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# EFFECT OF PLANT HORMONES IN DIFFERENT STAGES OF IN VITRO MICROPROPAGATION OF PAULOWNIA SHAN TONG (P. TOMENTOSA & P. FORTUNEI)

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**Abstract:**
In vitro propagation conditions of Paulownia Shan Tong (P. tomentosa & P. fortunei), especially effect of 6-benzylamino purine (BAP) in multiplication media and effect of indole-3-butyric acid (IBA) in rooting media were studied. Shoot proliferation was induced by the mean of MS medium containing different concentrations of BAP (0, 0.25, 0.5 and 1 mg/$L^{-1}$) and supplementation of the culture medium by 1.0 mg/$L^{-1}$ of BAP induced the highest mean length of shoots / explants (4,38 cm) and number of nodes per shoot (5,86). 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/$L^{-1}$). 98 % of shoot cuttings produced roots when they were cultured in the medium with 0.5 mg/$L^{-1}$ IBA. The highest number of roots per microcutting was 5.2 and the maximum root length was 6,383 cm.

**Keywords:** Paulownia tree, proliferation media, rooting media, BAP, IBA.

## INTRODUCTION

A new, very perspective plant, Paulownia has been introduced into Central Asian nurseries production the last few years. This genus includes nine species and numerous interspecific hybrids. The most common species being: Paulownia tomentosa, P. fortunei, P. elongata, and hybrids P. tomentosa & P. fortunei and P. elongata & P. fortunei. P. tomentosa is the most temperature tolerant species. P. fortunei is known for its straight trunk and narrow crown. P. elongata is a fast-growing species with a straight trunk, medium crown and suitable for warm climates. Hybrid Paulownia Shan Tong (P. tomentosa & P. fortunei) combines features of mother plants, and as a result, it is also the perfect plant for plantation in Central Asian climate[1,4]. Paulownia plants have many applications. Paulownia wood is used for making musical instruments, boxes, chests, lightweight skis, furniture, delivery containers, moldings, doors and windows. It is suitable for beehive construction, veneer, particleboard, picture frames, toys, fishing net floats, and for shoes. It was successfully tested for making high-strength composite boards used in construction. Paulownia wood is good for making paper pulp. Due to these qualities, Paulownia species are among the most important forestry crops in the world[2,6,9].

The use of in vitro propagation techniques provides a supply of healthy, homogenous planting material for Paulownia. Trees planted from seed often show an altered growth habit and may be more susceptible to pests and diseases. Usually, only primary and axillary shoot meristems are used as explants for in vitro cultured Paulownia plants in order to ensuretrue clonal propagation [3,7,8].

## MATERIAL AND METHODS

### Plant material collection and surface sterilization

Explants were collected from tissues of mature Paulownia Shan Tong (P. tomentosa & P. fortunei) plants. The shoot tips and nodal segments were cut and collected in a beaker containing water to avoid desiccation and then brought to the laboratory.

For the surface sterilization, the shoots were kept in running tap water for about 30 min with few drops of liquid detergent Tween 20. After washing with detergent the explants were thoroughly rinsed with distilled water for 4-5 times to remove any traces of detergent remaining in explants. After these treatments, explants were taken

inside the laminar air flow for further sterilization. Explants were surface sterilized with freshly prepared 0.1% w/v aqueous solution of $HgCl_2$ for 3 min. Then, they were washed for three times with autoclaved double-distilled water.

### Culture medium

Single or double nodal explants were inoculated onto MS basal medium [5]. Different concentrations of plant growth regulators were tested for shoot induction. MS medium was supplemented with 0,25 mg/l, 0.5 mg/l, 1.0 mg/l, and without BAP. 3% sucrose was used as carbon source and pH was adjusted to 5.7 before autoclave. The media was solidified using 8 mg/l and autoclaved at 121°C for 15 min.

### Culture condition and in vitro shoots proliferation

The cultures were incubated at 16 h photoperiod with light intensity of 3000 lux using florescent tube lights and temperature of 25± 2°C for 4 weeks. After successful initiation of the shoot, newly formed shoots were excised and again leaf were trimmed and single nodes were sub-cultured on the medium with the same concentration of BAP. Sub-culture process was performed 4-5 times.

### Root induction 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.

After rooting process, regenerated seedlings were transplanted to potting mixture composed of peat:perlite (3:1) in plastic pots by covering with polyethylene bags to provide for high humidity and maintained in the greenhouse. The trays were kept in shady place, covered with transparent polythene sheet and were watered daily. After 10-12 days when the plants were fully acclimatized to the outdoor conditions, they were again transplanted individually in clay pots containing compost and soil (1:1 ratio) and watered on alternate days.

## RESULTS AND DISCUSSION

Effect of different concentrations of BAP (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 1). Data indicated that, supplementation of the culture medium by 1.0 mg/$L^{-1}$ of BAP induced the highest mean length of shoots / explants (4,38 cm) and number of nodes per shoot (5,86) figure 1A. The study yield that the medium free from BAP showed mean length of shoots per explants (2.53cm) and a number of nodes per shoot (3,5). As it is shown, the best result obtained when 1,0 mg/$L^{-1}$ BAP was used in multiplication media.

Table 1. Effect of different concentrations of BAP on shoot multiplication of Paulownia Shan Tong (P. tomentosa & P. fortune) in MS medium.

| BAP concentratios (mg/ $L^{-1}$) | Shoot length (cm) | Number of nodes /shoot |
|---|---|---|
| 0 | 2,53 ±0,274 | 3,5 ±0,234 |
| 0,25 | 2,245±0,279 | 3,96 ±0,421 |
| 0,5 | 2,896±0,204 | 4,63 ±0,324 |
| 1 | 4,38±0,689 | 5,86±0,563 |

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 2). 98 % 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 5.2 and the maximum root length was 6,383 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.

Table 2: Effect of IBA on in vitro rooting of Paulownia Shan Tong (P. tomentosa & P. fortune) on MS medium

| IBA concentrations (mg $L^{-1}$) | Percentage response (%) | Number of roots/shoots | Root length (cm) |
|---|---|---|---|
| 0 | 0 | 0 ±0 | 0 ±0 |
| 0,25 | 55 | 2,3 ±0,643 | 3,342±0,47 |
| 0,5 | 98 | 5,2 ±0,368 | 6,383 ±0,34 |
| 1 | 83 | 4,4 ±0,258 | 4,787 ±0,054 |
| 2 | 68 | 4±0,986 | 3,919±0,450 |


**Figure 1.** Shoot proliferation of Paulownia Shan Tong (P. tomentosa & P. fortune) explants in MS medium

Plants were very efficiently acclimatized with a 90% success rate. Ventilation of the mini greenhouses was also crucial in the hardening stage because this prevented fungal infections. Abundant watering favored the fast growth of the plants(Fig.1C).

## CONCLUSION

In that research shoot proliferation was induced by the mean of MS medium containing different concentrations of BAP (0, 0.25, 0.5 and 1 mg/$L^{-1}$) and supplementation of the culture medium by 1.0 mg/$L^{-1}$ of BAP induced the highest mean length of shoots / explants (4,38 cm) and number of nodes per shoot (5,86). 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/$L^{-1}$). 98 % of shoot cuttings produced roots when they were cultured in the medium with 0.5 mg/$L^{-1}$ IBA. The highest number of roots per microcutting was 5.2 and the maximum root length was 6,383 cm.

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