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ORIGINAL ARTICLE

Responses of Auxin Derivatives on Rooting and Sprouting Behavior of *Excoecaria agallocha* L. Stem Cuttings

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In our study, the auxin effect on rooting and sprouting behavior of stem cuttings of *Excoecaria agallocha* L. has been studied. Initially stem cuttings were pretreated to remove the phenol content of cutting and then the stem cuttings that were devoid from phenols were subjected to hormonal treatment with auxins including indole-3-butyric acid (IBA) 2000ppm, indole-3-pyruvic acid (IPA) 2000ppm, naphthalene acetic acid (NAA) 2000 ppm and IBA+NAA combination 2000 ppm. The root length, root number, rooting and sprouting percentage, number of leaves per cutting, leaf area and photosynthetic pigments were analyzed on 40, 50 and 60 days after planting. Besides this, peroxidase isoenzyme pattern of root and leaf was also analyzed. All the growth attributes have shown an increasing trend at all stages of growth with auxin treatments. Among the auxin treatments, IBA 2000ppm vastly enhances rooting and sprouting behaviour of blinding eye mangrove. The isoenzyme analysis for peroxidase clearly showed that peroxidase (POX) highly supported root initiation and root elongation processes in *Excoecaria agallocha*.

Key words: auxin derivatives, Excoecaria agallocha L., mangrove, peroxidase isoenzyme, stem cutting

Mangroves are one among the world's productive ecosystems, widely distributed in the intertidal zone of the tropical and subtropical regions of the globe (Tomlinson, 1986; Hogarth, 1999). Mangroves are generally treated as even-aged forests, primarily developing after an eruption or establishment of mudflats and such an uneven-aged mangrove forest is represented by the area covered (diameter distribution) by both trees and saplings (Saenger, 2002; Trettin *et al.*, 2016). Taxonomically, mangroves create a varied flowering plants (angiosperms) that exhibits a set of distinct physiological adaptations (Tomlinson, 1986); conserving the coastal line from natural calamities including cyclones, tidal thrust, and tsunamis, and also providing a wide variety of goods and services used by coastal people (Zhang *et al.*, 2006). The mangroves significance for coastal communities and humans has been well documented throughout the tropics (Sandilyan and Kathiresan, 2012).

The mangroves counteract encroachment by the seas by monitoring soil erosion and thereby stabilizing the sea shoreline. Mangrove habitat has been under serve destruction globally at alarming levels and reclamation (Kathiresan and Bingham, 2001). However, Mangrove ecosystems being unique habitats of the coastal wetland areas are facing intense pressure due to improper destruction by humans for various developmental purposes. Such levels of eradication and habitat fragmentation raise concerns about the conservation of mangrove diversity. The site of work done was pichavaram mangrove situated on the southeast coast of Tamil Nadu, India. The mangroves of pichavaram are threatened largely due to many natural and anthropogenic pressures. Grazing is the one reasons for the degradation of mangroves in the peripheral area of Pichavaram (Selvam *et al.*, 2004).

Excoecaria agallocha L. (Euphorbiaceae) known as "milk mangrove" grows in sandy soil or hard sandy mud near the terrestrial fringers of mangrove vegetation. All plant parts secrete white latex, which causes temporary blindness if enters in the eyes and hence, called as "blinding tree". It is traditionally used to healing sores and the smoke of its bark has property of cure leprosy (Ghani, 2003). Clinical trials carried out on this plant have shown its potentiality against anticancer, antibacterial anti-HIV, and other antiviral properties (Peter and Sivasothi, 1999). The vegetative reproduction of *E. agallocha* is necessary because, this species is threatened by human intervention i.e. encroachment upon the land for cultivation and shrimp culture, for the exploitation of timber, fuel, and fodder and other uses. Generally, *E. agallocha* reproduces through seeds. Sudden depletion of the growing stock, and post-dispersal predation on seeds by crabs, poor flowering and seed setting necessitate asexual method like vegetative propagation of such species for re-establishment in degraded forest areas (Das *et al.*, 1997a, b).

