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Research Article

Vegetative propagation of salt excreting mangrove species *Aegiceras corniculatum, Avicennia marina* and *Avicennia officinalis*

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Abstract

Mangroves are specially adopted plant species along the coastal region. The present work aimed to standardize the regenerative protocol for conservation through vegetative propagation. Salt excreting mangrove species *Aegiceras corniculatum, Avicennia marina* and *Avicennia officinalis* were selected and propagated through stem cuttings. Three different growth promoters like IAA, IBA and NAA individually and in combination with eighteen different concentrations were tried to standardize this regenerative protocol. From this study, the best rooting was observed from *Aegiceras corniculatum* on NAA 5000 mgl^{-1} and IBA 2500 mgl^{-1}.Whereas *Avicennia marina* responded on IBA 5000 mgl^{-1} and IAA 5000 mgl^{-1}. And NAA 5000 mgl^{-1} was found to be best for propagation of *Avicennia officinalis*. Hence the study concluded that this regenerative and alternative protocol may helps to conserve these medicinal and commercially important mangroves.

Keywords: Mangroves, vegetative propagation, Growth regulators, Stem cuttings.

Introduction

Mangrove forests are among the world's most productive ecosystems. However, they exist in the conditions of high salinity, extreme tides, heavy winds, high temperatures and muddy anaerobic soils (Kathiresan, 1991). The mangroves and their associated plants have various economic values and environmental functions (Kathiresan K, and Qasim, 2005). Though it has important role in environment, mangrove forests continue to disappear all over the world they are destroyed by man-made pressures and degraded by environmental stress factors. Some estimates put global loss rates at one million hectares per year, with mangroves in some regions in danger of complete collapse (Kathiresan and Bingham, 2001). Also the mangrove forests are degraded and threatened worldwide, due to anthropogenic events (Silva and Amarasinghe, 2010). It is an urgent need to develop fast and economically viable technique to conserve mangroves. The vegetative propagated plants are mostly identical with the parent plant (Hartmann and Kester, 1968). This technique would be ideal in supply of the planting materials around the year and the parent plant is usually reproduced exactly without genetic modifications. Hence the present study designed to standardize the protocol for conservation mangroves through vegetative propagation technique.

Materials and Methods

Leafy stem cuttings were cut from 3-5 years old plants of mangroves species *Aegiceras corniculatum*, *Avicennia marina* and *Avicennia officinalis*. The cuttings were made in early morning and evening from the healthy mangroves by using sharp secateur. They were wrapped in moist cloth and transported to the

experimentation site. The whole plant materials were washed with tap water for 2 min. From these washed materials 25-50 cm was selected away from the growing tip each, with the stem diameter of 0.5 to 2.0 cm, having 2 to 3 leaves. In which, a new fresh cut was again made adjacent to the nodal region. Then the materials were kept for 3 min in hormones of 18 different concentrations and combinations - IBA 5000 $mg1^{-1}$, IBA 2500, IBA1250, IAA 5000, IAA 2500, IAA 1250, NAA 5000, NAA 2500, NAA 1250, IBA 5000 + IAA 5000, IBA 5000 + IAA 2500, IBA 5000 + IAA 1250, NAA 5000 + IBA 2500, NAA 5000 + IBA 1250, NAA 5000 + IAA 2500, NAA 5000 + IAA 1250, IBA 5000 +NAA 1250, IBA 5000 + NAA 2500 $mg1^{-1}$. The control was treated similarly in water without hormone.

For each hormone treatment, 20 cuttings were maintained for each species. The rooted cuttings were planted in polythene bags of 15 x 7 cm size containing coarse sand. The poly bags were kept under a mist chamber in a row like fashion, at 30 ± 2°C with relative humidity of 70-80% for 1 to 3 months. During this incubation period, the water was sprinkled every day in to the chamber not directly to the soil. The cuttings were monitored continuously for their controlled environmental conditions. After 1-3 months, the rooted cuttings were removed from the mist chamber and kept under shady conditions in the nursery. During the time, the rooted cuttings were watered with gradually increasing salinity from 15 to 35 $g1^{-1}$ for 15 days. After hardening under shady conditions, the planting stocks were transplanted to the field, especially during the monsoon season, with a suitable space in between the seedlings.

