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RHIZOBIAL POPULATIONS IN COASTAL SALINE SOILS OF BANGLADESH AND THEIR EFFECT ON LEGUME CROPS

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ABSTRACT

Khan MSI, Khan Zahed UM, Khanam D, Uddin MI (2013) Rhizobial populations in coastal saline soils of Bangladesh and their effect on legume crops. *Int. J. Expt. Agric.* 3(1), 22-26.

The experiment was conducted in three coastal saline Upazillas *viz.* Kalapara Upazilla of Patuakhali district, Amtali and Borguna Sadar Upazilla of Borguna district along with a non saline Upazilla, Babuganj of Barisal district of Bangladesh during 2006-2007 to quantify the *Rhizobium* population in saline soils and their effect on legume crop production. Rhizobial populations, nodulation and yield of legume crops were reduced due to toxic effect of salt present in coastal soil. In saline locations the highest heterotrophic bacteria (14.0 x 10^6) including *Rhizobium* (4.26 x 10^6) were counted during March, the flowering period of the legume crops. Bacterial populations in saline soils were much lower than non-saline soil (8.86 x 10^6 and 38.60 x 10^6, respectively). Salinity stress adversely affected on plant growth, nodulation, biomass and seed yield. Nodule production by the legumes was almost half in saline soil than in non-saline soil. Biomass and seed yield in saline locations reduced 28% and 34%, respectively over non-saline location.

Key words: *salinity, heterotrophic bacteria, rhizobium, nodule, legume, Ec*

INTRODUCTION

The salt-affected zone of Bangladesh is essentially the coastal zone. Over the last two decades the total coastal area of Bangladesh has expanded markedly from 0.83 million to 3.10 million hectares (SRDI 2000). Salinity is a great threat for agriculture in coastal areas. Grain legumes e.g. groundnut, cowpea, mungbean and grasspea are important crops in coastal areas but their average production rate is very low (859 t/ha only). Soil salinity and poor fertility is believed to be responsible for lower yield of pulses in these areas (Karim *et al*. 1982; Sattar 2005). Salinity stress is one of the main factors limiting legume productivity in arid and semi-arid regions, particularly when plant growth depends on symbiotic nitrogen fixation, since high salt concentrations in soil is also a negative factor for growth and activity of soil bacteria that establish symbiosis with legumes, collectively called rhizobia (Asraf and Harris, 2004). Among the factors that affect growth and survival of *Rhizobia*, there have been reports of the detrimental effects of salt-stress on plant growth, multiplication of the genera, nodulation and nitrogen fixation (Bottomley 1991; Cordovilla *et al*. 1995; Elsheikh and Wood, 1995; Hoben and Somasegaran, 1982). The harmful effect of salinity on *Rhizobia* may be due to direct specific ion effects or to the indirect effect of salinity by raising the pH value and decreasing osmotic potential. Strains of *Rhizobia* vary in their tolerance to salinity. It is also reported that *Rhizobium* is more tolerant toward salt than its host legume and therefore survives in the saline soil (Somasegaran and Hoben, 1995). Legumes and their symbiotic partners, the root nodule bacteria, *Rhizobia* work as a team. The full potential of the team can be achieved only when the proper *Rhizobia* are present in the soil for effective nodulation, so that plant can utilize the vast reservoir of free nitrogen in the atmosphere. When effective *Rhizobia* do not occur naturally in the soil, they can be provided by seed inoculation. In Bangladesh no research work has been done yet in this regard. So, the study has been undertaken to enumerate the native *Rhizobial* population in soil and their effect on legume crop production.

