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In vitro Propagation Protocol for the Conservation of *Sonneratia alba* Sm.

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Abstract

Sonneratia alba Sm. (apple mangrove) is a mangrove tree belonging to the family Lythraceae. The plant is known for its ecological, medicinal and economic significance. The present study describes a standardised protocol for the rapid clonal propagation of *Sonneratia alba* Sm. MS medium augmented with different concentrations of Benzyladenine (BA), Kinetin (KIN), 1-Naphthaleneacetic acid (NAA) and 2, 4-D, either alone or in combinations were used to study shoot multiplication, indirect organogenesis and rhizogenesis. Synthetic seed germination was also investigated. Maximum shoot multiplication was noticed on MS medium fortified with 4mg/l BA, 500 mg/l activated charcoal and 500 mg/l NaCl with maximum rhizogenesis. Light green callus was obtained from cultures containing 2 mg/l BA along with 2 mg/l NAA, 500 mg/l activated charcoal and 500mg/l NaCl. Synseed stored at 4°C exhibits maximum frequency of germination. Plantlets were nourished with Hoagland solution. The sprouted plantlets after acclimatisation transferred to pots with sterile sand, farmyard manure and 10g NaCl. This procedure can be used for the micropropagation and conservation of *Sonneratia alba* Sm.

Keywords: 1-Naphthaleneacetic acid, Rhizogenesis, Synseed, Acclimatisation

1. Introduction

Mangroves, the most productive ecosystem of the world are found in tropical and sub-tropical regions. They play a major role in the sustainable development of biodiversity. Mangroves are the groups with greater ecological significance that is by protecting the earth from natural calamities, pollution and soil erosion. Mangrove vegetation is also known for its economic importance. Importance of the mangrove ecosystem in fisheries has been reported early, with a high rate of primary production and they are able to sustain populations of fish, shell fish and wild life. They are good sources of medicines for treating several diseases. Mangrove ecosystem has declined worldwide at an alarming rate due to land reclamation and industrial discharges (Kathiresan and Bingham, 2001). To overcome this threat, proper conservation measures should be taken. In mangroves, seed viability is very poor and success rate of vegetative propagation is very low, so alternative methods should be adopted for the propagation of mangroves. Micropropagation method can act as a fruitful procedure for the conservation of mangrove ecosystem. But the literature reviews are very less as mangroves are recalcitrant for tissue culture study (Fukomoto *et al*., 2004). But one of the major consequences of tissue culture is after inoculation mangrove explant may turn brown/ black due to the presence of compounds like phenols and tannin. This issue can be rectified by adding activated charcoal in the culture medium.

Sonneratia alba Sm., belonging to the family Lythrceae is an important mangrove found near tidal creeks and sandy seashores. The plant helps in modifying soil for colonising other mangrove species and prevents soil erosion. The plant is used as medicine to treat cuts and bruises. The fruit is used to treat cough and intestinal parasites. *Soneratia alba*'s sepals shows significant anti-oxidant property (Kjer *et al*., 2009). *Soneratia alba* exhibits anti-microbial properties against certain microorganism (Saad *et al*., 2012).

Due to its ecological, economical and medicinal significance it is necessary to conserve *Sonneratia alba* Sm. Since it has a low seed viability, *in vitro* propagation studies is urgently required for the rapid multiplication of *Sonneratia alba* Sm. The present study aims to establish a standardised *in vitro* propagation protocol for *Sonneratia alba* through shoot multiplication, organogenesis and synthetic seed production.

2. Materials and Methods

Sonneratia alba Sm. collected from Asramam, Kollam and was grown for 6 months at green house of F.M.N. College, Kollam. 10g salt/ pot were added for the better growth of mother plant. Explants were collected from healthy grown 6 month old plants (Fig.1A). Shoot tips, nodal segments, internodal segments and leaves were selected as explants for inoculation. The healthy explants from the mother plant were washed in running tap water for 10-15 minutes and treated with 10% labolene in a conical flask for 20 minutes. The conical flask was kept under running tap water for 40 minutes. The explants were treated with 0.1% mercuric chloride solution inside the laminar air flow cabinet. Then it was washed with autoclaved double distilled water for 3 minutes. The shoot tip, nodal segments, internode segments and leaf segments were excised from the surface sterilized plantlets and used for inoculation.

The explants were then inoculated to MS medium (Murashiege and Skoog, 1962) with different concentrations of plant growth regulators (PGR's) such as Benzyladenine (BA), Kinetin (KIN), 1-Naphtha-

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leneacetic acid (NAA) and 2,4-D to induce shoot multiplication, indirect organogenesis and rhizogenesis (Fig.1 B-C). Activated charcoal and NaCl were added on to MS medium to absorb the exudates and to give a natural environment for the growth of explants. Sub-culturing was done in every 20 days.

