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RESEARCH ARTICLE **(Open Access)**

In vitro propagation of a forest tree Paulownia tomentosa (Thunb.) Steud. - A valuable medicinal tree species

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Abstract:

The micropropagation of *Paulownia tomentosa* (Thunb.) Steud. was achieved by culturing nodal explants in MS medium through adding growth regulators: 6-benzylamino purine (BAP), indole-3-butyric acid (IBA) alone or combined in order to initiate shoot bud. Shoot proliferation was induced by the mean of MS medium containing different concentrations of BAP (1or 2 mg L^{-1}) alone or in combination with IBA (0.25 or 0.5 mg L^{-1}). Shoot buds were also placed on MS medium and added with IBA (0.25, 0.5 and 1 mg L^{-1}). During rooting stage, grown shoots were placed in a medium containing IBA at different concentrations (0, 0.25, 0.5, 1 and 2 mg L^{-1}). The results showed that supplementation of the culture medium by 1.0 mg L^{-1} of BAP exhibited the highest percent response (100%) for shoot bud initiation. During the multiplication stage, the medium containing (1 mg L^{-1}) BA and (0.25 mg L^{-1}) IBA was the most effective treatment to promote shoot multiplication. However, the number of shoots produced is relatively low represented by 1.4 shoot per nodal explants. For this reason, adding IBA only to MS medium was crucial to produce a single shoot with several nodes. Therefore, the supplementation of the culture medium by 1.0 mg L^{-1} of IBA induced the highest mean length of shoots per explants (3.75cm) and number of nodes per shoot (6). Concerning rooting potentiality, the addition of 0.5 mg L^{-1} IBA to MS medium showed 100% rooting percentage. Rooted plantlets were transferred and successfully acclimatized in greenhouse. The micro-propagated plantlets appeared morphologically similar with the mother plants.

Keywords: AIB, BAP, micro-propagation, *Paulownia Tomentosa*.

1. Introduction

Paulownia tomentosa Steud. (empress tree), belongs to the genus Paulownia (Scrophulariaceae), which includes nine species of fast-growing trees. Paulownia is a deciduous tree capable of achieving very high growth rates under favorable conditions. The genus Paulownia is indigenous to China and East Asia. The tree is ornamental, widely distributed throughout China, Korea and Japan [1]. It's appreciated in Eastern Asia for its medicinal and timber uses [2]. Parts of the plant *P. tomentosa* (leaves, wood, and fruits) have been used in traditional Chinese herbal medicine for the treatment of tonsillitis, bronchitis, asthmatic attack, and bacterial infections such as enteritis or dysentery. In fact, the flower is the most important material used in folk medicine herbs [3, 4, 5]. However, extracts of *P. tomentosa* contains many bioactive compounds such as flavonoids and particularly Apigenin. The latter has been found to show a variety of pharmacological virtues, including hypotensive [6] anti-inflammatory [7],[8] antispasmodic [9], antioxidant [10] and vaso-relaxant [11]. Besides, Apigenin may exert its anti-tumorigenic effect *in vivo* not only via the inhibition of tumor cell proliferation, but also via the impairment of the invasive potential of tumor cells [12].

It is receiving increasing attention as a short rotation woody crop plant. The ability of Paulownia for afforestation [13] in contaminated mine sites has been possible thanks to its capacity to tolerate harsh environmental conditions on surface mines [14]

The tree is propagated through seed or root cuttings. Germination of Seedling is low and slows growth than root cuttings [15, 16].

The micropropagation seems to be a prospective method of mass-production of valuable cultivars. Some authors have already reported the method of paulownia micropropagation. It's generally achieved through shoot bud regeneration directly from leaf explants or via. the callus phase [17, 18]. Mass multiplication of *P. elongata* through nodal culture has also been reported [19]. Shoot regeneration from nodal and intermodal segments of *P. taiwaniana* and from hypocotyls and cotyledons of *P. tomentosa* have been reported [17, 20]. Somatic embryogenesis of *P. tomentosa* was also reported [21].

The application of micropropagation techniques in agro-forestry was essential because it offers a rapid way of producing cloned stock in order to contribute to the environmental afforestration with a high quality

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material which is genetically uniform, free from diseases and viruses. [22]

The goal of this study was to establish an efficient protocol for rapid *in vitro* propagation of *Paulownia tomentosa* tree from nodal explants by studying the effect of various combinations of growth regulators such as IBA and BAP.

