EFFECT OF EXPLANT SOURCE ON IN VITRO PROPAGATION OF *PAULOWNIA TOMENTOSA* STEUD.

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ABSTRACT

Shoot tip, leaf with petiole, nodal stem and root explants of in vitro germinated seedlings of Paulownia tomentosa Steud. were cultured on Murashige and Skoog (MS) medium combined with 1-naphthaleneaceticacid (NAA)/6-benzylaminopurine (BAP), indole3-aceticacid (IAA)/BAP or IAA/Kinetin. Nodal stem explants were found to be an excellent explant source to induce direct ogranogenesis. The highest number of shoot regeneration from stem explants was obtained on MS medium supplemented with 0.1 mg.l^{-1} IAA+3.0 mg.l^{-1} BAP. Rooting was induced on MS basal or MS containing NAA+IAA combinations. The rooted plantlets were transferred to potting soil and adopted to greenhouse conditions.

Introduction

For last decade following the economical crisis of the Republic of Turkey, in order to support the boomed furniture industry and plantation of the vanished woodlands, the government started to fund the forestation and the manufacture of high quality timber. The *Paulownia* spp that belong to the Scrophulariaceae family is one of the favourable trees. A risen popularity of Paulownia in Turkey is due to its many desirable features such as ornamental landscape use, forestry use, food source use, waste water control . Due to tolerance to temperate conditions and its fast-growing trunks, recently *P. tomentosa* is given the most attention in Gaziantep province of Turkey. In the majority of extreme weather (-10 °C - +45 °C), existence of wide arid lands and commercial timber manufacturing in Gaziantep, *Paulownia* spp took considerable interest as one of the most promising woody plant.

Since *P. tomentosa* Steud. has been habituated to drastic climate conditions of Southeastern Anatolia of Turkey for the last five years, it was chosen on the basis of adaptation to environment of Gaziantep province. In addition to this, because of its importance for growing timber industry in the region, it is required for fast and efficient propagation. In our laboratory conditions, in order to establish an efficient shoot multiplication system we considered that it was necessary to determine the capacity of regeneration from all parts of the plant and optimal composition of the regeneration media. Regeneration of *Paulownia* spp. using traditional methods consumes time and *in vivo* seed germination ratio is very low (1). *In vitro* propagation of *P. elongata, P. fortunei, P*. Henan 1 and *P. tomentosa* from leaves and petioles has been previously reported (1, 2). In this paper, we reported the most productive explant source among shoot tips, leaves and nodal stems of *P. tomentosa* Steud. dissected from *in vitro* grown seedlings and the optimal hormone combination for the reproduction of somaclonal plantlets.

Materials and Methods

Explant source and culture conditions

Seeds and the youngest leaves of *Paulownia tomentosa* Steud. were collected from the Botanical Garden of University of

Gaziantep (Gaziantep-Turkey) in 2001-2002 growing season. Plant materials and the loculicidal capsules of fruits containing seeds were first rinsed in running tap water for 30 min and dipped in 70 % of absolute ethanol for 10 seconds. After taking fruit capsules out of seeds, surface sterilization of seeds was carried out for 8 min and 4 min for plant materials and seeds, respectively, with 5 % NaOCl, followed by three rinses in sterile distilled water, each of 5 min, and then the plant tissue was drained on the sterile Watmann 3 MM paper aseptically. For in vitro culture experiments, the seeds were sown on two different basal MS-based media -one full strength MS media included 3 % (w/v) sucrose and the other half strength MS media included 1 % (w/v) sucrose in petri dishes (11.5 cm in diameter x 2 cm high) (3). Each petri dishes included 15-20 of seeds. When length of the seedlings were totally 3.5 - 4 cm (**Fig. 1**), they were dissected into shoot tips, leaf with petiole, nodal stem and roots and transferred to MS; the basal media combined with different combinations of hormones including 3 % (w/v) sucrose and solidified with 0.8 % (w/v) agar (Difco-Bacto). Each petri dish contained 4-5 of fully developed leaves with petioles or 2-3 pieces of nodal stems, shoot tips or roots. Hormones of basal MS medium comprised auxins (IAA, NAA) and cytokinins (BAP, Kinetin) were added individually or in various combinations (**Table 1, 2, 3**) prior to autoclave and the pH of the medium was adjusted to 5.7. Following autoclaving (121 °C, 15 min), twenty-five ml aliquots of the medium was dispended into each petri dishes (11.5 x 2 cm). The cultures were maintained at 26 ± 2 °C under white fluorescent tubes and 16/8 h (light/dark) photoperiod.

