The Role of Nitrobenzoic Acid Derivatives on Callus Induction and Plant Regeneration in *Paulownia Shan Tong*

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Abstract

Paulownia is a multipurpose tree species with a rapid growth which is cultivated in several temperate zones worldwide for its economic importance. The present study was undertaken to investigate the effects of different alkanolamine salts of 4-nitrobenzoic acid on *in vitro* callus induction and plant regenerating in *Paulownia shan tong*. The alkanolamine salts, namely methylethanolamine 4-nitrobenzoate (4-NO₂BA MMEA), diethanolamine 4-nitrobenzoate (4-NO₂BA DEA) and triethanolamine 4-nitrobenzoate (4-NO₂BA TEA) are new synthesized compounds with biological activity and low toxicity. The influence of title compounds on sprout length, number of internodes, number of leaves and number of ramifications has been studied at two different concentrations: 1 mg/L respectively 0.5 mg/L. The experimental data show that new tested compounds have a better growth effect on plant vegetable parties in report to control, the most eloquent results being obtained for 4-NO₂BA MMEA. For callus induction 2,4-D (2,4-dichlorophenoxyacetic acid) hormonal balances were attempted and the development of callus was examined at two different concentrations. An induced callus development was observed regarding callus dimensions for all explants inoculated on culture medium supplemented with 2,4-D.

Keyword: *Paulownia*; *in vitro* culture; callus; alkanolamine salts of 4-nitrobenzoic acid

INTRODUCTION

The genus *Paulownia*, one of the fastest growing and hardwood tree in the world, includes several species and natural hybrids that are native to China. The trees reach impressive dimensions in the first years of life exceeding 5 meters in height (Yadav *et al.*, 2013). The wood is strong, yet light weight, making it ideal for musical instruments, chests, boxes, furniture, lightweight skis, doors and windows, delivery containers (Akyildiz and Kol, 2010; Kiaei, 2012). Another usage of *Paulownia* wood is making paper pulp that is of higher quality comparable to *Eucalyptus* (Feria, *et al.*, 2013). Due to the mentioned qualities, *Paulownia* species are

among the most important forestry crops in the world. In the last decade, *Paulownia* becomes an important culture also in Romania where about 200 ha are annually cultivated (Stiriagricole.ro, 2015), being used acclimatized hybrids for specific weather conditions. Thus, *Paulownia shan tong*, a new paulownia variety, attracts attention by fast growing rate, strong resistance to disease and insect pest, strong resistance to drought and cold, large timber output, good material quality, strong renewal ability, comparatively with other *Paulownia* species (e.g. *elongata*).

Paulownia can be propagated through seeds, stem cutting roots or *in vitro* by tissue culture

(Burger, 1985). It was established that propagation by seeds or early seedling growth is slower and difficult than vegetative propagated plants produced from root and shoot cutting or rooted shoots from tissue culture. *In vitro* propagation technique provides healthy, homogeneous planting stock for a forestation and woody biomass production of *Paulownia*. Mass multiplication of *Paulownia elongata* through axillary shoots and nodal culture was reported (Bergmann and Moon, 1997) and also *in vitro* propagation of *Paulownia tomentosa* through shoot bud regeneration (Rao, Goh and Kumar, 1996).

Plant growth regulators play an essential role in micropropagation of plants, being commonly used in conditioning media for increasing multiplication rate and development of new plantlets. Among them, the auxins are the best studied inducers for somatic embryogenesis, an important pathway for plantlet regeneration. The synthetic auxin 2,4-D is frequently used to induce somatic embryogenesis in many plant species (Mordhorst *et al.*, 1998; Zimmerman 1993). Beside auxins, gibberellin and cytokinin may increase the multiplication rate in some plants. Thus, for *Paulownia tomentosa* and *fortunei* species, a better growth rate was observed when the culture medium was prepared with 6-benzylaminopurine (BAP) and gibberellic acid (GA3) (Danci *et al.*, 2015). Our previous work (Crisan *et al.*, 2014) reveal the auxin-like effect of monoethanolamine salt of 4-aminobenzoic acid on *Arabidopsis thaliana*, suggesting that alkanolamine salts of substituted benzoic acids could be potential candidates for new plant growth regulators.