2. Material and methods

2.1. Plant material

Nodal explants were collected from old plants of *Paulownia tomentosa (**Thumb**.)* Steud. maintained in the greenhouse, Department of Agronomy and Plant Biotechnology, National Agronomic Institute of Tunisia (INAT).

2.2 Material disinfection

After excision, nodal explants were primarily rinsed in running tap water. Further disinfection was carried out in the laminar airflow chamber by using ethanol for few seconds, and then explants were rinsed in sterile distilled water and soaked in 0.1% (w/v) $HgCl_2$ for 5 min. Then, the shoot tips were rinsed with sterile distilled water and surface sterilized with 10% (w/v) sodium hypochlorite for 5 min. Next, they were rinsed two times for 5 min with sterile distilled water. Sterilized nodal explants were used for *in vitro* studies as described below.

2.3 Culture media and growth conditions

The culture medium consisted of [23] medium (MS) salts and vitamins, and 3% (w/v) sucrose. The medium was gelled with 0.6% (w/v) agar (Sigma) and the pH was adjusted to 5.8 with 0.1 N NaOH or HCl before autoclaving at 120°C for 20 min under a pressure of 1.1 kg/cm². The cultures were incubated at 23 ± 1°C under 16/8h (light/dark cycle) photoperiod and irradiance (36 µmol m^{-2} s^{-1}) provided by cool-white fluorescent lamps.

2.4 Shoot bud initiaion

For shoot bud initiation, explants were cultured on MS medium supplemented with different concentrations of BAP and IBA either alone or combined with: 1 mg L^{-1} IBA, 1 mg L^{-1} BAP, mg L^{-1} IBA + 0.25 mg L^{-1} BAP, 0.25 mg L^{-1} IBA + 1 mg L^{-1} BAP. The MS medium without adding of growth regulators was served as control. After four weeks of culture, percent response was determined and direct shoot bud initiation from nodal explants was noticed.

2.5 Multiple shoots bud induction

In order to achieve multiple shoots bud regeneration, the synergistic effect of auxin-cytokinin was evaluated. So, nodal explants derived *in vitro* regenerated shoot buds as explants source were cultured on MS medium supplemented with different concentrations of IBA or BAP either alone or in combination: 1 mg L^{-1} IBA, 0.25 mg L^{-1} IBA, 0.5 mg L^{-1} IBA, 1 mg L^{-1} BAP + 0.25 mg L^{-1} IBA, 2 mg L^{-1} BAP, 2 mg L^{-1} BAP + 0.25 mg L^{-1} IBA, 1 mg L^{-1} BAP + 0.5 mg L^{-1} IBA, 2 mg L^{-1} BAP + 0.5 mg L^{-1} IBA . The MS medium free from growth regulators served as control. The total number of multiple shoots regenerated and shoots length were recorded.

2.6 In vitro rooting of elongated shoots and acclimatization

For root induction, shootlets produced from multiplication stage were transferred onto the rooting medium containing MS salts, vitamins and different concentrations of IBA (0, 0.25, 0.5, 1 and 2 mg L^{-1}) that were used individually. The MS medium free from growth regulators served as control. Data were computed through percentage of rooting, number and length of roots/shoots after four weeks of culture. The rooted plantlets were transferred to plastic pots filled with peat and covered with polythene bags for two weeks to ensure high humidity. They have been gradually removed from the greenhouse before subsequent transfer to the field. They grew under confined conditions before their transfer into the greenhouse soil.

2.7 Statistical analysis

Experiments were conducted as a completely randomized block design. Twenty-four explants were used per treatment in triplicates. Data were subjected to statistical analysis using the program package SAS [24]. The one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at the significance level of 5% was used to compare means.

3. Results and discussion

In the present study, nodal explants from mature *Paulownia tomentosa* plants were placed on MS medium supplemented with BAP or IBA either alone or in combination with shoot bud initiation. Shoot formation has already occurred in all induction media with a 100% percent initiation response. This result corroborates those of [25] who observed that

incubation of meristem tissus of *Paulownia taiwania* in MS medium recorded a percent response of 99%.

