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Growth of *Avicennia marina* and *Ceriops decandra* seedlings inoculated with halophilic azotobacters

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Abstract: *Inoculation of azotobacter has significant positive effects on the growth characteristics and pigments in mangrove seedlings of Avicennia marina and Ceriops decandra. The bacterial inoculation significantly increased the root dry biomass at the maximum of 75.8% at 30 gl^{-1} salinity in Ceriops decandra. But in Avicennia marina, the shoot dry biomass was increased significantly at the maximum of 56.12% at 30 gl^{-1} salinity. In general, the Azotobacter beijerinkii improved the growth characteristics better in both species of mangroves preferably at higher salinity levels in A. marina and at a range of salinity in C. decandra. The results recommend this for raising vigorous seedlings under nursery conditions.*

Key words: *Avicennia marina, Azotobacters, Biofertilizers, Ceriops decandra, Mangroves, Salinity*
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Introduction

Coastal mangroves are tropical and sub-tropical trees being deforested at an alarming scale (Honculada-Primavera, 1993; Kathiresan and Bingham, 2001) and they generally self-regenerate after clear cutting. In some instances, regeneration is aided by the propagules sown by the forest personnel (Aksornkoae *et al.*, 1984) but in semi-arid tropics, following clear-cutting the mangroves hardly ever regenerate (Cintron *et al.,* 1978) due to very low levels of nitrogen (Holguin *et al*., 1992). This apparent nutrient deficiency in mangroves is believed to be overcome by artificial inoculation with plant growth promoting bacteria, as do many other plant species (Bashan and Holguin,1997; Bashan *et al*., 1998; Glick, 1995). Nitrogen fixation in mangrove sediments, in the rhizosphere and associated aerial roots may provide the nitrogen necessary for plant growth (Zuberer and Silver, 1978; Toledo *et al*., 1995). The bacterial species that facilitate nitrogen fixation in association with mangrove development are not well characterized.

It was previously observed that mangroves and the crop seedlings grew better when inoculated with diazotrophic filamentous *Azotobacter* and *Azospirillum.* This beneficial effect was attributed to nitrogen fixation and phytohormone production by these bacteria under various salinity regimes (Ravikumar *et al*., 2002a; Ravikumar *et al*., 2002b; Ravikumar *et al*., 2004b; Ravikumar *et al*., 2005). Although mangrove seedlings might be benefited by being inoculated with plant growth promoting bacteria, soil salinity is a major constraint in making soil unfit for plant growth. Soil salinity appears to have a negative effect on the *Rhizobium* on the host plant (Bekki *et al.*, 1987) and on their symbiotic relation (Rai *et al.*, 1985; Craig *et al.*, 1991). Several authors have discussed the inhibitory effect of salt stress on root-nodule activity in legumes (Yousef and Sprent, 1983; Rai and Prasad, 1983; Velagaleti and Marsh, 1989). Some of these studies have described interactions between salt stress and N_2 fixation without considering the effect of salt on plants. Hence, the present study has been initiated to assess the ability of three salt tolerant *Azotobacter* strains, isolated from the mangrove soil on the growth of mangrove seedlings of *Ceriops decandra* and *Avicennia officinalis* under different salinity regimes.

Materials and Methods

The bacterial species of *Azotobacter chroococcum, A. vinelandii* and *A. beijerinckii* isolated from the Pichavaram mangrove environment (Lat.11°27' N; Long.79°47'E) were obtained from the Department of Marine Microbiology, Manonmaniam Sundaranar University, Rajakkamangalam , Tamil Nadu, India were inoculated separately in to 200 ml of Winogradsky's broth and were cultured at 28 ±2°C for 3 days in a shaker. The liquid culture was centrifuged at 12,000 rpm for 15 min in a centrifuge (Hewlett-Packard, USA). The pellet was suspended in phosphate buffer (pH 7.0) and washed repeatedly with the buffer and was resuspended in the same buffer solution.

To study the influence of bacterial species on the growth of mangrove seedlings, 100 ml of suspended culture (10^8 cells ml^{-1}) of *A. chroococcum, A. beijerinckii* and *A. vinelandii* were mixed

Table - 1: Effect of *Azotobacter* inoculation on the growth characteristics of *Ceriops decandra* seedlings under optimum salinity regimes

Bacterial species	Number of primary roots seedling^{-1}	Average root length seedling^{-1}	Root dry biomass (g seedling^{-1})	Shoot length (cm seedling^{-1})	Shoot dry biomass (g seedling^{-1})	Content of chlorophyll-a (mg g^{-1}) dry tissue	Content of chlorophyll-b (mg g^{-1}) dry tissue	Content of carotenoid (mg g^{-1}) dry tissue	Content of total chlorophyll (mg g^{-1}) dry tissue	Leaf area (cm^2 seedling^{-1})
Azotobacter chroococcum	5	7.22	0.074	3.76	0.023	1.425	0.615	0.341	21.153	8.5
(at 10 gl^{-1})	(8.6)	(22.2)	(45.94)							
Azotobacter beijerinkii	5.5	5.37	0.124	6.58	0.035	1.115	0.523	0.264	16.369	15.5
(at 30 gl^{-1})			(75.8)	(25.08)						(13.42)
Azotobacter vinelandii	5.57	5.38	0.079	5.27	0.027	1.376	0.591	0.318	19.635	11.59
(at 35 gl^{-1})	(28.18)	(2.970)	(74.68)	(13.28)						(19.24)

