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### Vegetative propagation of *Bruguiera gymnorrhiza*: An effort to conserve the rare degrading black mangrove of Indian West Coast

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#### Abstract

Climatic fluctuations and other anthropogenic activities are apprehending West Coast mangroves. The present work focuses on the vegetative propagation of *Bruguiera gymnorrhiza* through the cut-propagule method. The *B. gymnorrhiza* is a species having timber value and can be grown in half-submerged conditions along the edges of waterways and ponds. Vegetative propagation by the cut-propagule method is an alternate and cost-effective mode of enhancing the production of *Bruguiera*. The potting mixture comprised on-site soil, sand, coir dust and compost mixed in equal amounts. The average root length, the number of roots, leaves, chlorophyll content and vigor was determined. The propagules were also utilised for the study and the cut-propagule method was employed, the growth hormone IBA was found to be efficient in the development of propagules. The shoot length was high in the propagule treated with 2000ppm of IBA ( $20 \pm 1.0$  cm). Also, the highest root length was recorded at 2000ppm NAA ( $8.7 \pm 0.435$ cm). Other hormones such as NAA and a combination of NAA & IBA didn't have much effect when compared with IBA alone. This study will provide insight into the conservation of *B. gymnorrhiza* on the west coast of Kerala through cost-efficient means and large-scale multiplication can be done to unlock the pharmaceutical importance of the plant.

**Keywords:** Vegetative propagation, Hormones, *Bruguiera*, Growth performance, Conservation ecology, Habitat restoration

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## 1. Introduction

Mangroves are the primary level barrier to preventing seawater from entering the mainland. It plays a major role in controlling the water flow during storm surges and climate-change-induced sea level rise. This natural phenomenon is believed to increase the destruction in coastal areas or low-lying areas (Woodruff et al., 2013). Mangroves are proven to be efficient green barriers in coastal areas and reduce the coastal in-surges' impact by stabilizing the soil run-off and dissipating the waves (Guannelet al., 2015). The water storage and water exchange in the mangrove forests are very high, making it an excellent limiting buffer (Montgomery et al., 2019).

In Kerala, the low-lying areas are inundated by constant flooding and the people living near the coasts are at a high stake. The black mangrove or Kuttikandal in Kerala is predominantly found in the three districts of Kollam, Alappuzha and Cochin Vidyasagaran and Madhusoodanan (2014). And, during our exploration to find this species, we discovered the same species in the Thrissur district. *B. gymnorrhiza* mainly grows in the landward region, can be propagated well along the coastline, and requires low levels of salinity for its growth. Several anthropogenic activities have tremendously contributed to the loss of *B. gymnorrhiza* species across central Kerala. Currently, there is a very thin line of mangrove forest along the coast of central Kerala, this is very disturbing as it creates an imbalance in nature. Central Kerala makes up the maximum count of mangroves throughout the state, where Kollam has a distribution of 15 species followed by Alappuzha (14 species) and Cochin (13 species). In Cochin alone, being the economic artery of the state, the mangrove forests are destroyed for the creation of the International Container Transshipment Terminal (ICTT) and LNG Petronet terminal in Puthuvype (Sreelakshmi et al., 2020).

Vegetative propagation of mangroves can be done to mass multiply the plant and reduce the cost of conservation. *B. gymnorrhiza* also carries commercial importance as it can be effectively used for timber, furniture making, folk medicine, etc. Governments around the world are now focusing on the conservation of mangroves Marois and Mitsch (2015). The day-to-day applications of mangroves are enormous. Extensive afforestation and conservation of mangrove habitats are quintessential to developing plants and maintaining the pristine wetland ecosystem. The cost efficiency of mangrove propagation through vegetative methods makes it more reliable, and more new shoots can be produced from a single part. This study

aims to develop a cost-effective method to multiply the vegetative propagules of *B. gymnorrhiza* through hormone treatment methods and produce adaptable species on the west coast of Kerala and evaluate the efficacy of the propagules and stem cuttings by comparing the vigour of the propagules and stem cuttings under different hormone treatments Kathiresan and Ravikumar, (1995).