Data collection

The number of rooted cuttings in each species was recorded and the percentages of rooted cuttings were calculated using the following formula:

$$\text{Percentage of rooted cuttings} \frac{\text{Number of cuttings rooted in each species}}{\text{Total number of cuttings in each species}} \times 100$$

The number of roots in each cutting was counted and also the length of the roots was measured. From these, the average number of roots, the average length of roots and the rooting vigor (= average number of roots x average length of roots) were calculated. The data were treated statistically for analyze the variance to find the significance between the hormone treatments using Two - way ANOVA.

Results

An experiment was conducted to find the optimal hormone concentration and combinations for successful propagation of *Aegiceras corniculatum*, *Avicennia marina* and *Avicennia officinalis* through cutting technique. In this experiment, the effect of 18 hormonal treatments was tested on rooting of three mangrove species. The best hormone concentrations for the mangroves were identified and the results were shown in Fig.1.

Effect of hormones on rooting in the cuttings of *Aegiceras comiculatum*

From the species *Aegiceras comiculatum*, the maximum number of roots 9.50 was obtained from NAA 5000 + IBA 2500 $mg1^{-1}$ combination. And the best root length 2.80 cm observed from with NAA 5000 + IBA 1250 mg 1^{-1}. The rooting vigor 23.80 cm was found in NAA 5000 + IBA 1250 mg 1^{-1}, this effect was about 8-fold higher than the treatment with NAA 2500 mg 1^{-1}.There was no root formation observed in the control, the results are shown in Table 1.

Effect of hormones on rooting in the cuttings of *Avicennia marina*

Similarly number of root, length and rooting vigor were studied for the species *Avicennia marina*. From this species the higher number of roots 6.00 was recorded from the concentration and combination of IBA 5000 + IAA 5000 $mg1^{-1}$. The average length of roots 13.08 cm per cutting was observed with NAA 1250 mg 1^{-1} .The rooting vigor 27.77 cm was recorded in NAA 5000 + IBA 1250 mg 1^{-1}. This effect was about 5-fold higher than in the treatment with IAA 2500 mg 1^{-1}.There was no root formation observed in the control, the results are shown in Table 2.

Table 1. Hormone-induced effects on rooting performance in cuttings of *Aegiceras corniculatum*

No.	Treatment (mgl^{-1})	No. of roots (Mean±SE)	Length of roots (cm) (Mean ± SE)	Rooting vigour (cm)
1	IBA – 5000	6.50 ± 0.75	2.40 ± 0.04	15.99
2	IBA – 2500	5.00 ± 0.50	2.50 ± 0.60	12.70
3	IBA - 1250	3.00 ± 0.50	2.35 ± 0.37	7.05
4	IAA – 5000	2.50 ± 0.56	2.40±0.20	5.60
5	IAA – 2500	2.00 ± 0.36	1.80± 0.25	3.60
6	IAA – 1250	1.75 ± 0.42	2.13 ± 0.10	3.73
7	NAA – 5000	2.50 ± 0.25	2.24 ± 0.29	5.60
8	NAA – 2500	2.00 ± 0.00	1.45 ± 0.19	2.90
9	NAA – 1250	2.00± 0.50	1.58 ± 0.19	3.16
10	IBA 5000 + IAA 5000	6.75 ± 1.56	2.09 ± 0.20	14.11
11	IBA 5000 + IAA 2500	5.25 ± 1.19	2.03 ± 0.20	10.66
12	IBA 5000 + IAA 1250	5.50 ± 1.25	2.40 ± 0.11	13.20
13	NAA 5000 + IBA 2500	**9.50± 0.25**	2.22 ± 0.50	21.09
14	NAA 5000 +IBA 1250	8.50 ± 0.25	**2.80 ± 0.49**	**23.80**
15	NAA 5000 + IAA 2500	8.00± 0.50	2.55 ± 0.58	20.40
16	NAA 5000 + IAA 1250	5.75 ± 0.60	2.35 ± 0.06	13.51
17	IBA 5000 +NAA 1250	9.00± 0.00	2.45 ± 0.46	22.05
18	IBA 5000 +NAA 2500	5.25± 0.79	2.30 ± 0.07	12.08
19	Control	-	-	-

