Growth, nodulation, stem anatomy and nitrogen content of *Sesbania rostrata* grown in different salinity levels

D. C. Joshua, Saradha Ramani and M. S. Shaikh
Nuclear Agriculture Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085, India

Plant growth, stem and root nodulation, stem anatomy, acetylene reduction activity and nitrogen content were studied in *Sesbania rostrata* grown hydroponically in different levels of NaCl salinity. The number and fresh weight of root and stem nodules decreased with salinity. The thickness of the xylem tissue and the number of cell layers in the xylem decreased significantly with salinity but no significant difference in cell dimensions was observed. Salinity affected cell division, resulting in reduced growth. Salinity in the medium reduced acetylene reduction activity of nodules and the nitrogen content of plants.

SESBANIA ROSTRATA is a stem-nodulating legume which fixes 160–250 kg nitrogen/ha in 50–55 days[1]. This green manure plant withstands water logging and tolerates salinity[2,3]. *S. rostrata* was more tolerant to NaCl salinity than *S. aculeata* and *S. speciosa* when grown hydroponically[3]. Radiotracer studies showed that translocation of Na to the stem and leaves was much lower compared to the uptake by roots[3]. Since this salt-tolerant species produces both root and stem nodules, we studied the effect of NaCl salinity on the development and function of root and stem nodules. Such studies being difficult in soil-grown plants, the hydroponic system was used. The present study deals with the effect of salinity on nodulation, plant growth parameters, nitrogen content and stem anatomy. As far as the authors know, this is the first study on the effect of salinity on stem nodulation.

Seedlings of *S. rostrata* raised in moist sand were transferred to Hoaglands medium. After seven days in this complete medium, they were grown for 50 days hydroponically in minus N medium containing 0, 25, 50, 100, 150 and 200 mM NaCl. The roots and the stem were inoculated with a bacterial culture which induces both stem and root nodules[4]. Inoculation was done twice, viz. 20 days and 35 days after transferring to the minus N medium. Three plastic buckets (4.5 l volume), each with 5 plants, were used as replicates per treatment. Plants were grown in 7000 lux, 12 h day and 25 ± 2°C.

Plant height, fresh weight of shoot, and the number and fresh weight of root and stem nodules were recorded at 55 days growth. Acetylene reduction activity (ARA) of detached root and stem nodules (200 mg samples each) was estimated on the same day by gas chromatography using Porapak column T[5].

The percentage of nitrogen in the dry samples of shoot was assessed using Technicon Auto Analyser (Industrial Method No. 334-74 W/B of Technicon) after digesting with concentrated sulphuric acid and digestion mixture using Tecator block digestor at 340°C for 2 h.

Anatomy of the stem was studied from transverse sections of the stem taken at the same internode. Samples were taken from 0, 50, 100 and 200 mM NaCl treatments. The number of cell layers in the cortex and the xylem was recorded from five samples per treatment. The thickness of the cortex and the xylem, the diameter of the pith (almost hollow) and the dimensions (length and breadth) of cortical and xylem cells (five cells per sample) were recorded from five samples using an ocular micrometer. The ocular readings were converted to microns.

The data, except those on stem anatomy and ARA, represent the means of three different experiments.

Plant height and fresh weight decreased with increasing NaCl concentration in the medium (Table 1). A linear dose–response relation was observed, but the reduction in fresh weight was more compared to plant height reduction. The number of root nodules in the control and in NaCl treatments was less compared to the number of stem nodules; but they were twice as heavy as the stem nodules (Table 1). The number and fresh weight of both root and stem nodules decreased with increasing concentrations of NaCl (Table 1). A significant reduction in the number of root and stem nodules was observed with 100 mM NaCl. But fresh weight showed a significant reduction with 25 mM NaCl treatment onwards.

