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EFFECT OF DIFFERENT AUXINS ON *IN VITRO* ROOTING OF *PAULOWNIA ELONGATA* PROPAGATED PLANTS

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Summary: Paulownia (*Paulownia elongata* S. Y. Hu) is a fast growing hardwood species having a lot of applications in industry, where a lightweight wood is needed. An effective *in vitro* protocol for large-scale propagation of *P. elongata* was developed. Attempts were made to study the effect of different auxins: α-naphthalene acetic acid (NAA), indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) supplemented to full and half strength Murashige and Skoog (MS) nutrient medium on rooting of *P. elongata* propagated plants. It was established that culturing of plants on full MS media each containing one of the three auxins resulted in lower root formation in comparison with ½ MS medium. The highest percentage of rooting (100%) and the maximum number of roots per plant (5.1) were recorded on ½ MS medium supplemented with 0.5 mg/l IBA. The obtained results suggested that the three tested auxins could be used separately in ½ MS medium for rooting of *P. elongata* and IBA was more effective to produce plants with well developed roots. Rooted plants were successfully acclimatized to *ex vitro* conditions. Among the three tested substances, the highest survival percentage (90%), plant height (9.5 cm) and number of leaves (5.3) was observed in plants grown on a mixture of peat:*perlite* in a 2:1 ratio. Plants were successfully transferred to a greenhouse. They were characterized by rapid growth and normal development. The adapted plants did not exhibit any morphological variations when compared with the initial plants. The choice of optimal rooting medium and appropriate *auxin type* for adventitious root formation can improve the *ex vitro* acclimatization of micro plants.

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INTRODUCTION

Paulownia (*Paulownia elongata* S. Y. Hu) is a fast growing hardwood species of economical importance because of the high biomass production that could be used as an excellent source of feedstock for the biofuel industry, for pollution control (both air and soil), land reclamation and as a fast growing ornamental tree providing both shade and very attractive flowers (Melhuish et al., 1990; Wang and Shogren, 1992; Bergman, 1998). *Paulownia* species are rich in *phenolic compounds* and *flavonoids* distributed in different parts and tissues of the tree (Chang et al., 2002; Si et al., 2013). The useful application of *P. elongata* and its wide cultivation need a rapid clonal propagation since traditional methods are not efficient. In this context, micropropagation can be an effective alternative for mass production of selected genotypes. In recent years, many researchers have been reporting about the use of tissue culture techniques for propagation of different *Paulownia* species: *P. elongata* (Ipekci et al., 2001; Castillo-Martínez et al., 2012); *P. tomentosa* (Ozaslan et al., 2005; Çelik et al., 2008; Corredoira et al., 2008; Bahri and Bettaieb, 2013); *P. fortunei* (Kumar et al., 1998; Venkateswarlu et al., 2001) and *P. kawakamii* (Prakash and Kumar, 2002; Lobna et al., 2008). Root formation of multiple plants is an important step of the micropropagation protocol. The rooting and acclimatization stages of *P. elongata* plants are poorly described by several authors (Chang and Donald, 1992; Ipekci et al., 2001). *In vitro* propagated plants of *P. tomentosa* (Marcotrigiano and Stimart 1983), *P. elongata* (Chang and Donald 1992), and *P. fortunei* (Rao et al. 1993) were rooted on MS without auxin. The inclusion of auxin in the rooting medium was reported to increase root number of *P. elongata* plants (Chang and Donald, 1992). The combination of 0.2 mg/l NAA and 0.4 mg/l IBA, and 2% sucrose in MS medium during the rooting stage was found to improve rooting of *P. elongata* shoots. This rooting treatment resulted in a shorter rooting time (10 days), a greater number of roots per shoot, and shorter roots that were more easily manipulated during transfer to the greenhouse (Bergmann and Whetten, 1998). A novel technique of direct *ex vitro* rooting and acclimatization in floating perlite was proposed by *Clapa* et al. (2014). *Ex vitro* rooting in floating perlite beds proved to be efficient for all *Paulownia* species.

The aim of the present research was to improve the *in vitro* rooting and *ex vitro* acclimatization of *P. elongata* micropropagated plants.