In *Excoecaria agallocha* adventitious root formation is extremely difficult. But it can be achieved with the help of phytohormones application. Among various plant growth substances, auxin plays main role in root formation (Davis *et al.*, 1986; de Klerk *et al.*, 1999). They promote root initials (Nordstrom *et al.*, 1991; Nag *et al.*, 2001) and affect the newly formed roots in the expressive phase of root development (Bellamine *et al.*, 1998). Further, auxin along with peroxidase plays a major role in root initiation and elongation. Several studies have suggested a central role of peroxidase on adventitious root formation. The role of peroxidase in growth can be clearly studied by isoenzyme pattern. Numerous papers have reported auxin-induced peroxidase isoenzyme changes during adventitious rooting (Chen *et al.*, 2002a).

Keeping all these in view the auxin derivatives, Indole-3-butyric acid (IBA), Napthylacetic acid (NAA) and Indole-3-pyruvic acid (IPA) have been selected for the present study. The present studies dealt with the action of auxins (IBA, NAA and IPA) on rooting and sprouting of the stem cuttings of *E. agallocha.* Moreover, the isoenzyme pattern of peroxidase in both leaf and root of *E. agallocha* was also studied.

MATERIALS AND METHODS

Plant materials and hormonal treatment

Healthy and uniform stem cuttings (10-15cm in long) *Excoecaria agallocha* were assembled from Pichavaram in Chidambaram, Cuddalore district, Tamilnadu, India.

Initially, these cuttings were pretreated with sodium carbonate and sodium tungstate for 5-10 mins and treated cuttings were washed two to three times with distilled water. The stem cutting that was devoid from phenol was dipped for 30mins in IBA 2000 ppm, IPA 2000 ppm, NAA 2000 ppm and IBA+NAA combination 2000 ppm. After hormonal treatment plants were transferred to a plastic tray containing coarse sand and soil in 1:1 ratio and tray was kept in the mist chamber at 32/26°C (maximum and minimum) temperature and relative humidity (RH) was between 60-75% during the experimental period. The observations on the number of cuttings rooted, number of roots and sprouts formed on each cutting and their length, biomass were recorded on 40, 50 and 60 (DAP) respectively, for each treatment.

Pigment composition

Chlorophyll was extracted from leaves and determined according to the protocol previously given by Arnon (1949). Xanthophyll contents were quantified by the protocol explained by Neogy *et al.* (2001). The results were expressed in mg g^{-1} fresh weight (FW).

Extraction for enzyme activity

Fresh plant tissue, one gram weighed was homogenized in 1ml of an ice-cold solution containing 100mM phosphate buffer (pH7.8), 1mM EDTA, 0.5% (v/v) Triton X–100. Later, the homogenate collected was centrifuged for 30mins at 18,000 rpm. The eluent was stored at -20˚C for analysis of peroxidase isoenzyme.

Isoenzyme analysis

Isoenzymes were separated using 7.5% separating and 5% stacking polyacrylamide native gels. Electrophoresis was performed at 4°C under non-denaturing conditions as described by Laemmli (1970). Following electrophoretic separation, the gel was stained for peroxidase isoenzymes. Subsequently, gel was incubated in the staining solution for few minutes to get clear bands. Once the clear bands were appeared, the gel was washed with sterile water and photographed immediately. The staining solution was made ready by dissolving 500mg benzidine in 0.5ml of ethanol and 5ml of acetic acid, and 95ml of distilled water were added to it. The contents were mixed thoroughly and filtered using filter paper. 250µl of hydrogen peroxide was added before staining.

Statistical analysis

Each treatment data was analyzed statistically by using SPSS software (version 16.0, SPSS Inc., USA), with at least four replicates. A mean value (n=4) was calculated and (±) represents standard deviation (SD) of four replicates.

RESULTS

The results showed that the application of IBA, IPA, NAA and IBA+NAA combination resulted in the best root and shoot growth in stem cutting. (Table 1 and 2) show significant differences with respect to percent rooting, root number, root length, percent sprouting, number of leaves and leaf area in auxin treatments at different concentrations.