Values in paranthesis shows percentage over uninoculated control at respective salinity levels. Values are average of triplicates. All standard errors values are less than 10% of mean values Values between the bacterial species are significant at 5% level

Table - 2: Effect of *Azotobacter* inoculation on the growth characteristics of *Avicennia marina* seedlings under optimum salinity regimes

Bacterial species	Number of primary roots seedling^{-1}	Average root length seedling^{-1}	Root dry biomass (g seedling^{-1})	Shoot length (cm seedling^{-1})	Shoot dry biomass (g seedling^{-1})	Content of chlorophyll-a (mg g^{-1}) dry tissue	Content of chlorophyll-b (mg g^{-1}) dry tissue	Content of carotenoid (mg g^{-1}) dry tissue	Content of total chlorophyll (mg g^{-1}) dry tissue	Leaf area (cm^2 seedling^{-1})
Azotobacter chroococcum	5.75	7.97	0.162	22.75	0.766	0.902	0.391	0.218	12.922	40.41
(at 10 gl^{-1})		(30.1)	(19.75)				(20.97)	(16.5)	(20.0)	(29.77)
Azotobacter beijerinkii	8.5	7.95	0.198	21.3	0.752	1.112	0.50	0.265	16.159	24.65
(at 30 gl^{-1})		(16.35)	(49.49)	(42.7)	(56.12)	(2.97)	(9.4)	(2.64)	(5.29)	
Azotobacter vinelandii	6.5	6.1	0.109	23.48	0.639	1.129	0.491	0.271	16.19	20.42
(at 35 gl^{-1})		(17.8)	(45.0)	(3.44)	(31.3)		(0.61)	(16.6)	(23.6)	(7.1)

Values in paranthesis shows percentage over uninoculated control at respective salinity levels. Values are average of triplicates. All standard errors values are less than 10% of mean values Values between the bacterial species are significant at 5% level separately with one kg of sterilized soil and were kept in sterilized polybags. Propagules of mangrove plant species *viz., Ceriops decandra* and *Avicennia officinalis* were planted into the soil and were irrigated with sterile water (100 ml per bag per kg of soil with different salinity regimes from 0, 10, 20, 30 and 35 gl^{-1} NaCl salinity). The propagules without bacterial treatments were maintained as control. Ten propagules were maintained for each treatment. After 45 days, the growth characteristics of mangrove seedlings such as average root length, root biomass, and shoot biomass and leaf area were recorded. Levels of total chlorophylls, was measured by following the method of Arnon (1949). Triplicates were maintained for each treatment. The data were statistically analyzed by following the two-way analysis of variance for significance at 99% and 95% confidence levels. The optimum salinity suitable for pronounced enhancement of growth characteristics were taken for table representation.

Results and Discussion

The bacterial inoculation increased the root growth in *Ceriops decandra* treated with *Azotobacter beijerinckii* by enhancing root dry biomass at a maximum of 75.8% at 30 gl^{-1} NaCl salinity and the number of primary roots by 28.18% at 35 gl^{-1} NaCl salinity. The azotobacter stimulated shoot length and leaf area at a maximum by 25.08% and 19.24% respectively under 30 and 35 gl^{-1} NaCl salinity. However, none of the bacterial species enhanced the level of pigments in *Ceriops decandra* at varied salinity levels (Table 1).

The inoculation of *Azotobacter* species enhanced the root and shoot parameters in *Avicennia marina*. For instance, the root dry biomass was enhanced by 49.49% when treated with *A. beijerinckii* at 30 gl^{-1} NaCl salinity whereas the content of total chlorophyll (23.6%), shoot dry biomass (56.12%) and shoot length (42.7%) were enhanced by *Azotobacter vinelandii* respectively at 30 and 35 gl^{-1} NaCl salinity levels (Table 2). Earlier findings indicated that, the inoculation of any one of the *Azotobacter* species enhanced the growth parameters either in *Rhizophora apiculata* or in *R. mucronata* (Ravikumar *et al*., 2002a). In comparison, the level of photosynthetic pigments were enhanced in *Avicennia officinalis* either in low or in high salinity levels by the inoculation of *Azotobacter* species than in *Ceriops decandra* (Ravikumar *et al.*, 2007). Previous studies reported increased level of chlorophyll-a content (62.1%) in *Rhizophora* seedlings inoculated with *A. chroococcum* (Ravikumar *et al*., 2004b). It was also evident that, the inoculation of *Azotobacter* species improved the phytoplankton growth in terms of content of chlorophyll in *Penaeus indicus* culture water (Ravikumar *et al*., 2004a). In general, the inoculation of *A. beijerinckii* enhanced the maximum number of root and shoot growth parameters in both *Ceriops decandra* and *Avicennia officinalis* as compared to the inoculation of *A. chroococcum* and *A. vinelandii*. It is concluded from the present study that, inoculation of *A. beijerinckii* preferably at 30 and 35 gl^{-1} salinity would be more beneficial for raising vigorous seedlings in nursery for *C. decandra* and *A. officinalis.*

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