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Research Article
In -vitro callus induction from *Ceriops decandra*- A true mangrove viviparous

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Keywords

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Abstract

From this study a reproducible protocol was developed to induce callus from the mangrove species *Ceriops decandra*. The surface sterilized shoot explants were inoculated on MS medium fortified with different concentration and combination of growth regulators. The callus was initiated after 15 days of inoculation and the maximum Yellow colored compact callus biomass was obtained from 0.5mg/L of BAP and 2.5 mg/L of NAA after 23 days of inoculation. The moderate amounts of calli were observed from IAA and NAA and the minimum was obtained from IBA. Then the browning of callus biomass was gradually started from 27th day onwards. Due to the importance of its bioactive substances like tea compounds, this protocol was developed to enhance the secondary metabolites production of the important mangrove species *Ceriops decandra*.

Introduction

The secondary metabolites constitute a source of bioactive substances and presently scientific interest has increased due to the search for new drugs of plant origin. Metabolites, with some novel chemical structures which belong to a diversity of chemical classes have been characterized from mangroves and mangrove associates. These include both primary and secondary metabolites (Osbourne, 1996). Mangrove forests are among one of the world's most productive tropical ecosystems. They are endowed with rich and divers living resource that provides forestry and fishery products to large human population (Kathiresan, 2000). Mangroves are woody plants, usually found in inter-tidal areas between land and sea in tropical and sub-tropical latitudes in over 120 countries between 30° N and S latitude where they exist in conditions of high salinity, extreme tides, strong winds, high temperatures and muddy, anaerobic soils (Kathiresan and Bingham, 2001). Because of their environment, the mangroves are necessarily tolerant of high salt levels and have mechanisms to take up water, despite strong osmotic potentials. The mangroves and their associated plants have various economic values and environmental functions (Kathiresan and Qasim, 2005). Some also take up salts, but excrete them through specialized glands on the leaves. Others transfer salts into senescent leaves or store them in the bark or the wood. Still others simply become increasingly conservative in their water use as water salinity increases. Morphological specializations include profuse lateral roots that anchor the trees in the loose sediments, exposed aerial roots for gas exchange and viviparous water-dispersed propagules (Kathiresan *et al*., 2006; Kathiresan and Ravinkumar, 2010).

Number of mangrove plant secondary metabolites like gedunin, hydroquinone, xanthone, diterpenes, polyphenol etc., are currently used as anticancer agents in different cell line, however, not much study has been undertaken (Konishi *et al*., 1998; Konishi *et al*., 2000; Konoshima *et al*., 2001; Xu *et al*., 2005; Scheck *et al*., 2006; Uddin *et al*., 2007; Uddin *et al*., 2011).

Materials and Methods

Sterilization of glassware

The most important step in tissue culture techniques is sterilization. All the glassware such as beakers, conical flasks, measuring cylinders, Petridish and culture tubes were washed thoroughly with detergent (2% Teepol) in tap water and rinsed

with double distilled water twice and dried in hot air oven at 30°C. The distilled water and other accessories such as forceps, blade holder, etc., were autoclaved at 121°C for 15 minutes at 15 lb. After autoclaving, they were kept in hot air oven until use.

Stock solution and media preparation

The optimized MS medium was used for the present investigation. For the preparation of the basal MS medium, separate stock solution of macro nutrients, micro nutrients, iron supplements, vitamins and boric acid were prepared by dissolving required amount of chemicals in double distilled water and were stored at 4 ± 1°C in a refrigerator. Individual growth regulators such as BA, kinetin, NAA, IAA, IBA and 2,4-D were prepared and kept at 4 ± 1°C.

MS medium was prepared with all the stock solutions in appropriate proportions and the final volume was made up to required quantity by adding double distilled water, in which, sucrose 3% (W/V) was added to the medium as a source of carbon. Various concentrations and combinations of growth regulators were added to the medium before adjusting the pH to 5.8 using 1.0 N NaOH or 1.0N HCl and gelled with 0.8% agar and melting the agar in a boiling water bath. Then the media were distributed in the phyta jar and autoclaved at 121°C for 15 min at 15 lb. The autoclaved media were kept in inoculation room until use.