The root length of *Excoecaria agallocha* increased with the age of the plant. The root length was increased significantly to a large extent than the control in all the treatments. IBA 2000ppm treatment highly enhanced the root length when compared to IPA 2000ppm; IBA+NAA 2000ppm combination and NAA 2000ppm treated plant. IBA 2000ppm treatment highly increased the number of roots also when compared to the other treatment and control. The highest rooting rate (80%) on 60th day was noted in the cuttings treated with IBA 2000ppm followed by IPA 2000ppm and other treatments. The results revealed a profound influence of auxin derivatives on increasing the rooting and sprouting of *E. agallocha* (Fig.1).

Different sprouting rates were obtained for the cuttings exposed with different auxins (Tab 2). The cuttings subjected to IBA at 2000ppm had a sprouting rate of (80%) which was significantly higher when compared with control and other treatments. IBA treated plants shows an increased number of leaves per plant followed by IPA, IBA+NAA, and NAA. The leaf area increased with the age in the control and treated plants. IBA 2000ppm treated plant highly increased the leaf area, relative to other treatments and control.

The biomass of root and leaves per cutting shows an increasing trend with the age of treated and control cuttings (Tab.3). Among different auxin treated plants, IBA significantly increased fresh weight and dry weight of both roots and leaves when compared with other

treatments and control.

Auxin treated plants typically appear dark greener and this has been correlated with an enhancement of the chlorophyll content in *Excoecaria agallocha*. Hormonal treatment increased the xanthophyll content to a larger extent than the control one. Among different auxin treatments, the IBA concentration has shown increased photosynthetic pigments in all the stages of growth (Fig.2). The hormonal treatment significantly enhanced the chlorophyll and xanthophyll contents in *E. agallocha*. Among different auxin treatments, IBA shows more increased content of photosynthetic pigments.

The isoenzyme pattern of peroxidase showed a single peroxidase band in leaves treated with IBA, IPA, NAA and IBA+NAA combination, exhibiting a high intensity of band in NAA treatment with relative mobility value (Rm) of 0.62. While in the root two peroxidase bands were found in all auxin treatments as well as in control, in contrast, the NAA treated root alone showed a significantly increased intensity of band with Rm value 0.57(Fig.3). In auxin treated *E. agallocha* stem cutting the isoenzyme pattern of peroxidase showed a single peroxidase band in leaves. Whereas root has shown two peroxidase bands in all auxin treatments and control (Fig.3).