The presence of NaCl in the medium reduced the

Table 1. Plant height, fresh weight and number and fresh weight of nodules of *S. rostrata* grown in different salinity levels

NaCl treatment (mM)	Plant height (cm)	Fresh weight (g)	Root nodules/plant		Stem nodules/plant	
			Number	Fresh weight (mg)	Number	Fresh weight (mg)
0	86 ± 0 8	9 0 ± 0 3	40 ± 3	256 ± 16	82 ± 4	238 ± 12
25	82 ± 1 2	8 1 ± 0 4	37 ± 4	210 ± 17	79 ± 6	194 ± 18
50	81 ± 1 3	7 5 ± 0 4	37 ± 3	205 ± 18	78 ± 2	182 ± 13
100	66 ± 1 3	6 4 ± 0 4	27 ± 2	156 ± 14	47 ± 2	121 ± 15
150	52 ± 3 0	3,7 ± 0 3	17 ± 2	67 ± 9	20 ± 1	41 ± 5
200	43 ± 3 3	2 5 ± 0 2	6 ± 1	9 ± 3	10 ± 2	17 ± 4

ARA of root and stem nodules (Table 2). In root nodules this decrease was linear with increase in NaCl concentration, while for stem nodules the decrease beyond 100 mM NaCl was more and abrupt.

The percentage of nitrogen in the whole plant (stem and leaves) also decreased with NaCl, but compared to other parameters this reduction was not very drastic. However, the total nitrogen (mg) per plant estimated using plant dry weight and percentage nitrogen decreased significantly with increasing concentration of NaCl (Table 2). The nitrogen content per plant and NaCl concentration in the medium showed a linear dose response with NaCl in the medium.

Among the anatomical parameters studied, the xylem tissue was affected the most by salinity. Both the thickness of the xylem tissue and the number of cell layers decreased with increasing doses of NaCl (Table 3, Figure 1). The diameter of the pith also showed reduction. But no significant reduction in the dimensions of cells was observed either in the cortex or in the xylem (Table 3, Figure 1).

Our earlier studies on *Sesbania* species have shown *S. rostrata* to be more tolerant to salinity compared to *S. aculeata* and *S. speciosa*[3]. But growth reduction was observed with higher concentrations of NaCl in the medium. From the anatomical data in the present study, it is clear that salinity affected cell division rather than cell growth in dimension. This explains the reduced plant growth. The greater reduction in fresh weight compared to plant growth in height is due to the effect of salinity on cambial activity. The cambial activity was reduced by salinity, leading to reduction in the xylem tissue. The concentrations of NaCl used are not as high as that used by Serrato Valenti *et al.*[6], who found drastic changes in almost all the tissues from epidermis to stele in *Prosopis*. At tolerable levels of salinity cell division is affected in *S. rostrata*, resulting in reduced growth.

An inhibitory effect of stem nodulation on root nodule number was reported earlier in plants grown hydroponically in the normal nutrient medium[4]. With the initiation of stem nodules, the formation of root nodules ceased, leading to a reduced number of root nodules[4]. In the

Table 2. Acetylene reduction activity, percentage nitrogen and nitrogen/plant of *S. rostrata* grown in different levels of salinity

NaCl treatment (mM)	ARA (ethylene nM/mg fresh weight of nodule)		Nitrogen (%)	Nitrogen/plant (mg)
	Root nodules	Stem nodules		
0	873	1362	3.92 ± 0.06	45 57
25	—	1167	3.90 ± 0 09	40 83
50	642	1540	3.73 ± 0.06	36 48
100	343	1145	3 60 ± 0 12	29 84
150	31	72	3 32 ± 0.07	14 99
200	0	0	3.62 ± 0 04	11 93

ARA value of root nodules for 25 mM NaCl could not be taken.

