thus, propagation of *P. elongata* was generally higher in MS medium containing 2 mg/L BAP and 0.05 mg/L NAA Bergmann and Moon (1997) reported a maximum adventitious shoot production of 48 shoots per leaf from *P. elongata* after 4 weeks in culture. High frequency of plant regeneration from leaves, leaves with petioles, internodes and nodes excised from 3-month-old non-aseptically grown *P. elongata* was obtained on Murashige and Skoog medium and Woody Plant Medium, with appropriate supplements of growth regulators (Ipekçi et al., 2001).

Table 1. Effect of plant growth regulators on the micropropagation of *P. elongata*

№	Plant growth regulators, mg/L			Formed shoots, %	No shoots/explant, x ± SE	Shoot length, cm x ± SE
	BAP	NAA	IAA			
a/plants obtained from stem tips						
MS1	1	0	0	45	1.2 ± 0.18	1.5 ± 0.14
MS2	2	0	0	50	1.5 ± 0.11	1.1 ± 0.11
MS3	1	0.05	0	60	1.7 ± 0.27	2.4 ± 0.12
MS4	2	0.05	0	70	2.5 ± 0.34	2.1 ± 0.23
MS5	1	0	0.05	30	1.0 ± 0.16	1.3 ± 0.22
MS6	2	0	0.05	40	1.2 ± 0.15	1.0 ± 0.17
b/ plants obtained from nodal explants						
MS1	1	0	0	70	3.5 ± 0.34	1.9 ± 0.26
MS2	2	0	0	80	3.9 ± 0.33	1.2 ± 0.16
MS3	1	0.05	0	90	6.1 ± 0.62	2.1 ± 0.21
MS4	2	0.05	0	95	8.2 ± 0.66	1.8 ± 0.28
MS5	1	0	0.05	40	1.4 ± 0.15	1.1 ± 0.12
MS6	2	0	0.05	45	1.0 ± 0.14	0.8 ± 0.11

a b

Figure 2. Micropropagation of *P. elongata* plants on MS4 medium (2 mg/l BAP + 0.05 mg/l NAA): a) stem tips; b) nodal explants

Combination of BAP and IAA did not stimulate the propagation rate and production of vegetative buds of *P. elongata*. The number of buds produced from nodal explants and stem tips significantly decreased on MS5 medium (Table 1a, b); the yielded buds were usually poorly developed. On MS6 medium, the number of produced vegetative buds decreased and they exhibit delayed development. Percentage of induced vegetative buds derived from stem tips was generally lower in all MS media compared to nodal explants. Hence, the type of *P. elongata* explants had a significant influence on the multiplication rate. Similar results were reported in *P. elongata* and *P. tomentosa* by Ipekçi et al. (2001) and Ozaslan et al. (2005). A rapid rate of propagation depends on the subculturing of proliferating shoot culture. In our case, the plants were sub-cultured four times in MS4 medium, which was optimal for induction of a number of vegetative buds (Figure 3a, b).

The propagation profile for *P. elongata* and observed that the shoot multiplication rate gradually improved as the number of subcultures increased (Figure 4). It was suggested that the effect reflected a time-dependant adaptation of the explants to *in vitro* conditions as it was reported by Upadhyay et al. (1989).

4. Conclusions

Using this protocol, numerous cloned plants of *P. elongata* were obtained and successfully rooted. Results from this study indicate that propagation trough tissue culture would provide a useful alternative to seed propagation and allow large scale multiplication of desirable plants of *P. elongata*..

a b

Figure 3. Micropropagated *P. elongata* plants, subcultured on MS4 medium: a) stem tips; b) nodal explants

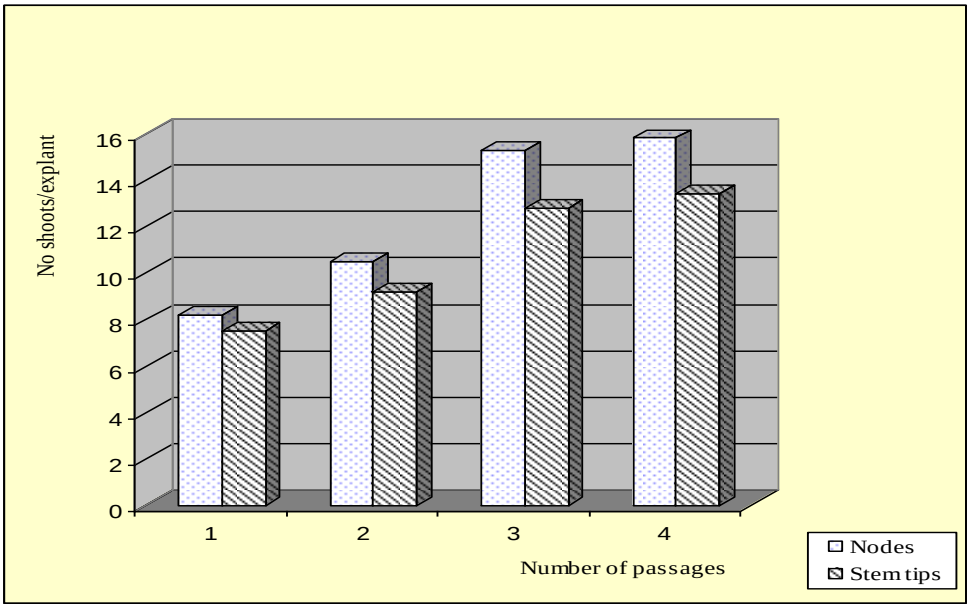

Figure 4. Multiplication of *P. elongate* shoots during subcultivatons

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