## 2. Materials and methods

### 2.1 Sampling

The plant samples were collected from the Cochin (10°01'43" N 76°12'49.76" E) and Thrissur (10°14'59.85" N 76°12'12.66" E) districts of Kerala. The propagules and mature stem cuttings were taken for the experiment. The mature propagules were collected from well-grown trees. The mature and good propagules were found to be fleshy, and shining, with deep red colour collars.

### 2.2 Preparation of samples

The propagules were cleaned thoroughly and cut into 2-5cm pieces. 20% stock solution of phenol removal solution was prepared by mixing 20g of sodium tungstate and 20g of sodium carbonate which was made up to 80ml and finally to 100ml with distilled water. The propagules were thoroughly washed with Bavistin fungicide before hormone treatment.

### 2.3 Experimental design

A complete randomized study was applied to the experiments, and the PSG College of Arts & Science greenhouse facility was employed. Hormones such as IBA, NAA, and IBA+NAA were used in three concentrations for all three experiments: T0 (Control), T1 (IBA 2000ppm), T2 (IBA 2500ppm), T3 (IBA 3000ppm), T4 (NAA 2000ppm), T5 (NAA 2500ppm), T6 (NAA 3000ppm), T7 (IBA+NAA 2000ppm), T8 (IBA+NAA 2500ppm), and T9 (IBA+NAA 3000ppm). After hormonal treatment, the propagule cuttings were transferred into a pot. The potting mixture is composed of original on-site soil, sand 1:1 (w/w), coir dust, and compost. A cutting containing the IBA hormone at 2500ppm with clay: sand 1:1 (w/w) potting mixture was used as control. The plant growth/survivability was determined by calculating the survival percentage, average number of roots, root length, root dry weight, and shoot parameters like number of shoots, number of leaves and chlorophyll content were analysed after 60 days of establishment by carefully removing them from the potting media. The

greenhouse had a temperature of  $35^{\circ}\pm 2^{\circ}\text{C}$ . Watering was done twice a day (morning and evening) throughout the incubation period.

## 2.4 Data analysis

The data analysis was performed using OriginPro 8.5. The mean and standard error were calculated for all parameters. The vigor scale for both root and shoot were designed according to Palihakkara and Ekanayake (2021).