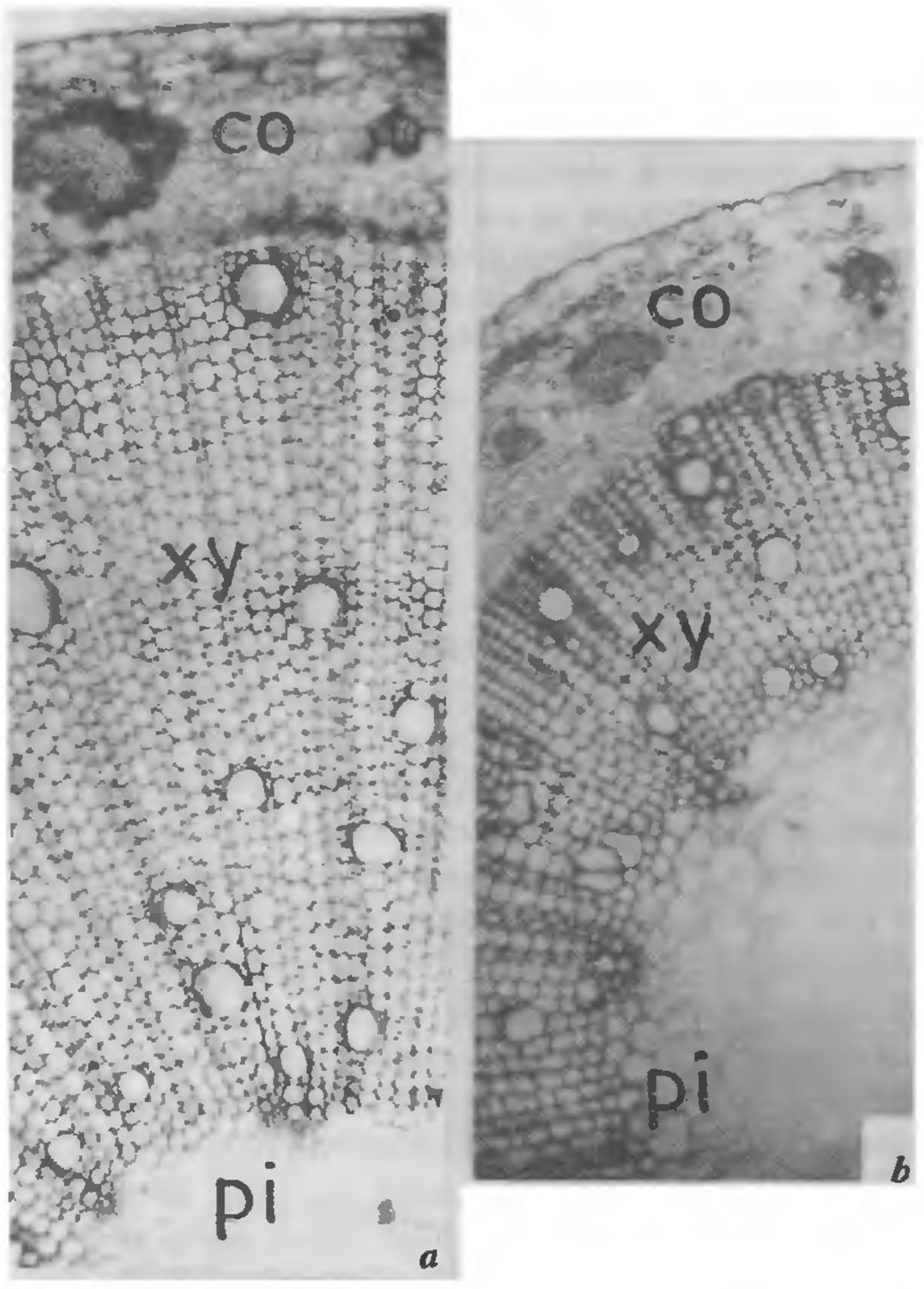

Figure 1. Transverse section of *S. rostrata* stem (×100); *a*, control. *b*, 200 mM NaCl; co = cortex, xy = xylem, pi = pith

Table 3. Stem anatomy of *S. rostrata* grown in different levels of salinity

Level of NaCl (mM)	Cortex			Xylem			Pith diameter (μm)
	Number of cell layers	Thickness (μm)	Cell dimension* (μm)	Number of cell layers	Thickness (μm)	Cell dimension* (μm)	
0	9 ± 0 5	231 ± 11	40 ± 2	42 ± 1 7	1007 ± 97	27 ± 0 9	983 ± 21
50	8 ± 0.2	195 ± 5	42 ± 2	36 ± 0.9	745 ± 23	24 ± 1.7	938 ± 39
100	8 ± 0.4	211 ± 6	40 ± 1	29 ± 0.5	614 ± 24	25 ± 0 5	883 ± 30
200	7 ± 0.2	213 ± 4	40 ± 1	20 ± 1.1	438 ± 22	26 ± 1 0	883 ± 36