The in vivo-grown leaves harvested from the Botanical Garden of the Gaziantep University, were cut from lamina including petiole in a leaf covering area approximately 9 cm^2 space and placed two pieces for each petri dishes containing basal MS medium. Composition of media and culture conditions were as described above.

Six to eight weeks after culture initiation, shoot formation from each explant was recorded. The means of adventitious shoots was estimated by number of total produced shoots from total explants (sum of each kind of explant) dividing to total explant number. The experiment was repeated at least twice and five to eight Petri dishes were used for each treatment.

Rooting and Potting of Plantlets

In two weeks, all of the shoots derived from explants produced roots on basal MS and basal MS supplemented with various concentrations of IAA and NAA. Six weeks after maintanence of plantlets in rooting media, regenerated seedlings were transplanted to potting mixture composed of torf:perlite:sand (1:1:1) in plastic pots by covering with polyethylene bags to provide for high humidity and maintained in the glasshouse. Polyethylene bags were removed two-three weeks after transfer. Roots of plantlets were immersed in 0.6 gr/lt of Benlate solution for a short time prior to transfer to soil.

Results and Discussion

Effects of explant sources and hormone combinations for reproduction of somaclonal plants

Half strength MS basal media supplemented with 1 % sucrose exhibited a germination efficiency of 35-40 % in comparison with full strength MS basal media supplemented with 3 % sucrose (20-25 %).

Direct shoot regeneration from petioles with leaf lamina of *in vivo*-grown plants was not observed but only non-embryogenic calli were formed (data not shown). This was in contrast with previous reports in which the best explant source for direct somatic embryogenesis was determined as petiolar ends of leaf explants (4, 5). The results of adventitious shoot regeneration as described below were obtained from *in*

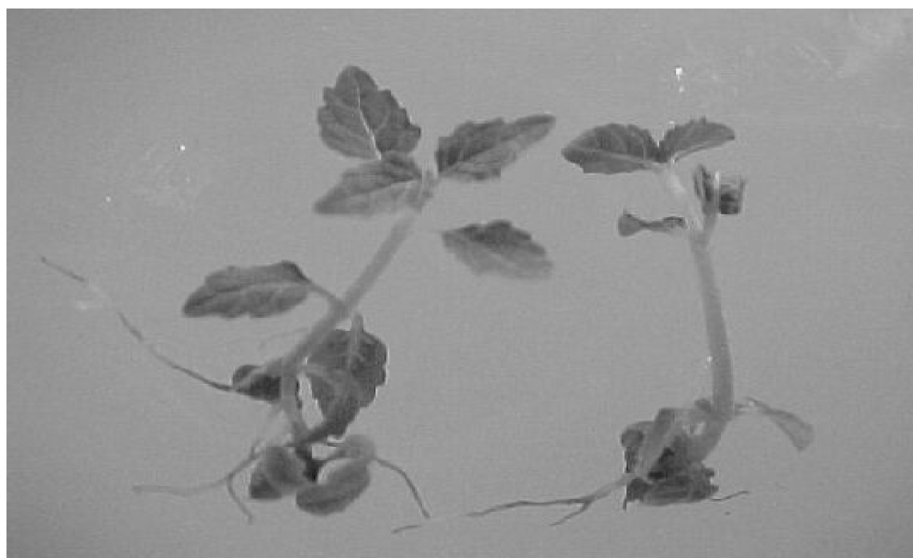

Fig. 1. Four weeks old *in vitro* germinated *P. tomentosa* plant that leaves, shoot tips, nodal stems and roots used as explant source

vitro-grown plants (**Fig. 1**). As shown in **Table 1** while plantlets formation from shoot tip explants occurred at reduced concentrations of NAA in combination with BAP, high level of regeneration was obtained in 5.0-7.0 mg.l^{-1} BAP. Rao et al (1996) reported that optimum shoot production from petioles of *P. fortunei* was obtained when MS medium supplemented with 4.5 mg.l^{-1} BAP and 0.8 mg.l^{-1} NAA was used (2). Chang and Donald (1992) used MS medium combined with 0.1 mg l^{-1} NAA + 0.5 mg l^{-1} BAP for regeneration of shoots from leaf callus of *P. Elongata*(6). Ratio of NAA/BAP was 1/5 in those works. The results in this study corroborated those of Rao et al (1996) and Chang and Donald (1992) indication that is the increased ratio of BAP and decreased ratio of NAA was significant to induce shoot for all explants (2, 6). Song et al. (1990) noticed that ratio of shoot multiplication from *P. catalpifolia* meristems was enhanced by increasing concentrations of BAP up to 8.0 mg l^{-1} (7). Either the combinations of KIN (Kinetin) and IAA or alone with an average 0 to 2 shoots per shoot tip was found to have nearly equal efficiency for adventitious shoot stimulation from shoot tips except 2.0 mg.l^{-1} IAA combined with 3.0 mg.l^{-1} kinetin with average of 6.6 adventitious shoots (**Table 3**). We found that combinations of 0.1 mg.l^{-1} IAA/7.0 mg.l^{-1} BAP (**Table 2**), and 0.1 mg.l^{-1} NAA/7.0 mg.l^{-1} BAP (Table 1) resulted a mean of 5.75 and 6.41 shoots per shoot tip explant (**Fig. 2**), respectively. Although Chang and Donald (1992) reported when shoot tips of *P. elongata* were cultured on medium containing 1.0 mg.l^{-1} BA, shoot production increased with 9.7 fold, in the present work 0.1 or 1 mg of BAP was unsuccessful to produce shoot from shoot tips (6).