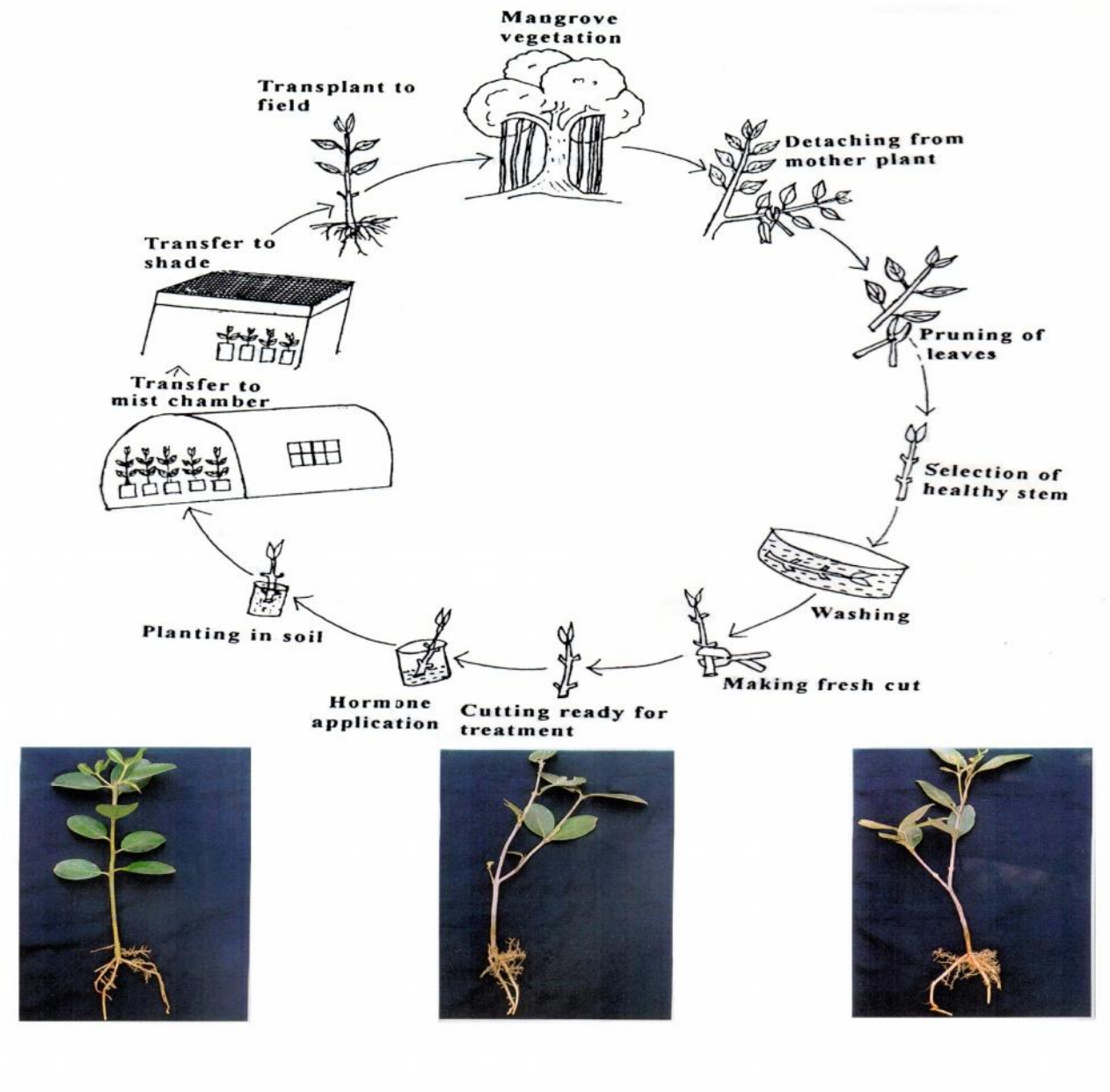
-No rooting; values are significant between hormonal treatments at 1% level, except no. of roots; Bold values are the maximum values.