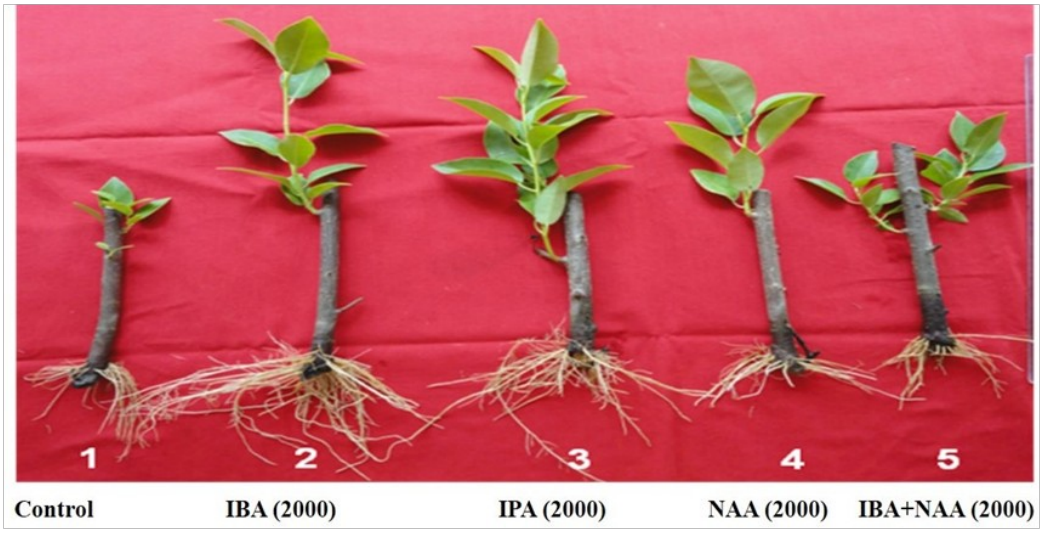

Figure 1. Effect of auxin on growth of *Excoecaria agallocha*

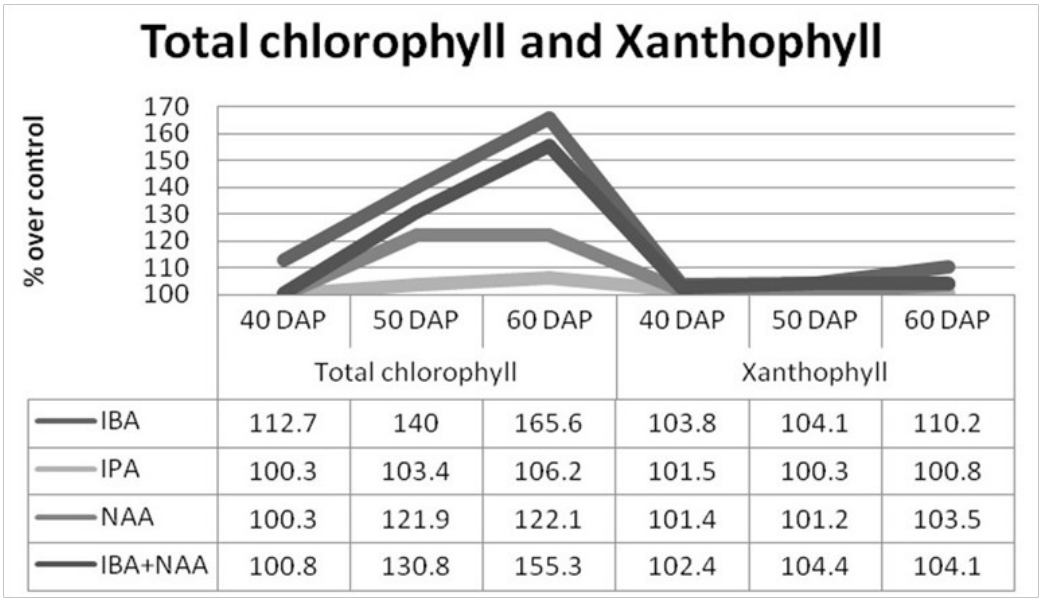

Figure 2. Effect of auxin on total chlorophyll and xanthophyll of *Excoecaria agallocha*

Table 1. Effect of auxin on the rooting of *E. Agallocha*

Treatment (ppm)	40th DAP			50th DAP			60th DAP		
	RL	RN	R%	RL	RN	R%	RL	RN	R%
Control	7.0 ± 0.189	18.0 ± 0.486	20.0	8.0 ± 0.240	17.0± 0.510	10.0	10.5 ± 0.357	20 ± 0.680	30.0
IBA (2000)	17.0 ± 0.459	36.0 ± 0.978	60.0	21.0 ± 0.630	46.0 ± 1.380	60.0	29.0 ± 0.298	56.0 ± 1.901	80.0
IPA (2000)	16.0 ± 0.432	28.0 ± 0.756	60.0	18.5 ± 0.555	28.0 ± 0840	50.0	25.0 ± 0.850	37.0 ± 1.258	60.0
NAA (2000)	10.0 ± 0.270	24.0 ± 0.648	30.0	11.5 ± 0.345	23.0 ± 0.690	20.0	18.0 ± 0.612	28.0 ± 0.952	40.0
IBA+NAA (20000)	8.5 ± 0.229	19.0 ± 0.513	40.0	12.0 ± 0.360	21.0 ± 0.630	30.0	20.0 ± 0.680	29.0 ± 0.986	50.0

RN - Rooting number, RL - Root length, R% - Rooting percentage, DAP-Days after planting

