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INFLUENCE OF NITROGEN SALTS ON GROWTH AND PHYSIOLOGICAL RESPONSES OF *RHIZOPHORA APICULATA* BLUME IN NON-AERATED WATER CULTURE

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ABSTRACT: Influence of two different forms of nitrogen on growth and physiological aspects of water-cultured seedlings of *Rhizophora apiculata* was studied. Of the two forms of nitrogen supplied to the growth medium, ammonium nitrogen was better than nitrate nitrogen by exhibiting increased dry matter production, shoot length, leaf area and also enhanced the contents of carotenoids, chlorophylls and their presence in photosystems and light harvesting protein complex.

KEY WORDS: Ammonium - mangrove - nitrate - photosynthesis - *Rhizophora apiculata*.

INTRODUCTION

Nitrogen is an important nutrient limiting the growth of halophytes in intertidal areas (Tyler, 1967; Stewart *et al.*, 1979). Application of nitrogenous chemicals has been reported to increase the biomass of marshy vegetation (Tyler, 1967; Valiela and Teal, 1974; Sullivan and Daiber, 1974; Bromme *et al.*, 1975; Naidoo, 1987). These studies imply the inadequate availability of specific forms of nitrogen to the plants for better assimilation and growth. The evidence reporting the specific forms of nitrogen utilised by the halophytes, is conflicting. Tyler (1967) showed increased growth response of seagrass meadows to ammonium chloride treatment, but not to sodium nitrate and considered that, these species may require ammonium as a source of nitrogen (N). But, Boto *et al.* (1985) contradicted that nitrate is the preferred source of N for the enhanced growth of *Avicennia* seedlings; and not the ammonium N. The present study has been made to find out the "Physiologically active" form of nitrogen for the growth in seedlings of *Rhizophora apiculata*, a predominant mangrove species in the study area.

MATERIALS AND METHODS

Propagules of *R. apiculata* Blume (26±2 cm in length) were collected from Pichavaram mangroves (11°27'N; 79°47'E) at southeast coast of India, during August 1992. The propagules were introduced into four litre plastic containers having Hoagland and Arnon (1950) nutrient solution with an optimal salinity of 15 g l^{-1}. Iron was supplied as ethylenediamine tetra acetic acid at 1 mg l^{-1}. The propagules were subjected to two different forms of N at the time of first exposure into nutrient solutions. Liquid culture was used since the plants absorb nutrients effectively from solution than soil (IPCS, 1992).

NITROGEN TREATMENT

Nitrogen was supplied as NH_4Cl or $NaNO_3$ at different concentrations of 0, 5, 10, 20, 30, 40, 50, 100, 150, 200 and 250 mg l^{-1}. NaCl content of treatments were maintained at a concentration of 15 g l^{-1} which was found optimal salinity for propagules of *R. apiculata* (Kathiresan and Thangam, 1990). Propagules were exposed to nitrogen treatments for eight days and control propagules had to rely entirely on internal nitrogen for growth. After the exposure, plants were reintroduced into nutrient medium devoid of any nitrogen. Nutrient solutions were replenished once in seven days. A completely randomised block of 22 treatments with five replicates each was maintained. Experimental set up was incubated at a temperature of 25±2°C with a light intensity of 330 μmol $m^{-2}s^{-1}$. After 90 days, the plants were analysed for dry matter accumulation, plant height, leaf area using Li-Cor leaf area meter 3100 (USA), tissue nitrate content (Cataldo *et al.*, 1975), leaf *in vivo* nitrate reductase activity (NRA) (Jaworski, 1971), NO_3 utilisation efficiency as the ratio between NRA and NO_3, chlorophylls (Arnon, 1949) and their fractions in photosystems I and II and light harvesting complex (LHC) (Krivosheeva *et al.*, 1991) and carotenoids of chloroplast (Ridley, 1977). The data are presented as mean and different treatments were statistically analysed and the least significant difference (LSD) at 5% level of probability is given.

RESULTS AND DISCUSSION

Both forms of nitrogen had significant effect on dry matter production. In general, shoot dry matter was greater than root in both the treatments. NH_4Cl increased the shoot dry matter by 133% and root by 68% at 100 mg l^{-1}. $NaNO_3$ enhanced the shoot dry matter by 198% and decreased the root dry matter by 25% at 100 mg l^{-1} (Fig.1). However, higher concentrations reduced the dry matter production whereas lower concentrations had no significant effect. The increase in dry matter production in optimal concentrations of both treatments reveals the efficiency of *R. apiculata* to assimilate both forms of nitrogen. However, ammonium-N was more efficiently utilised by this species than nitrate N by increasing the growth of both root and shoot systems.