MATERIALS AND METHODS

Plant material and culture conditions

Initial young plants of *P. elongata* were provided by company "Sortoizpitvane", Elena town, Bulgaria. To initiate rooting, the micropropagated plants were cultured on root development media: basal full and half strength Murashige and Skoog media with 0.7% agar, 2% sucrose and three types of auxins - α-naphthalene acetic acid, indoleacetic acid and indole-3-butyric acid applied at concentrations of 0, 0.5 and 1 mg/l. The auxins were applied separately as described in Table 1. The MS medium free of growth regulators served as control. For each induction medium, forty plants were cultured in tubes 150 x 20 mm (two plants per tube)

containing 8 ml medium. To determine the most appropriate rooting medium the following characteristics were recorded: percent of rooted plants, mean number of roots per plant and root length after three weeks of cultivation. All media were adjusted to pH 5.6 before autoclaving at 121°C for 20 min. The experiments were repeated twice. The data were subjected to statistical analysis (Sigma Stat 3.1 Systat Software, San Jose, California, USA).

Acclimatization of *in vitro* rooted plants

The rooted plants were rinsed carefully in tap water and transferred to plastic pots (8 x 6 cm) containing different peat substrates: Mix1 – peat:perlite (2:1), Mix2 - peat:soil:perlite (2:1:1) and Mix3 - peat:sand:perlite (2:1:1). The pots were kept in the shade, covered with clear plastic bags, and watered daily. After 2 weeks, when the plants were fully acclimatized to the outdoor conditions, the plastic bags were removed. Twenty plants were transplanted in each potting mixture. After 4 weeks, the percent of survived plants, plant height and number of leaves were measured. After 6 weeks, the fully acclimatized plants were transferred to a greenhouse.

Culture conditions

Cultures were incubated in a growth chamber at 22±2°C, 40 μmolm^{-2}s^{-1} illumination from cool-white luminescent lamps, 70% relative humidity and a 16/8h (light/dark) photoperiod. The adapted plants were grown *ex vitro* at 24±1°C, a 16/8h (light/dark) photoperiod, and 50 μmol m^{-2} s^{-1} light intensity.

RESULTS AND DISCUSSION

In vitro* rooting of *P. elongata

To study the efficacy of the auxin type on rooting of *P. elongata* cloned plants, various auxins - IBA, NAA and

Table 1. Effect of auxins on rooting of *P. elongata.*

No	Auxin [mg/l]	Rooted plants [%]	No of roots/plant	Root length [cm]
a) in full strength MS medium				
MS0	0	30	1.1 ± 0.2	0.8 ± 0.1
MS1	0.5 IBA	60	3.1 ± 0.3	1.5 ± 0.2
MS2	1.0 IBA	60	2.4 ± 0.4	1.9 ± 0.3
MS3	0.5 NAA	55	1.4 ± 0.2	1.2 ± 0.2
MS4	1.0 NAA	40	1.0 ± 0.2	2.6 ± 0.4
MS5	0.5 IAA	Callus	-	-
MS6	1.0 IAA	Callus	-	-
b) in half strength MS medium				
MS0	0	45	1.5 ± 0.3	1.2 ± 0.2
MS1	0.5 IBA	100	5.1 ± 0.7	2.8 ± 0.4
MS2	1.0 IBA	90	4.3 ± 0.8	1.5 ± 0.1
MS3	0.5 NAA	95	4.0 ± 0.9	2.4 ± 0.4
MS4	1.0 NAA	90	1.4 ± 0.2	3.6 ± 0.3
MS5	0.5 IAA	40	0.6 ± 0.1	0.4 ± 0.1
MS6	1.0 IAA	60	2.5 ± 0.6	0.5 ± 0.1