Table 2. Effect of auxin on the sprouting of *E. Agallocha*

Treatment (ppm)	40th DAP			50th DAP			60th DAP		
	Sprouting %	No. of leaves	Leaf area	Sprouting %	No. of leaves	Leaf area	Sprouting %	No. of leaves	Leaf area
Control	10.0	9.0 ± 0.243	2.1 ± 0.056	30.0	12.0 ± 0.360	2.6 ± 0.078	20.0	10.0 ± 0.340	3.0 ± 0.102
IBA (2000)	50.0	20.0 ± 0.540	9.3 ± 0.251	70.0	30.0 ± 0.900	12.3 ± 0.369	80.0	35.0 ± 1.190	14.9 ± 0.506
IPA (2000)	40.0	17.0 ± 0.459	6.9 ± 0.186	50.0	21.0 ± 0.630	7.3 ± 0.219	70.0	25.0 ± 0.850	9.8 ± 0.333
NAA (2000)	20.0	12.0 ± 0.324	5.8 ± 0.156	20.0	18.0 ± 0.540	7.0 ± 0.210	30.0	19.0 ± 0.646	8.3 ± 0.282
IBA+NAA (20000)	40.0	18.0 ± 0.486	7.3 ± 0.197	40.0	20.5 ± 0.615	86.0 ± 0.258	50.0	23.0 ± 0.782	10.5 ± 0.357

Table:3 Effect of auxin on the fresh and dry weight (leaf and root) of *E. agallocha* L.

Treatment (ppm)	40th DAP				50th DAP				60th DAP			
	Leaf		Root		Leaf		Root		Leaf		Root	
	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW
Control	1.05± 0.028	0.85± 0.022	2.99± 0.080	0.99± 0.026	2.02± 0.060	0.97± 0.029	2.80± 0.084	0.93± 0.027	2.10± 0.071	1.33± 0.043	2.82± 0.095	1.0± 0.034
IBA (2000)	3.37± 0.090	1.33± 0.036	4.04± 0.109	1.34± 0.036	5.96± 0.173	1.60± 0.048	6.60± 0.198	2.20± 0.066	6.45± 0.219	2.63± 0.089	6.79± 0.230	2.46± 0.083
1PA (2000)	3.10± 0.083	1.03± 0.027	5.20± 0.140	1.73± 0.046	5.19± 0.155	1.70± 0.051	5.63± 0.168	1.87± 0.056	5.46± 0.141	1.70± 0.056	5.95± 0.202	2.08± 0.070
NAA (2000)	2.35± 0.063	0.78± 0.021	5.16± 0.139	1.72± 0.036	4.09± 0.122	1.03± 0.030	5.10± 0.153	1.70± 0.051	4.85± 0.164	1.65± 0.056	5.26± 0.178	1.95± 0.066
1BA+NAA (2000)	2.49± 0.067	1.19± 0.032	4.93± 0.133	1.64± 0.044	4.91± 0.147	1.65± 0.049	5.25± 0.157	1.72± 0.051	5.19± 0.176	2.48± 0.084	5.41± 0.183	1.86± 0.063

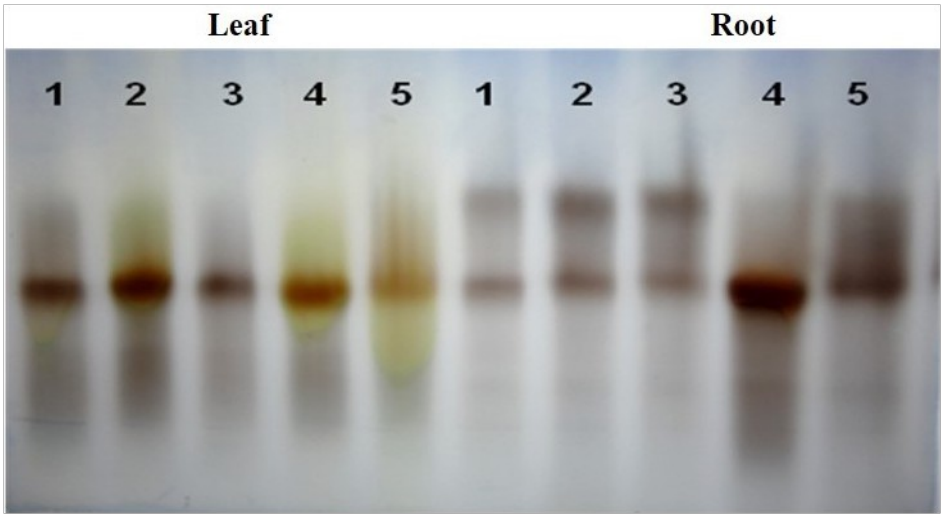

Figure 3. Effect of auxin on isoenzyme pattern of peroxidase. The lanes represent (1) Control (2) IBA (3) IPA (4) NAA and (5) IBA + NAA.