Surface sterilization

The young root explants of *Acanthus ilicifolius, Callophylum inophyllum, Excoecaria agallocha* were immersed in water immediately after collection. The root samples were washed with tap water to remove epiphytes and other unwanted external matters. And the explants were surface sterilized with different surface sterilizing agents. Therefore the explants were disinfected with a detergent solution (2% Teepol, Reckill and Colman, India) for 5 min. Then the explants were washed in 0.1% mercuric chloride for 1.5 min followed by 70% ethanol for 45 seconds.

Aseptic transfer of explants

Aseptic transfer of tissue was done in a laminar air-flow hood. The interior was swabbed with 95% ethanol before inoculation. The autoclaved equipment were flame sterilized 3 times before using them for tissue transfer using 95% ethanol taken in a coupling jar. After sterilization, all the explants were cut into small pieces (1.0 to 1.5 cm long) and were individually placed on MS medium supplemented with various concentration and combinations of cytokinins and auxins, 3.0% sucrose and 0.8% agar.

Results

This is the study mainly focused on to develop a protocol for enhance the secondary metabolites of *Ceriops decandra* through callus culture. In this study have tried to develop callus biomass from the shoot explants using different growth regulators alone (NAA, IAA and IBA) ranged from 0.5 mg/L – 5.0 mg/L and in combination BAP and NAA ranged from 0.5mg/L BAP and NAA ranged from 0.5mg/L to 3.0 mg/L. From this investigation the best callus biomass 950±0.00 mg fresh weight and 93 % response were obtained from BAP (0.5 mg/L) and NAA (2.5mg/L) combination. Followed by this the moderate amount 580±0.50 mg fresh callus biomass and 50% response were observed from the 5.0mg/L of IAA. Then the fresh weight 340±0.30 mg and 40% response were obtained from 4.0 mg/L of NAA. The minimum response 26% and lowest amount 180±0.50 of calli were observed from the growth regulator IBA 0.5mg/L the results were expressed in the Table 1 and Fig 1. Almost all the explants were responded on 15th days after inoculation. The maximum calli were observed from 23 after inoculation and the callus browning was observes from 27th day after inoculation. This is the study revealed that the BAP and NAA were found be the best combination and the best concentration was 0.5Mg/L BAP and 2.5 mg/L NAA.

Discussion

Plant tissue culture is an important tool not only for plant propagation and conservation but also it is used to enhance the plant bioactive compounds through cell culture and hairy root culture etc (Ravinder Singh and Kathiresan, 2013). This present study aimed to standardize the protocol for callus induction from the stem explant of *Ceriops decandra*. This is the report on callus induction from the mangrove species *Bruguiera sexangula* (Lour.) Poir in this study the callus biomass were initiated and achieved on MS medium fortified with amino acid, 2µM 2,4 dichlorophenoxy acetic acid and 2µM N-(2-chloro-4-pyridyl)-N-phenyl urea (Mimura *et al*., 1997; Vander Velde and Vander Velde, 2005) also this is the first tissue culture report on this species *Ceriops decandra*.

Table: 1. Effect of growth regulators on induction callus from *Ceriops decandra* in stem

MS medium (mg/l)	% of responses	Fresh weight of callus(mg)	Callus morphology
NAA			
0.5	47	450±0.20	Yellow Friable
1.0	30	250±0.70	Yellow Friable
2.0	25	190±0.80	Yellow Friable
3.0	21	230±0.40	Yellow Friable
4.0	40	340±0.30	Yellow Friable
5.0	16	090±0.00	Yellow Friable
IAA			
0.5	31	340±0.20	Yellow Friable
1.0	36	420±0.30	Yellow Friable
2.0	27	340±0.20	Yellow Friable
3.0	15	470±0.50	Yellow Friable
4.0	39	420±0.00	Yellow Friable
5.0	52	580±0.40	Yellow Friable
IBA			
0.5	10	30±0.10	Yellow Friable
1.0	9	50±0.40	Yellow Friable
2.0	13	140±0.50	Yellow Friable
3.0	19	120±0.00	Nodular Yellow
4.0	23	210±0.50	Yellow Friable
5.0	26	180±0.50	Yellow Friable
BAP+ NAA			
0.5 + 1.0	65	720±0.00	Yellow Friable
0.5 + 1.5	59	480±0.50	Yellow Compact
0.5 + 2.0	45	420±0.00	Yellow Compact
0.5 + 2.5	**93**	**950±0.00**	Yellow Compact
0.5 + 3.0	41	450±0.00	Yellow Compact

Data were expressed as fresh weight of callus, 50 explants were taken for each experiment, Each experiment was repeated five times.