The beneficial effect of the presence of the cytokinin and auxin (BAP and IAA) in the culture medium for shoot stimulation from leaf explants of *P. tomentosa* was demonstrated previously by Rao et al., (1996) (2). We investigated the optimal concentration of BAP and NAA or IAA required for initiation of shoot regeneration from leaves and found that low concentra-

TABLE 1

Effect of plant growth regulators on adventitious shoot regeneration from leaf, shoot tip and root explant of *P. tomentosa*

		Shoot Tip	Leaf	Root
NAA (mg)	BAP (mg)	Shoot formation Number of shoot regenerated	Shoot formation Number of shoot regenerated	Shoot formation Number of shoot regenerated
0.0	0.0	0.0	0.0	0.0
0.0	1.0	0.0	0.0	0.0
0.0	5.0	2.25	0.0	0.0
0.1	0.0	0.0	0.0	0.0
0.1	3.0	4.5	0.2	0.0
0.1	7.0	6.41	0.7	0.0
0.5	3.0	0.49	0.3	0.0
0.5	5.0	4.0	0.4	0.0
0.5	7.0	5.5	0.2	0.0
1.0	3.0	1.0	0.0	0.0
1.0	5.0	1.5	1.9	0.0
1.0	7.0	2.5	1.5	0.0
2.0	0.0	0.0	0.0	0.0
2.0	1.0	0.0	0.0	0.0
2.0	3.0	0.25	0.0	0.0
2.0	5.0	0.5	0.9	0.0
2.0	7.0	1.0	1.1	0.0

tion of BAP supplemented with combination of NAA and/or IAA caused low level of shoot regeneration, however the maximum number of adventitious plantlets was occured at BAP combined with IAA (Tables 1 and 2). Concentrations of BAP in combination with IAA affected to contribute positively to shoot initiation from leaves to different degrees but also caused more callus formation. None of the combinations of IAA and kinetin facilitated to shoot regeneration (Table 3).

All concentrations of IAA and BAP and KIN, either alone or in combinations induced shoot regeneration from stem explants (Table 1, 2 and 3). However a greater number of somaclonal plantlets formed at combinations of IAA/BAP (**Fig. 3**)

TABLE 2

Stimulation of adventitious shoot formation from *P. tomentosa* explants after 5 weeks culture on MS medium supplemented with different concentrations of plant growth regulators

		Adventitious shoot formation*		
IAA (mg)	BAP (mg)	Leaf Shoot Formation Number of shoot regenerated	Shoot tip Shoot Formation Number of shoot regenerated	Nodal Stem Shoot Formation Number of shoot regenerated
0.0	0.0	0.5	0.0	0.0
0.0	1.0	0.0	0.09	4.75
0.0	5.0	3.25	0.0	3.5
0.1	0.0	0.0	0.0	4.0
0.1	3.0	2.75	2.2	10.5
0.1	7.0	5.75	3.66	7.0
0.5	3.0	2.5	0.0	3.0
0.5	5.0	5.0	0.41	5.25
0.5	7.0	2.5	0.5	5.0
1.0	3.0	3.5	0.125	4.0
1.0	5.0	2.25	0.0	4.75
1.0	7.0	3.25	2.54	4.25
2.0	5.0	0.0	1.258	5.25

in comparisons with IAA/KIN. The medium in combination 0.1 $mg.l^{-1}$ of IAA and 3.0 $mg.l^{-1}$ of BAP caused the highest level of regeneration from stems with 10.5 adventitious shoots (Table 2).