IAA supplemented to the basal full and half strength MS media were tested. Significant variations due to the macro- and microelements content in the media were monitored in all traits studied (Table 1). In the control medium without any auxin supply, a low rooting rate of plants was induced. It was established that plants cultured on full MS media each containing one of the tested three auxins resulted in lower root formation in comparison with those cultured on ½ MS medium. This finding suggested that the rich mineral content of the medium was less effective for *in vitro* rooting of *P. elongata*. The optimal root induction treatment was full MS medium supplemented with 0.5 mg/l IBA. Roots were visible in 60% of the explants, where the number of roots per plant reached the highest mean value (3.1) among the tested auxins. The inclusion of NAA in the medium decreased the percent of rooting whereas IAA inhibited plant growth causing callus formation in the basal part of the plants that ceased root formation. Our observations revealed that the number of rooted plants was increased on ½ MS medium in the presence of the three auxins applied (Table 1). Rooting occurred 12 days after subcultivation of the plants. In 3 weeks, they intensively formed roots on ½ MS1 medium containing 0.5 mg/l IBA with a maximum number of roots per plant (5.1) and a mean length of 2.8 cm (Fig. 1a). In this case, the highest percentage of rooting (100%) was recorded. The rooting ½ MS medium supplemented with 1 mg/l IBA was also appropriate for rhizogenesis of *P. elongata* propagated plants (Fig. 1b). Rooting experiments showed that ½ MS medium containing NAA accelerated root formation, each plant forming about 4 roots on MS3 (Fig. 1c), while on MS4 only single long roots with many branches were observed (Fig. 1d). The mean number of roots per shoot decreased with increasing NAA concentration. On the other hand,

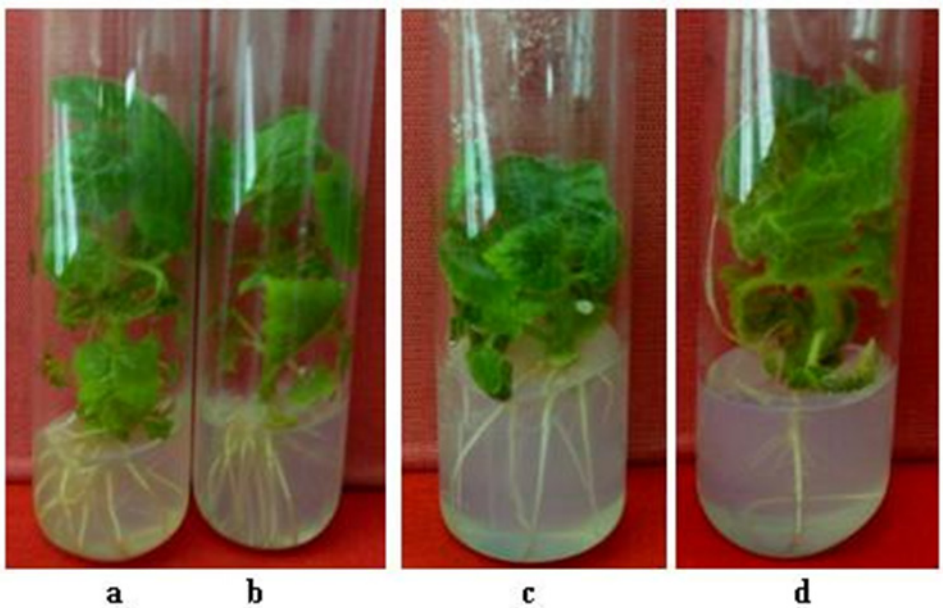

Figure 1. *In vitro* rooting of *P. elongata*: a) ½ MS + 0.5 mg/l IBA; b) ½ MS + 1 mg/l IBA; c) ½ MS + 0.5 mg/l NAA; d) ½ MS + 1 mg/l NAA.

Figure 2. *In vitro* rooting of *P. elongata*: a) ½ MS + 0.5 mg/l IAA; b) ½ MS + 1 mg/l IAA.

root length increased with increasing NAA concentration. Weak and single roots were observed on the medium supplemented with 0.5 mg/l IAA (Fig. 2a) whereas its increase to 1 mg/l caused the formation of a higher number of short roots (Fig. 2b). The obtained results suggest that all three investigated auxins could be used separately in ½ MS medium for rooting of *P. elongata*, IBA being more effective to produce plants with well developed roots. Rooting capacity was maintained in the next passages of cultivation on the optimal ½ MS1 medium. Ipekci et al. (2001) reported that the highest root formation efficiency (100%) of *P. elongata* was recorded on WPM medium supplemented with 1 mg/ml IBA. In contrast, Chang and Donald (1992) achieved plant rooting on MS medium without a plant growth regulator.