*Mean of length and breadth.

present experiment, the presence of NaCl in the medium further reduced their number and fresh weight. Reduced root nodulation with salinity was reported in *Vicia faba*[7], lucerne[8], mungbean and pea[9] and soybean[10]. Salinity-induced reduction in root nodulation could be due to the decreased growth and multiplication of rhizobia or decreased availability and susceptibility of root hairs[10]. But the reduced number and fresh weight of stem nodules in *S. rostrata* as observed in the present study is due to the indirect effect of NaCl through reduced plant growth.

The greater reduction in acetylene reduction activity (ARA) of root nodules compared to stem nodules may be due to the direct contact of nodules with salinity. Since the stem nodules are not directly exposed to NaCl, the reduced ARA could be the result of reduced plant growth. There was no significant reduction in the percentage of nitrogen with salinity. The total nitrogen content per plant decreased with salinity because of reduced plant growth.

In *S. rostrata*, although the stem nodulation may not be directly affected by NaCl, decreased availability of photosynthates and reduced plant growth become limiting factors for their normal development and function.

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Restriction site polymorphism in mitochondrial DNA of Indian major carps

B. K. Padhi and R. K. Mandal
Department of Biochemistry, Bose Institute, Calcutta 700 054, India

Restriction endonuclease analysis of mitochondrial DNA (mt-DNA) in *Labeo rohita* from the river Ganges and a hatchery-maintained population from a fish farm revealed polymorphism at *Hin*dIII site. Inter-specific differences in mt-DNA restriction patterns in Indian major carps (*Catla catla*, *L. calbasu* and *L. rohita*) were also compared. An extension of this study in different populations of these widely cultured fishes would be useful for genetic stock identification and genetic diversity documentation.

INDIAN major carps (IMCs) are commercially valuable fishes cultured widely in the inland waters of India. The natural populations of these fishes are declining in the Ganges[1]. In hatcheries, improper breeding practices of these fishes affect aquaculture productivity and their genetic diversity[2]. Therefore, documentation of intra-specific genetic diversity in these fishes has become a necessity to identify their genetic stock (i.e. genetically differentiated populations of a species) for the benefit of future breeding programme and for conserving their diverse 'gene pools'.

Restriction fragment length polymorphism (RFLP) of mt-DNA was used for genetic stock identification in many fishes[3]. Due to its small genome size (16–23 kb), predominantly maternal mode of inheritance, higher mutation rate than nuclear single-copy gene and lack of genetic recombination[4,5], mt-DNA has been a useful parameter for the above study. Moreover, mt-DNA RFLP reveals a good amount of intra-specific polymorphism and mt-DNA genotypes are strongly patterned geographically[5]. We, therefore, have initiated mt-DNA RFLP analysis in Indian major carps for genetic stock identification.

Three species of Indian major carps. *L. rohita* (rohu), *L. calbasu* (calbasu) and *Catla catla* (catla), were obtained live from Nilu Ghosh Fish farm (NG), Naihati, West Bengal. All these species were also collected from the river Ganges, rohu and catla at Farraka (Fr) (Murshidabad, West Bengal) and calbasu at Allahabad (Al), Uttar Pradesh. The fishes were sacrificed in the field. The liver tissue was dissected out into sterile polypropylene tubes containing TEK buffer (50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 200 mM KCl), kept in wet ice in a thermosflask and carried to the laboratory and deep-frozen at –70°C. The mt-DNA from the fresh and frozen tissue was isolated by the method described by Padhi and Mandal[6]. Restriction enzyme digestion, end-labelling with α-^{32}P-α-^{35}S-labelled dATP or dCTP and agarose gel (0.8 or 1%) electrophoresis were done following standard protocols[7]. After electrophoresis the