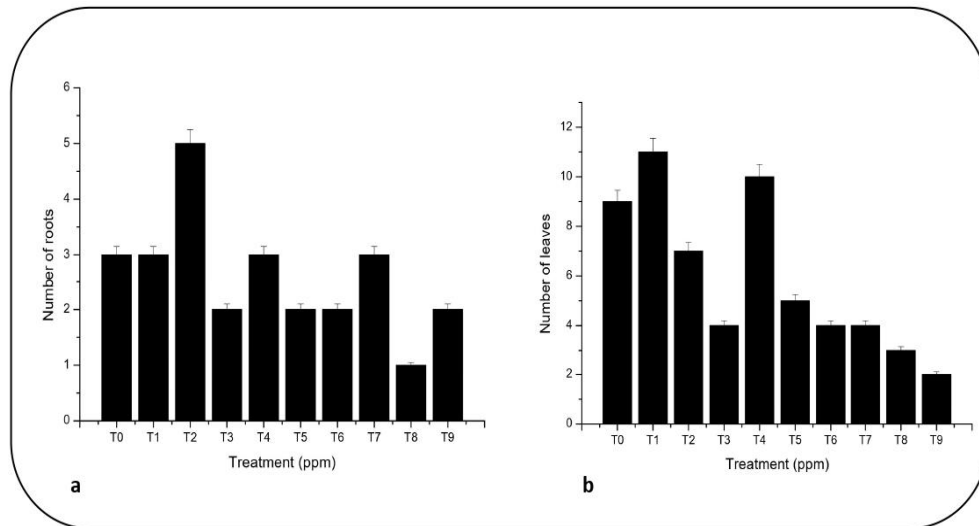
## 3. Results and Discussions

After 60 days the potting media was first tested for pH at a temperature of  $29.7^{\circ}\text{C}$  and was recorded at a range of 5.9-6.3. The best results of the propagule germination were shown in the pot with 2500ppm IBA. This was found to be optimum for the propagation of *B. gymnorrhiza* through propagules. The good root initiation as well as the number of roots arising out of the plant from the rest of the potting media was greater. The expression of plant propagule in IBA 2500ppm was more excellent than the control. The number of roots of 5, the average root length of  $4.9\pm 1.18$  cm (**Fig 1a**) and the number of leaves of 11 were recorded from the propagule treated with IBA at 2500ppm. Rooting was maximum in the propagule treated with IBA when compared with other hormones like NAA, and IBA+NAA. The length of shoots also greatly differed. The root and shoot vigor were also determined to understand the acquisition of nutrients by the plants. The shoot length was found to be high in propagules treated with IBA 2000ppm ( $20.0\pm 1.0$ cm) (**Fig 2d**) and more leaves were found in control pots ( $9\pm 0.45$ ) and plants with 2000ppm of IBA ( $11\pm 0.55$ ), 2500ppm of IBA ( $7\pm 0.35$ ), NAA 2000ppm ( $10\pm 0.5$ ) (**Fig 1b**). The chlorophyll content was also found to be highest in IBA at 2500ppm ( $3.01\text{mg.g}^{-1}$ ) (**Fig 2e**). The root vigor was high in plants treated with IBA 2500ppm. The dominant mangrove species on the west coast of Kerala are constantly affected by either salinity fluctuation or anthropogenic activities. Culturing mangroves through propagation techniques is a very strenuous task as its physiology should be understood completely. With mangroves having a wide range of salt resistance and flooding capacity each species behaves differently across the zones. The zonation of the mangroves is often influenced by factors like topography, substrate conditions salinity factors and the ability of the mangrove species to grow in various climatic conditions (Afri et al., 2021). Rooting and air-layering techniques are one of the major methods employed in vegetative propagation to produce new plants, although air-layering techniques are subjected to both biotic and abiotic stresses which are uncontrollable (Carlton and Moffler, 1978). So, we have

