Effect of Medium Composition on *In Vitro* Axillary Shoot Development of *Paulownia fortunei*

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ABSTRACT

Nodal explants harvested from *in vitro* grown *Paulownia fortunei* were cultured on Murashige – Skoog (MS) medium and Woody plant medium (WPM) containing 2mg/l kinetin. Percentage of axillary shoot development was better in MS medium (89%) than that in WPM medium (56%). A significantly higher mean shoot length (1.67cm) and shoot number (0.79cm) were observed in MS medium after 5 weeks. Culture survival rate was better in WPM medium (57%) than that in MS medium (48%). Rhizogenesis was possible in both MS and WPM media containing activated charcoal but devoid of growth regulators. Rooting percentage was comparatively higher in MS medium (75%) than that in WPM medium (35%).

KEYWORDS: ***In vitro* propagation, Murashige - Skooge medium, Nodal explants of *Paulownia*, Woody Plant medium.**

INTRODUCTION

Paulownia is an economically important genus in the family Scrophulariaceae, with 9 species of very adaptable and fast-growing timber tree (Zhu *et al.*, 1986). The nine species of *Paulownia* are very similar in appearance and timber properties. *Paulownia* varieties, which have been cultivated in the world, are *P. fortunei, P. kawakami, P. tomentosa, P. taiwaniana, P. elongate, etc. Paulownia* is naturally distributed ranging from tropical to cool temperate climates, on sites with average annual rainfall ranging from 500mm to over 2000mm. *Paulownia fortunei* is a fast-growing and dominant species under tropical and sub-tropical condition in China. The timber is one of the main export items in China (Shanqing *et al.*, 1988). In addition to that *Paulownia* has been naturalised in Japan, Australia, Brazil, Europe and United State of America (Rao *et al.*, 1996).

Paulownia has recently been introduced to Sri Lanka by *Paulownia* Plantation (pvt) Ltd, which is a BOI approved joint venture with Norway. The plan is to cultivate 1000 hectares of *Paulownia* at 100 hectares per year, mainly for the purpose of timber production and agro-forestation in Sri Lanka. Two nurseries have been established at Parakaduwa and Kalpitiya as a trial (Fernando and Fernando, 2002). As for environment, agro-forestation provides a reversal of the harmful effects caused by the depletion of natural forest (Fernando and Fernando, 2002).

Paulownia yields a multiple-use wood, its leaves and flowers are used in medicine, as fertilizer and fodder (Zhu *et al.*, 1986). In addition to that it also grows well in polluted areas and even seems to absorb pollutants. It protects the environment, because its broad leaves are effective filters of smoke and dust. Most of the organic honey produced in China comes from flowers of the *Paulownia* trees. Recently, there has been increased interest on this genus because of its possible use in reforestation in nutrient-poor soils (Marcotrigiano and Jagannathan, 1988). However, deep fertile soils are required for optimum growth of the plant. *Paulownia* may reach 40-50m in height and have a diameter of greater than 2m when mature, but trees of this size under cultivation are rare. *Paulownia* requires intensive management if grown for timber production.

Paulownia is mainly propagated by seeds and also by root and stem cuttings (Zhu *et al.*, 1986). Vegetative propagation of *Paulownia* has many advantages over seedling production. *Paulownia* seeds germinate slowly, and early seedling growth is slower than that of plants derived from roots or shoots cuttings or from rooted shoots from tissue culture (Bergmann and Moon, 1997). Therefore, an efficient protocol for adventitious shoot production is essential for the establishment of a successful clonal propagation method of *Paulownia.*

Micropropagation of *Paulownia* using tissue culture techniques holds promise as an alternative solution for the conventional propagation methods. Previous workers have successfully regenerated shoots from nodal and internodal stem segments of one-year-old *Paulownia thaiwaniana*, especially if young stem tissue was used. Several workers have demonstrated *in vitro* adventitious shoot formation of *Paulownia* (Bergmann and Moon, 1997; Rao *et al.*, 1996; Kumar *et al.*, 1998).