MATERIALS AND METHODS

The experiment was conducted in three coastal saline Upazillas i.e. Kalapara Upazilla of Patuakhali district, Amtali and Borguna Sadar Upazilla of Borguna district. Babugonj Upazilla of Barisal district was considered as check. The hypsography of the region is Ganges Tidal Floodplain (AEZ-13). The lands are almost level with very large basins. Most of the lands (75%) are medium high. Twenty soil samples from each location were collected in every month during December to March, the legume crop growing period and their physiological, chemical and microbiological analyses were done. Total bacteria as well as Rhizobia in the soil were made following the drop plate method (Appunu and Dhar, 2006; Vincent 1970). In this method the highly diluted suspension of collected soil samples were prepared in sterile water in such a manner that the liquid contains only a limited number of cell organisms. A countable range of plate count is 30-300 cells/ml. To achieve this concentration, 10 g soil of each sample were diluted in 90 ml sterile water, then 8 screw cap test tubes containing 9 ml sterile water were set out to make serial dilution for each sample. One ml diluted soil sample was diluted in steps, tenfold each time (10^{-1} through 10^{-8}) and was vigorously shaken. Then Nutrient Agar (NA) plates and Yeast Manitol Agar (YMA) containing Congo red plates were prepared to count heterotrophic bacteria (HB) and *Rhizobium,* respectively. The mixture of NA and YMA were sterilized in autoclave at 121^0C under 15 PSI for 20 minutes for 500 ml volume. Immediately after autoclaving 15-20 ml melted mixtures were poured onto each sterilized petri dishes to solidify. Then the plates were allowed to cool overnight in refrigerator at 4^0C. Three days old plates incubated at 37^0C for two hours were used for bacterial growth. The plates were marked four equal sectors on the out side bottom of the Petri dishes to replicate one dilution. For each dilution 1 ml suspension was taken aseptically using calibrated pipette. Only one drop was placed onto the marked sector of NA and YMA containing Petri dishes. Sterile individual pipettes were used in every step and were calibrated 0.05 ml per drop. After drying up the drops, the plates were incubated at 28^0C for 3-5 days for their maximum growth. At the end of incubation period total colonies in nutrient agar plates for total HB and only whitest transparent colonies in Congo red containing YMA plates for *Rhizobium* were counted and average value were taken from marked 4 sectors for each dilution. Then the viable cells were calculated by the following formula (Somasegaran and Hoben, 1995):

$$\text{Number of cells/g} = \frac{\text{(Number of colonies) X (Dilution factor)}}{\text{(Volume per drop)}}$$

A survey on nodule production potential and yield contributing characters of different legume field crops was made in 20 spots of each location. Ten plants each of cowpea, mungbean and groundnut were collected at maximum nodulation period and senescence stage from each spots. Plants were selected randomly and were lifted out along with soil from a radius of approximately 15 cm around the plant to a depth of 20 cm carefully and then adherent soils were removed from root materials with hand. The nodules were separated, counted and weighed immediately. Incase of yield parameters the plants were brought in the laboratory to record plant height, pods/plant, seeds/pod, shoot-biomass and grain yield. The recorded data on various characters of the crop were statistically analyzed using M-stat package program (Freed 1992) to find out the significance of variation resulting from the experimental treatments. The differences between treatments means were compared by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Soil characteristics

Analytical results of the collected samples (Table 1) show that a widespread deficiency of nitrogen and phosphorus were found in the soils. Localized zinc deficiency was also observed in these areas. All other nutrients were in excess of plant requirements, particularly sulphur, magnesium and calcium. Iron and boron contents were also high in this region. The organic matter content of the soil ranges from 1.78 to 2.45%, which is not sufficient for microbial activity as well as successful crop production (SRDI 2000).

Table 1. Nutrient status of collected soil samples of southern saline areas during March 2006-2007

Location	OM	N	P	K	Ca	Mg	Na	S	Zn	B	Fe
	(%)	(%)	(ppm)	(meq/100g)				(ppm)			
Kalapara	2.12	0.11	4.90	0.39	6.53	5.40	1.67	360.32	1.13	0.92	180.0
Amtali	2.45	0.13	1.98	0.13	6.27	3.32	1.10	362.14	1.24	0.77	64.22
Borguna	2.33	0.12	5.53	0.11	6.77	3.92	1.34	348.04	1.28	0.80	83.00
Nutrient status	M	L	VL-L	M-VH	H	VH	H	VH	M	VH	VH

L= Low, VL= Very low, M= Medium, VH= Very high

Soil salinity and pH

Salinity and pH of the soil during crop growing period was also estimated and presented in Table 2. During legume crop growing period, salinity remained minimum (2.10 to 4.41 dS/m) in December. It rose over time and reached to the maximum in April (8.17 to 12.99 dS/m). It may be due to continuous pulling up of capillary saline water. This process continues until monsoon rain starts. Most of the legume crops (mungbean, cowpea and groundnut) are sown during late Rabi i.e. mid January to mid February. So, germination, early vegetative growth and nodulation of the crops hampered due to toxic effect of salt in the soil. The pH of the soils ranged from 4.7 to 6.3, which is much lower, compared to saline characteristics of the soils. Low soil pH may be the indication of the presence of buried acid sulphate layer.