There is a few reports of shoot regeneration from leaves or petioles of *P. tomentosa* and/or other *Paulownia* species (2, 4, 8). Bergman and Moon (1997) described petioles as an excellent explant source for adventitious shoot production within NAA/BAP combinations in *P. elongata, P. fortunei* and *P.* Henan 1(1). In contrast, nodal stem explants of *P.tomentosa* exhibited most effective shoot regeneration efficiency in our laboratory. This difference can be due to be the fact that the forest trees for acclimatization can adjust internal hormones depent upon a changing external environment hence somaclonal productivity may be affected. Shoot tips were medium

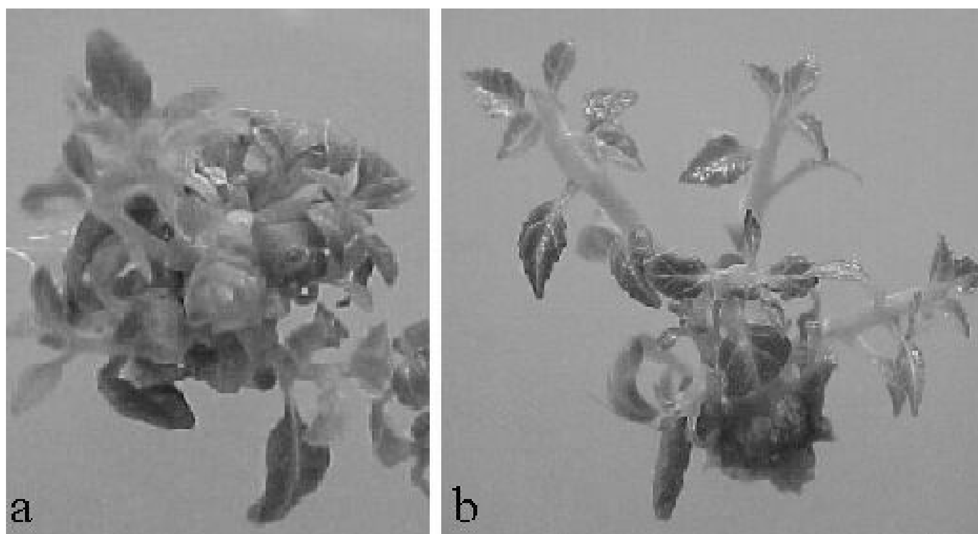

Fig. 2. Direct adventitious shoots from shoot tips of *P. tomentosa* on MS medium supplemented with 0.5 mg l^{-1} IAA and 5.0 mg l^{-1} BAP (a), and 0.5 mg l^{-1} NAA and 5.0 mg l^{-1} BAP (b).

TABLE 3

Stimulation of adventitious shoot formation from *P. tomentosa* explants after 5 weeks culture on MS medium supplemented with different concentrations of plant growth regulators

		Adventitious shoot formation*		
IAA (mg)	Kinetin (mg)	Shoot Tip Shoot Formation Number of shoot regenerated	Leaf Shoot Formation Number of shoot regenerated	Nodal Stem Shoot Formation Number of shoot regenerated
0.0	0.0	0.0	0.0	0.0
0.0	1.0	1.0	0.0	5.5
0.1	0.0	1.75	0.0	1.75
0.1	1.0	1.0	0.0	3.5
0.1	3.0	2.0	0.0	2.5
0.1	7.0	1.0	0.0	2.0
0.5	0.0	0.0	0.0	2.5
0.5	1.0	2.0	0.0	1.5
0.5	5.0	0.0	0.0	2.1
0.5	7.0	1.0	0.0	1.3
2.0	1.0	0.5	0.0	3.05
2.0	3.0	6.6	0.0	5.8
2.0	5.0	2.0	0.0	8.0

explant with regarding to embryogenic capacity whereas leaf explants with petioles produced relatively low number of shoots. While the best response was obtained from stems, root discs were the most inefficient material on shoot regeneration. In an earlier study of *P.tomentosa,* it was shown that the high frequency regeneration of shoots could be induced in leaf explants and the best response was on medium supplemented with 1.8 mg.l^{-1} IAA/11.3 mg.l^{-1} BAP (2). The difference between present results in our experiments and previous workers may arise from various factors such as physiological age and ontogeny of organs, harvesting periods of explant from plant, size of explant and growth conditions of plants used as explant source (i. e., greenhouse, field, *in vitro*). Regarding drought, calcareous and alkali soil (pH 7.9-8.4) and moderate territorial climate of Gaziantep province, we conclude that all these factors mentioned above might influence the balance of endogenous hormones of *P. tomentosa* hence somaclonal productivity can be affected.