High frequency direct regeneration of shoots was induced in leaf cultures of *P. tomentosa, P. fortunei* × *P. tomentosa and P. kawakami.* The optimum culture medium for the leaf explants derived from shoot culture was MS medium supplemented with 2mg/l indole-3-acitic acid (IAA) and 11mg/l benzyladenine (BA). Up to 40 shoots were obtained over a 4-month culture period from each leaf explant. Rooting occurred spontaneously in the shoots that were about 1cm when subcultured on phytohormone free MS medium (Rao *et al.*, 1996). The plantlets could be transplanted and some of them flowered in the greenhouse one year after transplanting (Rao *et al.*, 1996). *In vitro* adventitious shoot formation has been demonstrated in petioles and laminar of *P. elongata, P. fortunei* and *P.* 'Henan 1' (Bergmann and Moon, 1997). Fully expanded dark green, thick, older leaves exhibited greater callus and shoot production than young leaves. The growth regulator concentration required for maximum shoot production differed among

clones, but all required 0.2 or 0.5 mg/l α-Naphthalene Acetic Acid (NAA) and 5.0 or 7.0 mg/l BA (Bergmann and Moon, 1997). Axillary shoots were also initiated in the nodal explants. The internodes and roots became swollen but no other response was seen even after 21 days in culture (Rao *et al.*, 1996). In most cases, MS was the preferred basal medium supplemented with NAA as the auxin and BA as the cytokinin.

The present study aimed to investigate the effect of medium composition on development of shoots and roots from nodal explants of *Paulownia fortunei.*

MATERIALS AND METHODS

Experiments were carried out in the tissue culture laboratory of the Plant Science Department of Rubber Research Institute at Agalawatta. Nodal explants were obtained from one year old *in vitro* grown *Pawlownia fortunei* plants. Plants were cut in to 1.5cm pieces with one or two nodes in each leaving at least 0.5cm petiolar stub on each axil. The nodes were placed with their cut ends partially embedded in contact with the medium.

Culture and Media Incubation Condition

The basal media used for the experiment were Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) and Woody Plant Medium – WPM (Lloyd and McCown, 1981) with 2mg/l kinetin. The media p^H was adjusted to 5.7 by adding NaOH or HCl. Surface sterilisation of explants was not carried out as they were harvested from *in vitro* grown *Paulownia* seedlings. Nodal explants were cultured on MS medium and WPM medium separately placing one explant per each test tube. The experiment was repeated thrice with 30 explants per medium. Cultures were incubated at 25 ± 2^0C under a 16 hour photoperiod at irradiance of 100 $\mu Em^{-2}S^{-1}$ using cool-white florescence light in growth room.

Assessment of Growth of Explants

Number of shoots and shoot length were recorded weekly from the date of establishment. Root length was recorded after 2weeks from the date of transferring in to hormone free medium containing activated charcoal. Apart from direct shoot and root formation, callus formation was also recorded.

Excised shoots were transferred in to hormone free MS and WPM media containing activated charcoal (2g/l), after 35 days from the date of establishment (Hapuachchi, 2003). All experiments were of the Completely Randomised Design (CRD). Data were analysed by analysis of variance using Statistical Analysis System (SAS, 1991).

RESULTS

Developing shoot buds were visible after 6 days of culture in both media. Of the two media tested, culture survival percentage was comparatively high in WPM medium (57%) compared to that in MS medium (48%) at the end of 5th week from culture establishment. However, of the survived explants, a higher percentage of axillary shoot development (>1cm) occurred in MS medium (89%) than that in WPM medium (56%) (Table 1). Most of the explants which did not developed were dried up and ultimately dead in the MS medium. Although, in WPM medium, much of explants did not get develop even after one month from culture, but they exhibited in live condition.

Table 1. Axillary shoot development percentage (>1cm) of explants cultured in MS and WPM media at weekly interval

Treatment	% shoot development (>1cm)				
	1st week	2nd week	3rd week	4th week	5th week
MS	0	48	55	81	89
WPM	0	15	28	44	56

Table 2: Mean shoot number, shoot length and rooting percentage (>1cm) of *Paulownia fortunei* in MS and WPM media at weekly interval

Culture media	Shoot number	Shoot length (cm)					Rooting (%)
		1st week	2nd week	3rd week	4th week	5th week	
MS	1.88^a	0.02	0.46^a	0.77^a	1.12^a	1.67^a	75
WPM	1.51^b	0	0.18^b	0.37^b	0.57^b	0.79^b	35

Means with different letters are significantly different at P>0.05 according to Duncan's New Multiple Range Test.

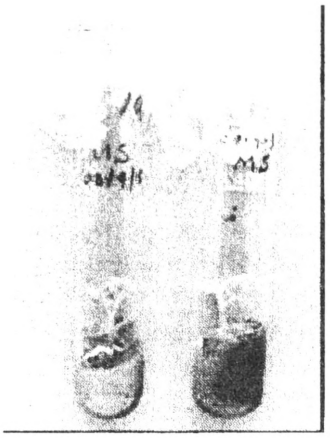

Plate 1: Shoot development of *Paulownia* nodal explants in MS media after 5 weeks.