Taking into account the present and future prospects of *Paulownia* species, and the scarcity of researches on *in vitro* micropropagation of *Paulownia shan tong*, this study aims to investigate, for the first time, the effect of different alkanolamine salts of 4-nitrobenzoic acid as plant growth regulators on the callus induction and plant regeneration in *Paulownia shan tong*.

MATERIALS AND METHODS

The new compound, methylethanolamine 4-nitrobenzoate (4-NO₂BA MMEA), diethanolamine 4-nitrobenzoate (4-NO₂BA DEA) and triethanolamine 4-nitrobenzoate (4-NO₂BA TEA), were obtained by the Institute of Chemistry Timisoara of Romanian Academy, via proton

exchange reaction, from 1:1 molar amounts of 4-nitrobenzoic acid and different alkanolamine, in acetone solvent. These compounds are based on biologically active components with low toxicity (Chicu *et al.*, 2008). Alkanolamines are chosen as cations, because are less toxic comparatively with aliphatic amines and heterocyclic amine (Chicu, Herrmann and Berking, 2000), are cheap, commercially available and environmentally tolerable, some of them naturally occurring.

The internodes excised from healthy plant of *Paulownia shan tong*, collected from a culture of monitored fields were used as explants. They were cleaned in more steps: the material was washed in soapy water, rinsed with tap water, washed in chlorinated water and then the process was passed in sterile area, under laminar air flow hood conditions. The material was sterilized in ethanol in a concentration of 70% for 1 minute and 2% HgCl₂ for two minutes followed by five washes with sterile distilled water. Murashige and Skoog medium (MS) (Murashige and Skoog, 1962) supplemented with 3% (Weight/Volume) sucrose and 0.6% (w/v) agar was used as culture medium for the experiments. For micropropagation various plant growth regulators with different concentrations were studied, singly and in combinations, and the best response was obtained by a combination of BAP and GA3. After obtaining a stable culture, the MS medium was supplemented with the new synthesized compounds (NO₂BA MMEA, NO₂BA DEA and NO₂BA TEA) at two different concentrations each one (C1 and C2), 0.5mg/L and 1 mg/L and callus induction and plant regeneration were determined. The experimental variants were: MS0 – the control variant; H1 – MS with 4-NO₂BA MMEA; H2 – MS with 4-NO₂BA DEA; H3 – MS with 4-NO₂BA TEA and 2,4D – MS with 2,4-dichlorophenoxyacetic acid. All the cultures were kept in the growth room at 24°C under a 16/8 h light /dark regime. After four weeks, sprout length, number of internodes, number of leaves, number of ramifications and callus dimensions were measured, for each characteristic a number of 11 plants were measured for each cultivated variant (H1 - C1, C2; H2 – C1, C2; H3 – C1, C2; 2,4D – C1,C2; MS0). All the experiments were repeated three times. The average ($\bar{x}$) and standard deviation (SD) program were used to quantify the effect of different treatment.

RESULTS AND DISCUSSION

In the present study, the effects of different alkanolamine salts of 4-nitrobenzoic acid on *in vitro* callus induction and plant regeneration in *Paulownia shan tong* were evaluated by employing internodes as explants. Nodal parts are considered one of the most suitable explants for *in vitro* improvements because these explants would preserve the genetic identity of the parent genotype. Data show that the induction of callus, differentiation and organogenesis are accomplished by differential application of compounds such as NO₂BA MMEA, NO₂BA DEA, NO₂BA TEA and 2,4-D in the culture medium. Thus, the plantlets cultivated on MS with new alkanolamine salts exhibited a better morphogenic response for all compounds in comparison with the control (MS0), inducing the plant regeneration, callusing has not been observed at those cultivated variants. The sprout length, number of internodes, number of leaves and number of ramifications were the examined parties of the plants, for each characteristic a number of 11 plants were measured for each cultivated variant and the results are presented in Table 1. Although 2,4-D is a synthetic auxin, its role in inducing organogenesis and plant regeneration was not observed in our experiments. Efficacy of phytohormone 2,4-D has been reported for callus induction in *Paulownia shan tong*. Callus growth was not prominently increased and did not show significant variations (C1 - 1.075±0.417; C2 - 1.04±0.21) along with the change of concentration.