In vitro buds after grown upto a size of 0.5cm in MS medium is dipped in alginate and then transferred to 2.5% w/v solution of $CaCl_2$. This results in formation of single bud calcium alginate beads. The beads are agitated in solution on shaker for about 30 min. After pouring off the solution beads were washed in double distilled water. Synthetic seeds were stored in sealed sterile petri dishes. Rooted *in vitro* plantlets were transferred to sugar free MS medium and then to autoclaved vermiculite in plastic cups. Plantlets were nourished with Hoagland's solution. Sprouted plantlets were transferred to pots containing sterile sand, farm yard manure, 10g NaCl, and watered regularly.

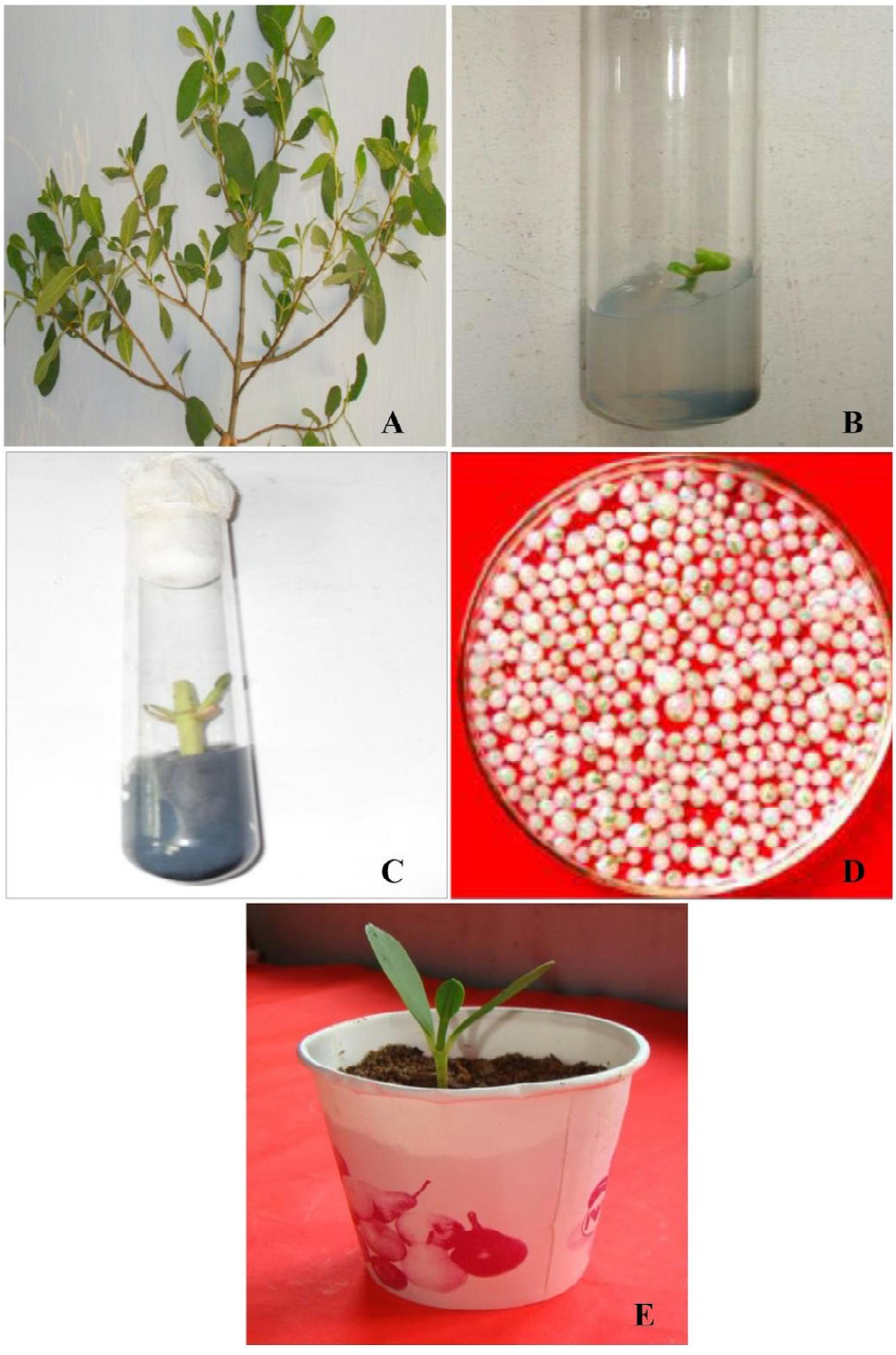

Fig. 1. (A) twig of *Sonneratia alba*, (B-C) establishment of in vitro culture, (D) encapsulated buds, (E) hardened *in vitro* cultured plantlet of *Sonneratia alba*