Table 2. Hormone-induced effects on rooting performance in cuttings of *Avicennia marina*

No.	Treatment (mgl^{-1})	No. of roots (Mean ± SE)	Length of roots (cm) (Mean ± SE)	Rooting vigour (cm)
1	IBA – 5000	4.50 ± 0.25	3.04 ± 0.11	13.68
2	IBA – 2500	1.50 ± 0.25	7.25 ± 0.11	10.88
3	IBA – 1250	1.33 ± 0.21	7.75 ± 1.65	10.31
4	IAA – 5000	1.33 ± 0.21	9.43 ± 1.45	12.54
5	IAA – 2500	1.50 ± 0.25	3.43 ± 0.54	5.15
6	IAA – 1250	1.50 ± 0.03	11.05 ± 0.18	16.58
7	NAA – 5000	2.00±0.41	10.97± 1.08	21.94
8	NAA – 2500	3.50 ± 0.25	4.45 ± 0.66	15.58
9	NAA – 1250	1.50 ± 0.25	**13.08 ± 1.12**	19.62
10	IBA 5000 + IAA 5000	**6.00 ± 0.50**	2.92 ± 0.00	17.52
11	IBA 5000 + IAA 2500	3.33 ± 0.24	6.49 ± 1.39	21.61
12	IBA 5000 + IAA 1250	3.50 ± 0.25	3.83 ± 0.35	13.37
13	NAA 5000 +IBA 2500	2.00 ± 0.00	6.50+ 0.08	13.00
14	NAA 5000 +IBA 1250	3.33 ± 0.47	8.34 ± 1.71	27.77
15	NAA 5000 +IAA 2500	1.67 ± 0.24	10.54± 1.27	17.60
16	NAA 5000 +IAA 1250	1.50 ± 0.25	12.75± 0.13	19.13
17	IBA 5000 + NAA 1250	1.50 ± 0.25	11.52± 0.46	17.28
18	IBA 5000 + NAA 2500	3.33± 0.24	4.45± 0.66	14.81
19	Control	-	-	-

- No rooting; Values are significant between hormonal treatments at **1%** level, except no. of roots; Bold values are the maximum values.

Fig.1 Vegetative propagation of salt excreting mangrove species *Aegiceras corniculatum, Avicennia marina* and *Avicennia officinalis* through stem cutting.



Aegiceras corniculatum, ***Avicennia officinalis*** ***Avicennia marina***

Effect of hormones on rooting in the cuttings of *Avicennia officinalis*

In this investigation NAA 5000 mg 1^{-1} was found to be the best for rooting and in which the maximum number of roots 4.67 observed in *Avicennia officinalis*. The length of root 12.00 cm was recorded in the treatment with NAA 5000 + IAA 1250 mg l^{-1}. And the rooting vigor 33.65 cm was recorded in NAA 5000 + IAA 2500 mg1^{-1}. This effect was about 5-fold higher than in the treatment with NAA 1250 mg l^{-1}.There was no root formation observed in the control, the results are shown in Table 3.