As shown in table 1, the mean shoot length and number of nodes per explants differ significantly through the treatments. Nevertheless, the difference in shoot number was not significant between different IBA and BAP levels. Indeed, the nodal explants produced approximately two shoots.

The MS basal medium with 1 mg L^{-1} BAP was the best representative for shoot induction from nodal explants of *Paulownia tomentosa*. [26] Indicated that supplementation of *Paulownia kawakamii* culture medium by 1 mg L^{-1} of BA favoured shootlets initiation compared to the control. However, this concentration gave the best results of shootlets number per explants (2.6) and the highest length of shootlets (35.37 mm). Furthermore, [27] demonstrated that *in vitro* propagation by nodal segments in *Ficus benghalensis*L.tree responded better than the shoot tip explants. Nevertheless 90% of nodal explants cultures produced shoots on MMSI (modified MS medium with half strength of major salts) with 0.5 mg L^{-1} BA.

Table 1. Effect of various concentrations of IBA and BAP on shoot initiation of *Paulownia tomentosa*

MS + IBA and BAP concentrations (mg L-1)	***number of shoots/ explants***	***Shoot length (cm)***	***Number of nodes /shoot***
Control	*1,3 ± 0,483 a*	*1,750± 0,236 c*	*3,6 ± 0,516 c*
1 IBA	*1,2 ± 0,422 a*	*2,175± 0,355 b*	*3,1 ± 0,316 d*
1 BAP	*1,5 ± 0,527 a*	*2,775 ± 0,381 a*	*5,4 ± 0,658 a*
1 IBA + 0,25 BAP	*1,3 ± 0,483 a*	*1,025 ± 0,079 d*	*1,4 ± 0,459 e*
0,25 IBA+ 1 BAP	*1,4 ± 0,516 a*	*2,525 ± 0,432 a*	*4,05 ± 0,438 b*

Values within columns followed by different letters are significantly different according to Duncan test at $P \leq 0.05$

Shoot multiplication of mature trees of *Paulownia tomentosa* from nodal explants was achieved on MS medium supplemented with different concentrations of cytokinin (BA) plus auxin (IBA) either alone or in combination. The medium containing BA (1 mg L^{-1}) and IBA (0.25 mg L^{-1}) was the most effective treatment to promote shoot multiplication (1.4 shoot per nodal explants, with an average length and number of shoots by 1.26 cm and 4.28 nodes, respectively.) (Table 2). [17] reported that optimum shoot multiplication of sub-cultured shoot tips resulted in a medium with 1 mg L^{-1} BA and 0 or 0.1 mg L^{-1} IBA. [28] showed that supplementation of the culture medium by 1 mg L^{-1} of BAP and 0.1 mg L^{-1} of NAA gave the best results of shootlets number in *Paulownia tomentosa* per explants (4-6). [29] demonstrated that the highest number of shoot regeneration from stem explants of *Paulownia tomentosa* was obtained on MS medium supplemented with 3mg/l BAP+0.1 mg L^{-1} IAA. According to [30], the use of BA at 3 mg L^{-1} + IBA at 0.05 mg L^{-1} in the culture medium of *Acacia meamsii* induced the highest mean number of shoots per explants (3.51). The inclusion of a low concentration of IBA (0.25 mg L^{-1}) into BA-supplemented medium strongly suggests an interactive effect between the cytokinin and auxin on shoot proliferation. A concentration of IBA at 0.5 mg L^{-1} resulted in a significant reduction in the shoot number and length. This result is in accordance with observations in *oroxylum indicum.* Indeed, [31] observed an approximately three-fold increase in the shoot number following the inclusion of a low concentration of IAA (2.85µM). Besides, shoot proliferation was inhibited by higher auxin levels and by media lacking BA.

In this study, 2 mg L^{-1} of BA concentration inhibited proliferation and growth of auxiliary shoots. This result disagrees with [30]. They showed that BA at 2.0 mg L^{-1} increased shoots number. As shown in table 2, the increase of the levels of BA from 1 mg L^{-1} to 2 mg L^{-1} in the basal medium decreased significantly the average number (0.2) and length of shoots (0.05 cm). However, an inverse relationship was observed between BA concentrations and shoot length. Adding BA to the culture medium at higher concentration (0.6–1.0 mg/l) stimulated formation of numerous shoots of *Sorbus aucuparia*. Nevertheless, these shoots were short. Besides, MS nutrient medium supplemented with low concentrations of BA (0.2–0.4 mg L^{-1}) plus IBA (0.1 mg L^{-1}) promoted effectively the formation of longer shoots in nodal segments of mature trees [32]. According to these results, we can conclude that explants of *Paulownia tomentosa* grown in culture medium with cytokinin BA exhibited low number of shoots (1.4). An apical dominance may explain these findings.