Ex vitro plant acclimatization

Acclimatization of transplanted plants to *ex vitro* conditions is one of most important stages in *micropropagation* protocols for many plant species. Different substrates used for adaptation of *P. elongata* influenced significantly the percent of plant survival (Table 2). The rooted plants were successfully acclimatized to *ex vitro* conditions. It was found that plants survived on 90% peat

Table 2. Effect of mixture substrate on survival of *P. elongata* plants during *ex vitro* acclimatization.

Peat mixture	Plant survival [%]	Plant height [cm]	No of leaves/ plant
Mix1 - peat:perlite (2:1)	90	9.5 ± 0.5	5.3 ± 0.7
Mix2 - peat:soil:perlite (2:1:1)	60	6.4 ± 0.6	4.2 ± 0.5
Mix3 - soil:sand:perlite (2:1:1)	70	6.1 ± 0.3	4.4 ± 0.2

based potting mixture containing peat and perlite in a 2:1 ratio. This mixture was found to be optimal also when the number of leaves per explant and the mean length of plants were measured. Our data revealed that different peat mixtures influenced plant height and number of leaves (Table 2). The highest plants (9.5 cm) as well as the greatest number of leaves (5.3) were obtained on mixture Mix 1. The *P. elongata* plants grew visibly in height and the roots were well developed during *ex vitro* acclimatization (Fig. 3a). It was found that 60% and 70% of adapted plants survived after transplanting on Mix 2 and Mix 3, respectively, and the plants exhibited lower values for the investigated characteristics. The appropriate humidity and temperature during the period of adaptation also influenced significantly the survival of the initial plants. After removing the plastic bags, the plants grew up fast and within 4 weeks they were ready to be transplanted into the greenhouse. About 95% of the plants developed normally. They were characterized by fast growth and big green leaves (Fig. 3b, c). A similar requirement was reported by Bergmann and Whetten (1998) who confirmed the high adaptability of *P. elongata*. The adapted plants did not exhibit any morphological variations when compared with initial plants. It was also established that when *P. elongata* plants were transferred to pots with a mixture of peat:perlite in a 3:1 ratio they showed a survival rate of 70–80% (Ipekci and Gozukirmizi, 2004).

Plant growth and development can easily be disturbed by a change in the environmental conditions after the *ex vitro* transfer and so, plants need a period of acclimatization. Many plants can die during this period (Fila et al., 1998). Acclimatization depends on the development of adventitious roots and this is affected by the substrate type and the physical parameters of *ex vitro* conditions. Acclimatization was evaluated by the percentage of the survived plants, plant height and number of leaves and it was seriously affected by the quality of the substrates. The aeration in the root substrate is very important for *ex vitro* acclimatization. It is suggested that the peat mixture, which contains peat and perlite, improves aeration and reduces water retention leading to root growth.

Figure 3. *Ex vitro* acclimated *P. elongata*: a) mix of peat:perlite (2:1); b) and c) rapid growth in the greenhouse conditions.

However, the peat mixture, which had an equal proportion of soil and sand showed a lower survival rate (about 60%). In contrast, the peat mix showed a great survival rate and good plant performance. Peat and perlite have a good porosity and allow the roots to penetrate quickly, suggesting more absorption of nutrients (Ipekci et al., 2004). Information of the requirements for root formation in *P. elongata* species and the selection of potting mixture can improve the *ex vitro* acclimatization of microplants.

In conclusion, the optimal culture conditions for *in vitro* rooting of *P. elongata* allowing fast and mass induction of roots were discussed. The half strength MS medium induced more effectively roots than the full MS medium. It was found that IBA was the most appropriate auxin for rooting induction and development. On ½ MS medium containing 0.5 mg/l IBA the rooting of plants sufficed for 100% and the maximum number of roots was 5.2. The plants revealed good adaptability *ex vitro* on a potting mixture consisting of peat:perlite (2:1) and 95% of them developed normally after being transferred into the greenhouse. The choice of optimal rooting medium and appropriate auxin for adventitious root formation can improve the *ex vitro* acclimatization of *P. elongata* plants.

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