Root length was recorded a maximum of 45% in NO_3 treatment at 100 mg l^{-1} and 27% in NH_4 treatment at 40 mg l^{-1}. The increased root length was not found associated with root dry matter accumulation which may be attributed to the lack of correlation between the growth and thickness of root. Shoot length was promoted by 78% in 20 mg l^{-1} treatment of NH_4 and 100% in NO_3 at 100 mg l^{-1}. Shoot length was significantly increased in all concentrations of N salts studied. Sullivan and Daiber (1974) also reported increased shoot growth to ammonium nitrate treatment. Total leaf area was increased to 176% in NO_3 treatment at 30 mg l^{-1} and 277% in NH_4 treatment at 20 mg l^{-1} (Fig.2). Similar promoting effect was reported in *B. gymnorhiza* (Naidoo, 1990). High level of nitrate reductase activity was reported in several halophytes (Stewart *et al.*, 1972; 1973; 1979). However, in the present study in both treatments, NRA was found to be insensitive to added nitrogen owing to decreased nitrogen uptake because of competitive inhibition of ammonium ions by sodium (Naidoo, 1987), chlorides, deficiency of O_2 in the rhizosphere and by metabolic

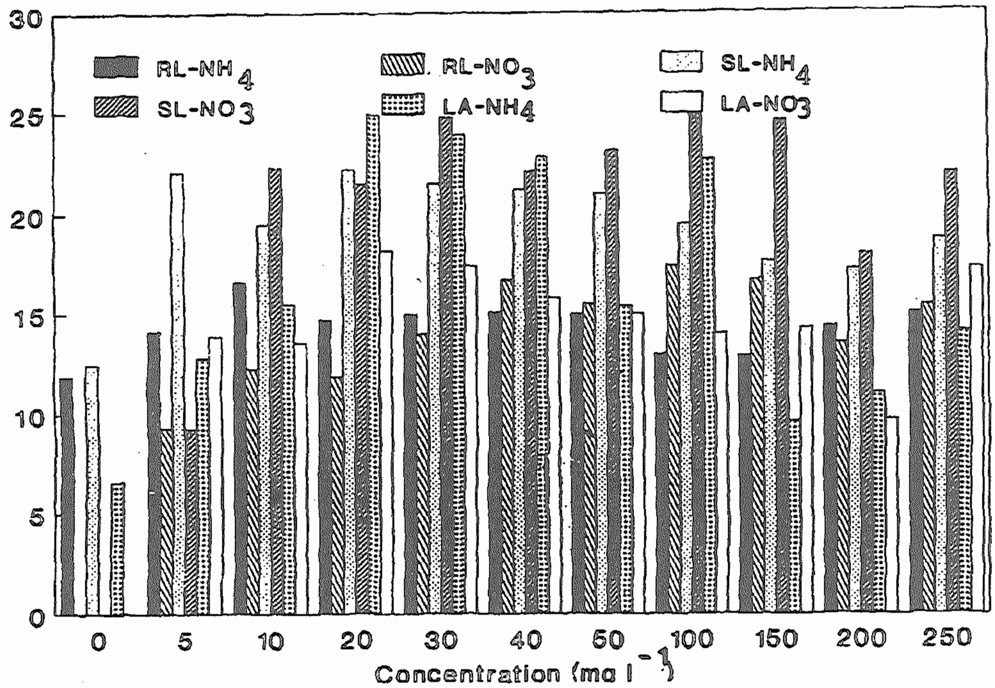

Fig.1. Root length (RL;cm) Shoot length (SL;cm) and Leaf area (LA;cm^2) as influenced by nitrogen treatments in seedlings of *R. apiculata*.