For this case, effect of different concentrations of IBA (0, 0.25, 0.5 and 1 mg L^{-1}) on the *in vitro* shootlets formation of *Paulownia tomentosa* showed a significant difference between the treatments (Table 3). Data indicated that, supplementation of the culture medium by 1.0 mg L^{-1} of IBA induced the highest mean length of shoots / explants (3.75cm) and number of nodes per shoot (6) figure 1A. The study yield that

the medium free from IBA showed mean length of shoots per explants (2.25cm) and a number of nodes per shoot (5) which indicates that <u>*Paulownia tomentosa*</u> is rich in endogenous auxin.

Table 2. Effect of BAP and IBA concentrations on shoot multiplication of *Paulownia tomentosa*

MS + IBA and BAP concentrations (*mg L^{-1}****)***	***Number of shoots/ explants***	***Shoot length (cm)***	***Number of nodes /shoot***
1 BAP + 0,25 IBA	*1,40 ±0,52 a*	*1,26±0,21 a*	*4,28±0,84 a*
2 BAP	*0,2 ±0,63 b*	*0,05 ±0,16 c*	*0,2 ±0,63 c*
2BAP + 0.25 IBA	*0,7 ±0,95 b*	*0,27±0,43 b*	*0,95 ±1,26 b*
1BAP + 0,5 IBA	*0 ± 0 c*	*0 ±0 c*	*0 ±0 c*
2BAP + 0,5 IBA	*0 ± 0 c*	*0 ±0 c*	*0 ±0 c*

Values within columns followed by different letters are significantly different according to Duncan test at $P \leq 0.05$

Table 3. Effect of different concentrations of IBA on shoot multiplication of *Paulownia tomentosa*

MS + IBA concentrations(mg L-1)	***Shoot length (cm)***	***Number of nodes /shoot***
0	*2,25 ±0,274 b*	*5,000 ±0,632 b*
1	*3,75 ±0,689 a*	*6,000 ±0,632 a*
0,25	*1,583 ±0,279c*	*4 ±0,632 c*
0,5	*1,917 ±0,204 b*	*4,167 ±0,408 c*

Values within columns followed by different letters are significantly different according to Duncan test at $P \leq 0.05$

For root induction, elongated shoots were transferred into MS medium supplemented with various concentrations of IBA (0, 0.25, 0.5, 1 and 2 mg L^{-1}) (Table 4). One hundred per cent of shoot cuttings produced roots when they were cultured in the medium with 0.5 mg L^{-1} IBA. In this experiment, the highest number of roots per microcutting was 2.8 and the maximum root length was 2.48 cm (Fig. 1B). However, 0.25 mg L^{-1} IBA gave the lowest percentage of rooting (60%) and the number and length of roots decreased significantly compared to the best medium. These results agree with those obtained by [26]. They found that the addition of IAA (1 mg L^{-1}) to microcuttings of *Paulownia* Kowakamii led to the highest percentage of rooting (100 %) compared to culture medium free from growth regulators and which contains low concentration of IAA. This gave the lowest percentage of rooting (53.33 % and 46.67 %, respectively).

In the same context, [33] reported that the adding both of IBA and IAA at 0.5 mg/l in half strength MS medium of *Averrhoa Carambola* showed the best rooting response (88%). MS medium free from IBA could not form any root. This result is consistent with those of [34] who reported that the excised shoots of *Paulownia tomentosa* did not root on culture medium without growth regulator.

[35] found that root induction in shoots of *Paulownia tomentosa* occurred without auxin treatment, but rooting frequencies significantly increased in IBA treated shoots. The rooting experiments conducted in our study revealed that the presence of an exogenous auxin was essential for *in vitro* root induction of micro shoots. At higher concentration of IBA, the rooting was reduced but this reduction doesn't affect significantly the number of roots. About 80% and 70% of the shoots were rooted in a medium containing respectively 1 and 2 mg L^{-1} of IBA within 4 weeks of culture. [36] reported, for *Melissa officanalis*, that addition of IBA at 1 mg L^{-1} induced rooting in 64% of shoots.