DISCUSSION

Auxin showed to regulate different aspects of plant growth performance by affecting numerous physiological processes like cell division, cell elongation, and cell differentiation (Woodward and Bartel, 2005). Although, exogenous auxin application induced rooting has previously been reported by many workers in other plant species (Davies, 1996; Blakely *et al.*, 1988). Exogenous application of IBA to the base of cuttings positively impacted rooting percentage, and other root related traits in our study. A similar result was obtained in *Cynometra iripa, Heritiera fomes* (Basak *et al.*, 1995). The rooting in the cutting of another mangrove *Avicennia alba* shows a similar type of response (Reddy *et al.*, 1994). In soybean hypocotyls auxin (IBA, IAA, and NAA) effectively promotes the rooting (Chou *et al.*, 2010). The differential root regenerating ability of auxins either alone or blend might depend on their respective capability to synthesize proteins necessary for the regeneration and elongation of roots (Ghosh, 1974; Basak *et al.*, 1999).

Our finding also suggests the pretreatment of IBA could be very effective in enhancing the sprouting of *Excoecaria agallocha.* Similar results were also observed in *Jatropha curcas* (Kochhar *et al.*, 2008). Different concentrations of auxin significant difference over control with regard to the number of leaves developed per cutting. The results reported by Chalapathi *et al.* (1997) in *Stevia* followed a similar trend. The possible reason for such an increase may be due to the activation of shoot growth by counteracting ABA levels of buds with probably increased the number of nodes that lead to the development of more number of leaves. Generally, auxin plays a significant role in the elongation of petiole, midrib and major lateral veins of the leaves. The leaf production was increased in auxin treated plants. Similar results were reported in *Cotinus coggygria* (Pacholczak *et al.*, 2005). The interesting observation is that the shoots are formed much earlier than roots in *E. agallocha*. This earlier Shoot formation is a result of reserved carbohydrates and shoots start producing auxin, which moves basipetally and accumulates in the lower portion of the cuttings. When the concentration reached a threshold, endogenous auxins at the extreme basal end start becoming metabolized and signal the process of root initiation.

The increased fresh and dry mass of root and leaf per cutting was recorded in the cuttings treated with IBA followed by other treatments. A similar effect has been observed in *Psoralea corylifolia* (Faisal and Anis, 2006), *Azadirachta indica* and *Pongamia pinnata* (Palanisamy *et al.* 1998).

IBA increased the vegetative growth and pigment concentration in maize (Kaya *et al.*, 2006). A similar result was also noticed in grapevine cuttings. In our study increased photosynthetic contents of leaves was

more in IBA treated cuttings, and this might have altered the synthesis and translocation of assimilates (Kaur *et al.*, 2002). Moreover, the exogenous IBA serves as a balanced storage form of IAA, since IBA can be converted back to IAA and thus can be gradually released when required by the plant (Normanly, 2010; Woodward and Bartel, 2005).

Regarding POX activity, similar result was noticed in *Jatropha curcas* cutting, where it shows two peroxidase bands at the phase of root elongation in auxin treatment (Kochhar *et al.*, 2008). In soybean hypocotyls, the activity of both anionic POX and cationic POX was significantly repressed by exogenous auxins at the inductive phase. The anionic POXs are most significantly elevated in IBA-treated tissues when compared with control (Chou *et al.*, 2010). The role of peroxidase in the rooting of poplar cuttings has been pointed out earlier by Gunes (2000). The present studies suggest that peroxidase helps in auxin catabolism and in prompting the root initiation process. A similar role can be done by IAA-oxidase also but IAA oxidase plays a part only for stimulating and initiating the roots/root primordial; peroxidase is involved in both the processes i.e., root initiation and elongation.

CONCLUSIONS

Our study revealed that auxin derivative IBA alone showed a profound influence in increasing rooting and sprouting of *E. agallocha* stem cuttings, relative to other treatments and control. Moreover, increasing number isoenzyme peroxidase bands formed in roots clearly showed the role of peroxidase during rooting initiation and elongation. Taken together, the study chosen might help in understanding mechanisms by which auxin derivatives promoting plant growth performance under *in vitro* or *in vivo* conditions.

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