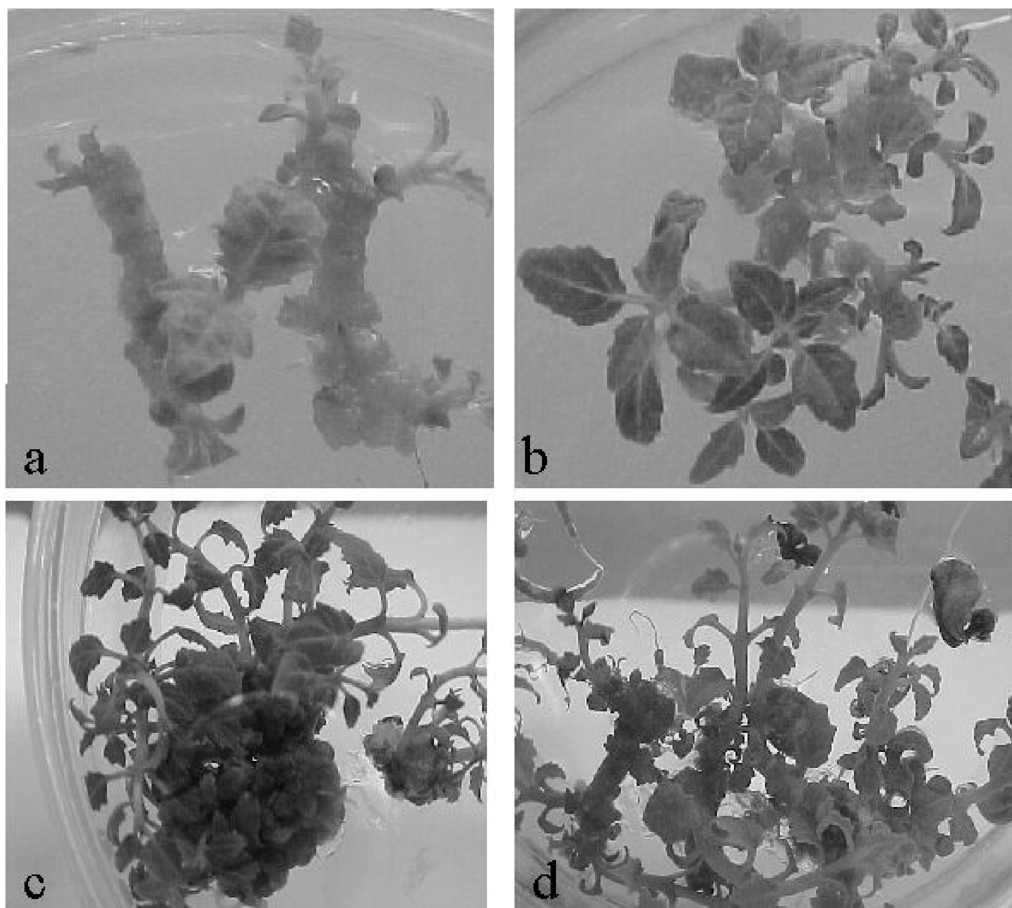

Fig. 3. Direct somatic embriogenesis from stem explants of *P. tomentosa* on MS medium supplemented with 0.5 mg l^{-1} IAA and 5.0 mg l^{-1} BAP. The photograps were taken 2 weeks (a), 4 weeks (b) and ,7 weeks (c) and 8 weeks (d) after culturing.

Rooting and Potting of Shoots

All of the shoots derived explants produced roots on basal MS and basal MS containing IAA and NAA combinations in two weeks (data not shown). The highest proliferation of roots was obtained in combination of 0.5 mg.l^{-1} IAA + 1.0 mg.l^{-1} NAA, likewise the longest root formation was achieved with 2.0 mg.l^{-1} IAA + 1.0 mg.l^{-1} NAA combinations. Following root development after normal plantlets were transferred ex vitro, more than 95 % of the acclimatised plants successfully survived. The whole regeneration process to soil from seed germination via *in vitro* culture required about 6 months.

Conclusions

Paulownia spp are becoming more popular in Turkey for its use in paper industry, honey production and ornamental landscape planning. The South Eastern Region of Turkey has extreme weather conditions and there are arid lands where *P. tomentosa* could be cultivated. *In vitro* clonal propagation is known to be the most effective method in *Paulownia* spp. An efficient re-

generation system of *P. tomentosa* Steud. was established using nod and shoot tip explants of 3-4 weeks old *in vitro* germinated seedlings in our laboratory. We were able to regenerate approximately 100 new plantlets from a 5-8 cm long seedling within 8-10 weeks.

Acknowledgements

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