The morphogenic response of the explants to alkanolamine salts of 4-nitrobenzoic acid is related in Figure 1. Similar values for sprout length, better than control were observed in all cultivation variants with exogenous supply of salts, at 0.5 mg/L concentration. But the maximum sprout length was noted for H1, at 1 mg/L concentration (Figure 1a). Regarding the number of internodes, best results were observed in H1 and H2 variants at 1 mg/L concentration (Figure 1b). The maximum number of leaves was observed in H1 variant at 1 mg/L concentration (Figure 1c). The number of ramifications has the best results on H1 variant, 1 mg/L concentration and the minimum number of ramifications at 0.5 mg/L concentration (Figure 1c). Therefore, among the tested compounds, NO₂BA MMEA (1 mg/L) showed the best results for all analyzed parties.

In Figure 2 are represented the results in what regards callus induction, in this case there is a small difference between the two concentrations, better results were observed using 0.5 mg/L 2,4-D.

CONCLUSIONS

The present study concluded that culture medium MS supplemented with different alkanolamine salts (H1 – H3) exhibited better morphogenesis at all tried concentrations, in comparison with synthetic auxin 2,4-D. Controlling *in vitro* morphogenesis should play important role at the applied level in developing new *Paulownia* cultivars and reducing the cost of micropropagation. Our study introduces new biological active compounds with low toxicity, efficient in inducing plant regenera-

Tab. 1. Effects of NO₂BA MMEA, NO₂BA DEA, NO₂BA TEA and 2,4-D on callus induction and plant regeneration in *Paulownia shan tong*

Cultivation variants	Concentrations mg/L	A* (cm) x±SD	B* x±SD	C* x±SD	D* x±SD	E* (cm) x±SD
H1	0.5	2.15±0.431	3.5±0.848	7.45±2.192	1.1±0.282	
	1	2.31±0.433	5.25±0.777	9.85±2.192	2±0	
H2	0.5	2.14±0.968	4.06±1.329	7.22±3.146	1.47±0.226	
	1	2.01±0.777	4.81±2.191	8.16±3.62	1.65±0.558	
H3	0.5	2.1±0.494	4.34±1.103	8.37±2.828	1.46±0.049	
	1	1.85±0.445	4.38±1.844	6.68±1.322	1.43±0	
MS0		1.59±0.275	2.84±0.579	5.43±0.968	1.06±0	
2,4D	0.5					1.075±0.417
	1					1.04±0.21

*A – sprout length; B – no. of internodes; C – no. of leaves; D – no. of ramifications; E – callus dimensions.

Figure 1. Effects of NO_2BA MMEA, NO_2BA DEA, NO_2BA TEA in variants H1-H3 on induction of sprout length (a), numbers of internodes (b), number of leaves (c) and number of ramifications (d)

Figure 2. Effects of 2,4-D on callus induction

tion in *Paulownia shan tong*. Thus, H1-H3 culture variants present better results for growth of plant vegetable parties (sprout length, number of internodes, number of leaves and number of ramifications) in report to control. The best results for all analyzed parties were noted for H1 (NO₂BA MMEA - 1 mg/L). MS medium improved with 2,4-D showed an induced callus development, with a small difference between the two concentrations in what regards callus dimensions. Therefore, these experimental results could contribute to optimize the culture treatments regarding morphological quality, induction of organogenesis, callus and plant regeneration.

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