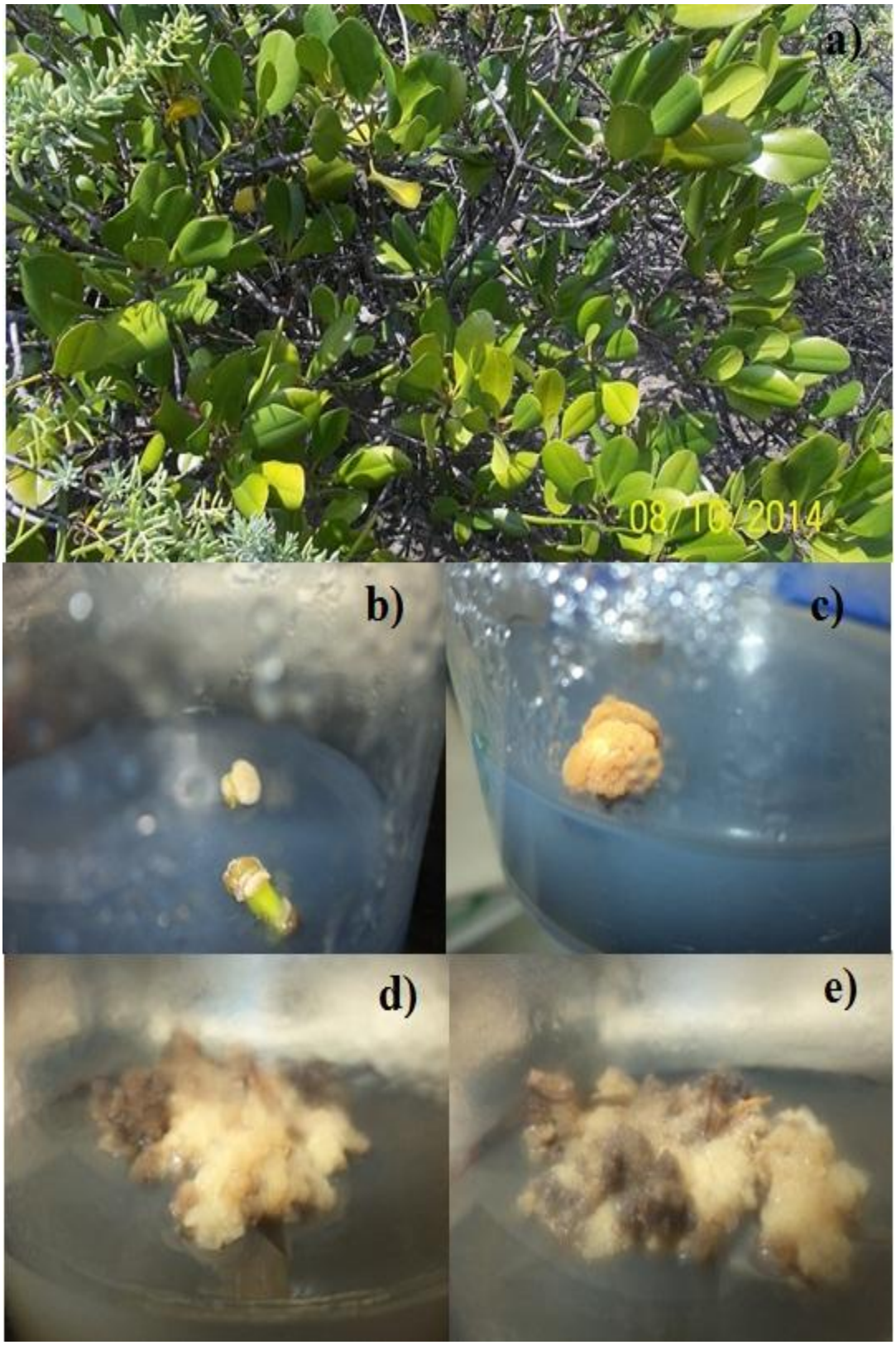

Figure 1. a) *Ceriops decandra***,** b) Calli initiated on 15th day, c) 23rd day Calli, d) 27th day Calli , e) Browning of calli after 27th day.

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