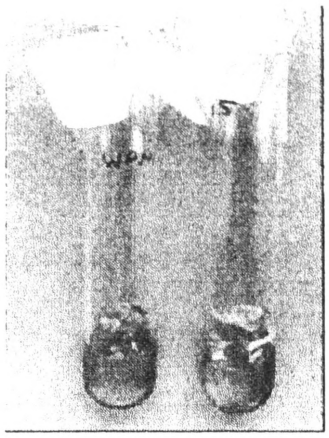

Plate 2: Shoot development of *Paulownia* nodal explants in WPM medium after 5 weeks.

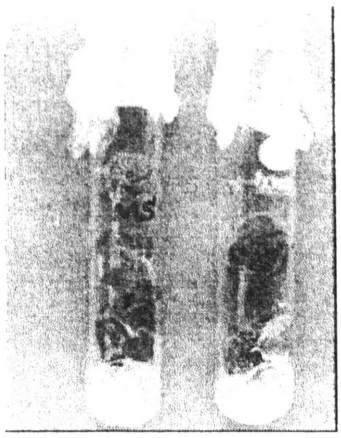

Plate 3: Six weeks old plantlet of *Paulownia fortunei.*

DISCUSSION

Previous research on Paulownia tissue culture did not address the effect of media composition on axillary shoot proliferation of *Paulownia* plants. This work showed the effect of the culture medium for axillary shoot proliferation of *Paulownia fortunei.*

According to Burger *et. al.* (1985) the greatest shoot elongation percentage was obtained from the nodal explants when they were cultured on modified MS medium supplemented with 1.0mg/l BAP and 0.1mg/l NAA. George (1993) demonstrated that auxin is required for the induction of callus in *Paulownia* and 2, 4-D is most employed for callus formation. But in this experiment, auxin was not used. MS and WPM media showed a great improvement in shoot and root development when they were supplemented with 2mg/l kinetin. This suggests that the media composition plays a significant role in production of adventitious shoots, roots and their further development.

The maximum shoot elongation percentage was observed in MS medium. The adventitious shoot production in MS medium was higher than that in WPM. Shoot length was also higher in MS medium than in WPM medium. Shoots remained stunted and leaf expansion was low in WPM medium compared to MS medium.

It has been shown that the cells at the leaf base and closer to the node have a high potential for shoot regeneration of Carnation in MS medium (Nontaswatsri *et al.*, 2002). In the present study, axillary shoot initiation was high in nodal explants but no callus formation was observed on petioler end in MS medium compared to that in WPM medium.

According to Quraishi and Mishra (1998), a significant and gradual increase in shoot number of *Cleistanthus collinus* may have been due to the loss of apical dominance caused by removing shoots at the end of each transfer. If the shoots were removed and subcultured on to a same fresh medium, it would give rise to more shoots (Marcotrigiano and Stimart, 1983). However, further subculturing was not attempted in the present study.

Higher root development percentage was also seen in MS medium. In the present investigation, no growth regulators needed for direct root formation of *Paulownia fortunei.* Once the shoots were sub cultured on to a medium containing activated charcoal without hormones, root formation occurred. Activated charcoal might have absorbed the growth regulators supplied exogenously from cultures and hence it would enhance the formation of roots. It suggests that, composition of the MS medium greatly effects shoot and root development of *Paulownia.*

Only 2-3 shoots per nodal explant were obtained in the present study. The regeneration capacity generally depends on the type of genotype, age of the explant and on the media composition. Although, the shoot proliferation rate was higher in MS medium than the WPM medium, culture survival rate was not satisfactory in MS medium. It might be due to difference in the salt concentrations in these two media.

Woody plant medium had not been used in the previous studies of *Paulownia* spp. All the workers used MS medium with different combinations of auxins and cytokinins.

The protocol described here for proliferation of axillary shoots from *in vitro* grown nodal explants is suitable for large scale propagation of selected elite trees. Explants will not be a limiting factor in the method described here because, nodes are available in abundant numbers and can be easily maintained compared to hypocotyls and cotyledonary explants (Marcotrigiano and Stimart, 1983) used in some of the previous studies with *Paulownia* spp. This plant has a greater potential for teaching *in vitro* propagation techniques because of ease of culture and quick response to culture media (Burger *et al.*, 1985).

There are relatively few reports available on shoot bud regeneration from nodal explants of *Paulownia.* Somaclonal variations could be a problem when adventitious shoot production is achieved through callus. However, direct axillary shoot proliferation does not involve callus formation or direct / indirect organogenesis and hence no chance of getting somaclonal variations.

CONCLUSIONS

In vitro axillary shoot development of *Paulownia fortunei* was better in MS medium supplemented with 2mg/l kinetin than that in WPM medium. However, culture survival rate was better in WPM than in MS medium. Rhizogenesis was possible in both MS and WPM media containing activated charcoal but devoid of growth regulators.

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