Table 4: Effect of IBA on *in vitro* rooting of *Paulownia tomentosa*

MS+ IBA concentrations (*mg L^{-1}****)***	***Percentage response (%)***	***Number of roots/shoot***	***Root length (cm)***
0	*0*	*0 ±0 c*	*0±0 d*
0,25	*60*	*1,1 ±0,994 b*	*0,653 ±0,572 c*
0,5	*100*	*2,8 ±0,422 a*	*2,483 ±0, 13 a*
1	*80*	*2,4 ±1,265 a*	*1,787 ±1,011 b*
2	*70*	*2 ±1,491 a*	*1,119±0,850 c*

Values within columns followed by different letters are significantly different according to Duncan test at $P \leq 0.05$

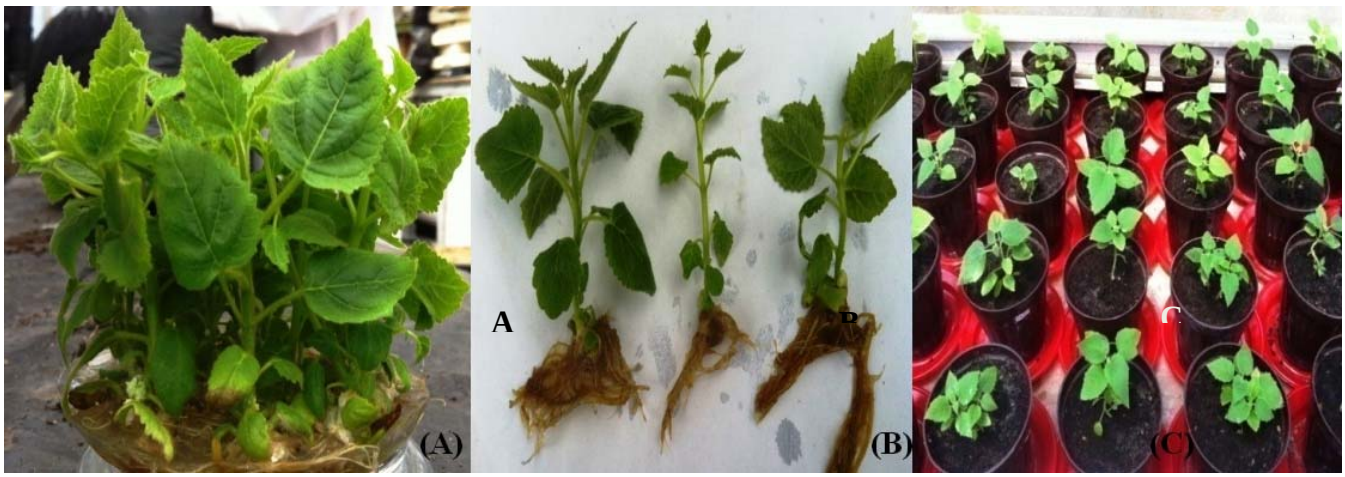

Figure 1. *In vitro* regeneration of *Paulownia tomentosa*. **A.** Multiple shoots formation from nodal explants. **B.** Rooted vitroplants. **C.** *In vitro* regenerated plantlets transferred to pots.

The plantlets with well-developed roots were transferred into plastic pots containing peat and had been maintained under controlled conditions for two weeks. Then, they were transferred into the soil under greenhouse conditions with a survival rate of 100%. No variation was observed in the morphology of the plantlets. *Ex vitro* plantlets showed healthy and uniform population with similar morphological characters to the donor (Fig. 1C).

4. Conclusion

In conclusion, the present study described an *in vitro* propagation protocol through nodal stem segments of *Paulownia tomentosa* aiming at initiating shoots and regenerating plants. In this experiment, the use of MS medium supplemented with BA at 1 mg L^{-1} was the best induction media. During multiplication stage, MS medium added to IBA at 1 mg L^{-1} was the most effective treatment for promoting shoot multiplication. 100% rooting were obtained through adding 0.5 mg L^{-1} IBA to the MS medium.

5. References

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