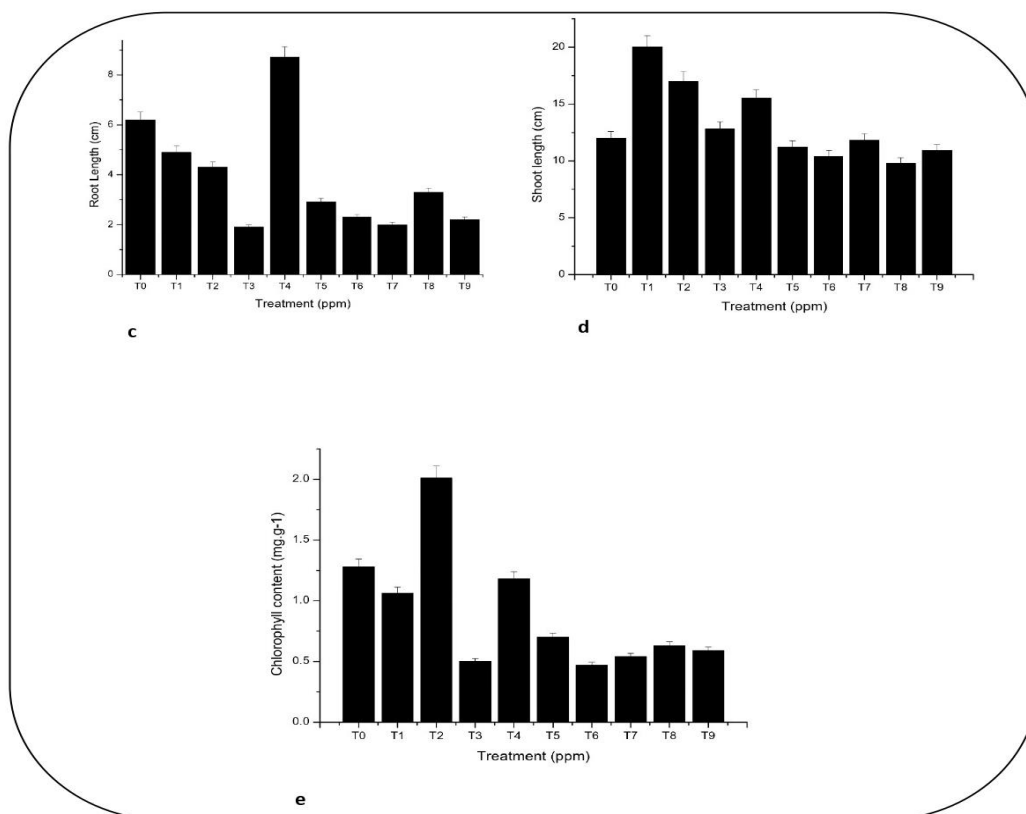
employed direct propagation through propagules by breaking their dormancy through growth hormones and IBA has proved to be the best hormone for this in *B. gymnorrhiza* when compared to that of NAA and combination of IBA & NAA. In *Excoecaria agallocha* a greater number of adventitious roots emerged from 2500ppm of IBA and 5000ppm of IBA (Basaket *et al.*, 2000). Rooting is mainly initiated by the carbohydrates stored as reserve foods (Rauter, 1983; Haissig, 1974). Breaking seed dormancy in the propagules of *B. gymnorrhiza* through mechanical methods is very critical and often leads to the deterioration of the propagules as it is delicate in nature, this species can be propagated by cut-propagule method (Kusmana *et al.*, 2018). In *A. officinalis* the highest rooting was observed in the 0.5g IBA (Govindan and Kathiresan, 2014). *B. gymnorrhiza* propagules had no significant effect on NPK fertilizer induction on the shoot and root growth while the *A. marina* had shown enhanced rooting and growth by IBA treatment (Silva and Amarasinghe, 2013). The *B. gymnorrhiza* survived well in a pH range of 5.8-6.3 and at this level, it does exhibit high-level phenol and other secondary metabolites (Allen and Duke, 2006).

#### 4. Conclusion

The cut-propagule method of vegetable propagation is a practical and effective technique for the conservation and restoration of *B. gymnorrhiza*, as it can produce healthy and vigorous seeds with high survival rates in the field. However, the growth performance of *B. gymnorrhiza* cut-propagule seeds can vary depending on the type of cut-propagule, and cannot be affected by the application of root-promoting hormones and NPK fertilizers. Other mangrove species may respond differently to vegetative growth by cut-propagation or cut-root methods, depending on their biology and ecology. Some species can benefit from hormones and fertilizers, while others do not. The vegetative spread of mangroves has advantages and disadvantages over sexual reproduction. It can overcome the limitation of the availability and germination of propagation, but can also reduce the genetic diversity and resistance of mangrove species. Therefore, a balance is needed between the two reproductive methods for the sustainable management of mangroves.



**Fig. 1 a)** Treatment with different concentrations of growth hormone and the number of roots with standard error; **b)** Treatment with different concentrations of growth hormone and the number of leaves with standard error.



**Fig. 2c)** Length of roots under different hormone concentrations; **d)** Shoot length under different hormone concentrations; **e)** Variation of chlorophyll content under different hormone concentrations.

## 5. Author Contribution

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by Sreeram S and A Arunprasad. The first draft of the manuscript was written by Sreeram S and V Sankara Vel, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## 6. Acknowledgement

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## 7. Conflict of interest

The authors declare no conflict of interest.

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