3. Results and Discussion

The present investigation, established a protocol for rapid clonal propagation of *Sonneratia alba*. Observations were taken after 15 days of inoculation. The synthetic cytokinin BA was highly effective for the microporpagation of *Sonneratia alba* (Table 1). The cytokinin BA is routinely added to tissue culture media to stimulate shoot proliferation (Thomas and Blakesley, 1987). The cultures containing BA (4mg/l) along with 500mg/l activated charcoal and 500mg/l NaCl shows best result. In *Sonneratia alba* cultures in MS medium supplemented with 4mg/l BA, 500mg/l activated charcoal and 500mg/l NaCl induced rhizogenesis along with shoot bud formation. Vartak.V and Shindikar M, (2008) also observed similar type of rhizogenesis in *Bruguiera cylindrica*. Activated charcoal was used to remove secondary metabolite exudation from the medium. The exudation imparts brownish colour to the medium, this can be avoided by the addition of activated charcoal along with the media.

In *Sonneratia alba* internodal segments cultured on 2mg/l 2, 4-D, 500mg/l AC and 500mg/l NaCl produced brownish callus. Light green coloured callus was obtained from nodal segments cultured on MS medium supplemented with 2mg/l BA along with 2mg/l NAA, 500mg/l activated charcoal and 500mg/l NaCl. The green colouration is an indication of shoot organogenesis. The BA along with NAA, showed more callusing than 2, 4-D. Blackening of the callus was noticed after 45 days of culture. This may be due to oxidation of phenolic compounds.

Synseeds were produced by encapsulating *in vitro* buds in calcium alginate beads (Fig. 1D). Seeds were germinated in MS basal medium and nutrient free agar gels. They retained germination capacity for more than 3 months when stored at 4°C. After rooting, plantlets were hardened in sterile soil, supplemented with salt and 1/10th strong Hoagland's solution (Fig. 1E).

The conservation of mangroves is an imperative need to prevent further deterioration. Conventional propagation methods possess problems in mangroves due to its low seed viability, high pollen sterility and poor vegetative propagation. If conventional breeding programmes are supplemented and augmented with unconventional methodology like tissue culture, great success in mangrove propagation may be achieved (Kathiresan and Ravikumar, 1997). The present study describes a reproducible method for the micropropagation of *Sonerratia alba* Sm. and by providing more attention to these area help in restoration of mangrove vegetation in the world.

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Table 1. Effect of different hormones on shoot multiplication, indirect organogenesis & rhizogenesis

Hormonal Concentration (Mg/L)				Activated Charcoal (Mg/L)	NaCl (Mg/L)	Explant	No.of Shoot (Mean±SE)	No.of Root (Mean±SE)	Intensity of Callus	Colour of Callus
2,4-D	BA	KIN	NAA							
1.5	-	-	-	500	500	Node	-	-	++	Light white
2	-	-	-	500	500	Internode	-	-	++	Brown
-	1	-	-	-	-	Node	-	-	-	-
-	2	-	-	-	-	Node	1±0.3162	-	-	-
-	3	-	-	-	-	Node	1±0.3984	-	-	-
-	4	-	-	-	-	Node	2±0.398	-	-	-
-	5	-	-	-	-	Node	1±0.3731	-	-	-
-	2	2	-	-	-	Node	1±0.2828	-	-	-
-	3	3	-	-	-	Node	1±0.4240	-	-	-
-	4	4	-	-	-	Node	3±0.4471	-	-	-
-	5	5	-	-	-	Node	2±0.4123	-	-	-
-	2	2	-	500	-	Node	0	-	-	-
-	3	3	-	500	-	Node	3±0.316	-	-	-
-	4	4	-	500	-	Node	1±0.344	-	-	-
-	5	5	-	500	-	Node	1±0.4471	-	-	-
-	2	-	-	500	500	Shoot tip	1±0.398	-	-	-
-	3	-	-	500	500	Shoot tip	2±0.4680	-	-	-
-	4	-	-	500	500	Shoot tip	1±0.2828	-	-	-
-	3	-	-	500	500	Node	2±0.4245	2±0.447	-	-
-	4	-	-	500	500	Node	2±0.5477	4±0.1732	-	-
-	5	-	-	500	500	Node	2±0.4693	3±0.3744	-	-
-	2	-	2	500	500	Node	-	-	++	Light green

+, ++, +++ indicate increasing order of intensity of callusing

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