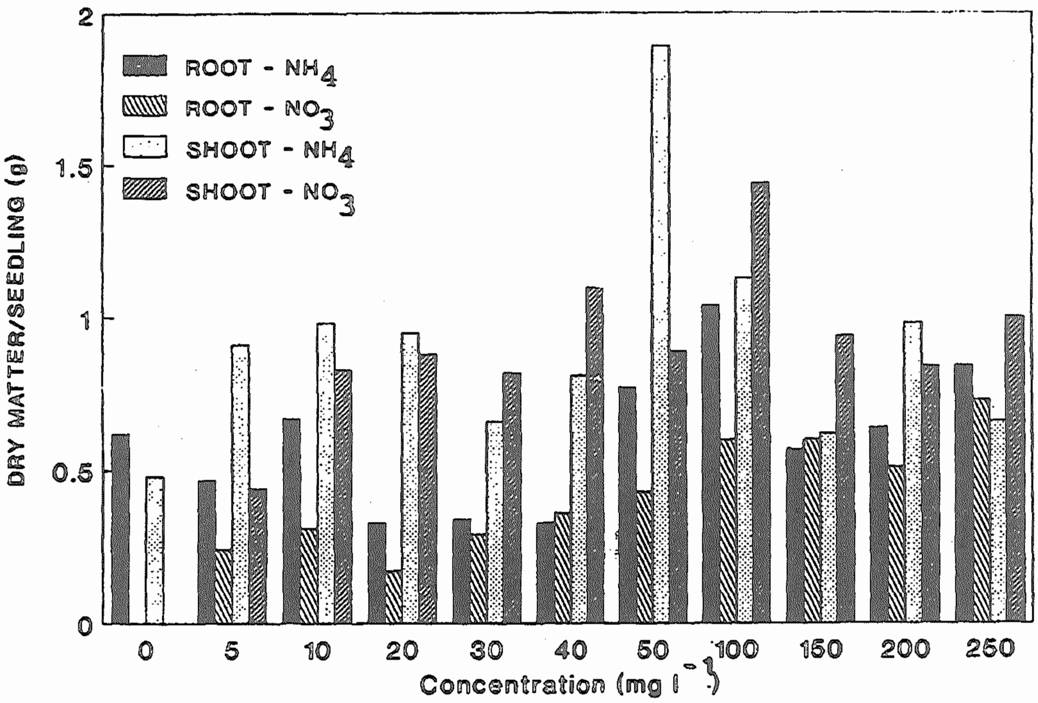

Fig. 2. Dry matter accumulation in shoot and root growth of *R. apiculata* seedlings as influenced by nitrogen treatments.

poisons such as hydrogen sulphide (Mooris, 1980). Total tissue nitrate content also followed a similar pattern.

The content of photosynthetic pigments increased in seedlings treated with both forms of N. Nitrate treatment increased the chlorophylls (a+b) content to 49%, reaction centre and light harvesting complex (LHC) chlorophyll to 96 and 29% at 50 mg l^{-1}. Likewise, NH_4 treatment also promoted the chlorophyll (a+b), reaction centre and LHC chlorophyll to 74,99 and 63% respectively at 100 mg l^{-1} (Table I).

Table I. Leaf pigments of *R. apiculata* seedlings as influenced by ammonium and nitrate form of nitrogen.

Concentration of chemical (mg l^{-1})	Chlorophyll (a+b) (mg g^{-1} FW)		Reaction centre chlorophyll (mg g^{-1} FW)		LHC chlorophyll (mg g^{-1} FW)		Carotenoids (μ mol g^{-1} FW)	
	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N
Control	0.892	1.021	0.275	0.300	0.316	0.316	0.182	0.205
5	1.363*	1.360*	0.552*	0.549*	0.407*	0.400	0.266*	0.280*
10	1.330*	1.553*	0.533*	0.526*	0.399*	0.514*	0.256*	0.318*
20	1.089*	1.285*	0.400*	0.402*	0.345*	0.442*	0.194	0.260*
30	1.631*	1.325*	0.612*	0.440*	0.509*	0.443*	0.292*	0.256*
40	1.270*	1.103	0.507*	0.340*	0.382*	0.381	0.228*	0.227
50	1.334*	1.521*	0.514*	0.587*	0.410*	0.467*	0.253*	0.286*
100	1.553*	1.244*	0.537*	0.450*	0.508*	0.397	0.301*	0.228
150	1.314*	1.152*	0.485*	0.390*	0.415*	0.381	0.252*	0.225
200	1.570*	1.426*	0.638*	0.601*	0.466*	0.413*	0.286*	0.252*
250	1.429*	1.211*	0.602*	0.399*	0.414*	0.403	0.250*	0.241*
LSD 5%	0.110	0.090	0.050	0.033	0.030	0.051	0.025	0.035

*Significant at 5% level
SD values are less than 10% of mean values.

Content of carotenoids were also enhanced by 40 and 66% in NO_3 and NH_4 treatments at 50 and 100 mg l^{-1}. Thus an increase in chlorophyll content in protein complexes represents a well organised LHC (also it is evident by higher amount of carotenoids), hence, promotion in primary reactions of chloroplast in the treated seedlings might have taken place. Of the two forms of nitrogen studied, NH_4-N was found to be better in accumulating photosynthetic pigments than NO_3-N. Ammonium treatment enhanced the dry matter accumulation, shoot length, nitrate utilisation efficiency, content of photosynthetic pigments than the propagules treated with NO_3-N. Similar response of many halophytes to added nitrogen especially to NH_4-N is well documented in *Avicennia marina* (Naidoo, 1987) and a sea grass meadow (Tyler, 1967). This is in accordance with the natural habitat of mangrove which contains ammonium as a primary source of dissolved inorganic nitrogen due to the reduced conditions of soil as a result of water logging and anaerobic sediments

(Naidoo, 1987). It is concluded from the study that the propagules of *R. apiculata* can be treated with NH_4Cl prior to planting in nursery or in natural condition for better establishment and rooting and hence suggested for artificial regeneration of the mangroves.

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