Table 3. Hormone-induced effects on rooting performance in cuttings of *Avicennia officinalis*

No.	Treatment (mgl-i)	No. of roots (Mean ± SE)	Length of roots (cm) (Mean ± SE)	Rooting vigour (cm)
1	IBA – 5000	2.00 ± 0.00	6.63 ± 0.19	13.26
2	IBA – 2500	1.67 ± 0.24	5.68 ± 1.91	9.49
3	IBA -1250	2.00 ± 0.00	6.38 ± 0.07	12.76
4	IAA -5000	2.00 ± 0.00	6.38 ±0.07	12.76
5	IAA -2500	1.00 ± 0.00	8.25 ± 0.38	8.25
6	IAA -1250	1.00 ± 0.00	8.75 ± 0.13	8.75
7	**NAA – 5000**	**4.67 ± 0.63**	**5.16 ± 0.42**	**24.10**
8	NAA – 2500	2.00 ± 0.00	5.63 ± 0.19	11.26
9	NAA – 1250	1.00 ± 0.00	6.90 ± 0.05	6.90
10	IBA 5000 +IAA 5000	2.00 ± 0.00	7.13 ± 0.19	14.26
11	IBA 5000 +IAA 2500	2.50 ± 0.25	8.29 ± 0.48	20.73
12	IBA 5000 +IAA 1250	3.33 ± 0.24	5.72 ± 1.03	19.05
13	NAA 5000 +IBA 2500	1.67 ± 0.24	5.48 ± 1.08	9.15
14	NAA 5000 +IBA 1250	2.50 ± 0.25	7.92 ± 0.46	19.80
15	NAA 5000 +IAA 2500	3.67 ± 0.24	2.78 ± 1.39	33.65
16	NAA 5000 +IAA 1250	1.00 ± 0.00	12.00 ± 0.25	12.00
17	IBA 5000 + NAA 1250	1.00 ± 0.00	11.25 ± 0.38	11.25
18	IBA 5000 + NAA 2500	2.00 ± 0.00	5.16 ± 0.42	10.32
19	Control	-	-	-

- No rooting; Values are significant between hormonal treatments at 1 % level, except root length; Bold values are the maximum values.

Discussion

The efficient and regenerative protocol was developed for the salt excreting mangrove species *Aegiceras corniculatum, Avicennia marina* and *Avicennia officinalis* through cutting method. In mangroves, there were few reports on propagation through cuttings of stem and propagules. An attempt was taken on stem cuttings to produce roots in *Avicennia marina,* and *A. officinalis* from the Godavari mangroves (Kesava Reddy *et al*. 1994).Similarly Bask et al. (1995) standardized stem cutting techniques for five mangrove and associated species *Bruguiera parviflora, Cynometra iripa, Excoecaria agallocha, Heritiera fomes* and *Tliespesia populnea.* Successful rooting was observed from the stem cuttings of two mangrove species - *Heritiera fomes* and *H. littoralis* from the Mahanadi Delta (Das *et al*. 1997). Rao (1998) observed rooting of cuttings made from the viviparous hypocotyl of *Rhizophora mucronata*. Rooting of stem cuttings were succeeded for the species *Cerbera manghas* and *Merope angulate* (Thatoi *et al*. 2000). Eganathan et al. (2000a) recorded the rooting of cuttings for *Rhizophora apiculata, Rhizophora* hybrid and *R. mucronata*. Simultaneously rooting of stem cuttings for three more mangrove species - *Excoecaria agallocha , Heritiera fomes* and *Intsia bijuga* and the effect of season on rooting of cuttings, were reported by Eganathan et al. (2000b). Eganathan and Srinivasa Rao (2001) have given general protocols for rooting of stem cuttings in some mangrove species. Very recently an efficient protocol was established for *in vitro* clonal propagation for *Rhizophora annamalayana* Kathir. only endemic mangrove species to India (Kathiresan Kandasamy, Ravinder Singh Chinnappan, 2013).

Much more detailed study of the stem cutting technique for the phytohormonal treatments are very much necessary for conservation of mangrove species. Many more species of mangroves have not been attempted so far for the technique. Hence, in this work the vegetative propagation of mangroves have been attempted, for re-establishment in the denuded and potential areas through the stem-cutting technique, by-passing the very sensitive seedling phase.

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