Table 2. Salinity and pH of coastal saline soil during legume growing period

Location	December/06		January/07		February/07		March/07		April/07	
	Ec (dS/m)	pH	Ec (dS/m)	pH	Ec (dS/m)	pH	Ec (dS/m)	pH	Ec (dS/m)	pH
Kalapara	4.41	6.2	5.98	5.8	7.46	6.3	8.41	5.9	12.9	6.1
Amtali	3.95	5.5	4.15	5.5	5.11	5.2	5.76	5.5	9.08	5.3
Borguna	2.10	4.8	3.60	4.9	5.48	5.1	6.67	4.8	8.17	5.0
Average	3.49	5.5	4.58	5.4	6.02	5.5	7.95	5.4	10.1	5.5

Bacterial population in soil

Results show that lower numbers of total heterotrophic bacteria (TBH) including *Rhizobium* (Rh) were found during December-February, at the time of germination and early vegetative stage of legume crops, in both saline and non-saline location. It was due to excessive moisture and low temperature of the soil. But, during March, when soil temperature and salinity turned to rise and remained 18^0-29^0C and 7.95 dS/m, average heterotrophic bacteria (14 x 10^6) of which *Rhizobium* (4.26 x 10^6) reached in maximum numbers. The bacterial population turned to reduce from April, when average salinity remained 10.1 dS/m and temperature, water and salinity stress reached in pick (Table 3). The bacterial populations remained always higher in non-saline soil than saline soil. The occurrence of THB as well as *Rhizobia* were 38.60 x 10^6 and 12.15 x 10^6, respectively in Babugonj, the non-saline location was the highest over saline locations (Table 4). Among the saline locations, the soils of Borguna contained the highest number of THB (9.74 x 10^6) of which 21.98% were *Rhizobia* followed by Amtali soils (8.96 x 10^6 and 28.68%, respectively). The occurrence of THB (7.89 x 10^6) including *Rhizobia* (21.04%) was the lowest and in the soils of Kalapara location as it contained higher salinity. It was reported that high temperature is often associated with saline soils. Survival and growth of many species under salt stress was found dependent upon temperature. It is also reported that increasing salt concentration may have a detrimental effect on soil microbial populations as a result of direct toxicity in soil and the rhizosphere of the plant. The results are supported by Tate 1995; Elsheikh and Wood, (1989).

Table 3. Bacterial populations (in million) in a cowpea field of coastal saline and non-saline soils during November to April

Location		November		December		January		February		March		April	
		THB	Rh	THB	Rh	THB	Rh	THB	Rh	THB	Rh	THB	Rh
Kalapara	Saline	2.4	0.5	2.9	0.8	3.4	0.3	11.4	3.36	13.0	4.25	9.70	3.8
Amtali		1.9	0.4	2.1	0.4	7.5	1.5	10.8	3.23	13.6	4.75	10.1	3.9
Borguna		2.3	0.5	2.6	0.5	6.5	1.5	13.1	3.40	15.5	3.80	12.4	3.3
Average		2.2	0.46	2.5	0.57	5.8	1.10	11.7	3.33	14.0	4.26	10.7	3.7
Babugonj	Non-saline	16.8	3.62	20.5	5.25	27.5	7.12	49.6	17.3	54.6	21.5	41.0	19.5

THB = Total Heterotrophic Bacteria, Rh = *Rhizobium*

Table 4. *Rhizobium* populations in a cowpea field of coastal saline and non-saline soils during maximum nodulation period

Location		Total Heterotrophic Bacteria (million)	*Rhizobium*	
			(million)	(%)
Kalapara	Saline	7.89	1.74	21.04
Amtali		8.96	2.57	28.68
Borguna		9.74	2.05	21.98
Average		8.86	2.12	23.90
Babugonj	Non-saline	38.60	12.15	31.47

Nodulation in saline soil

A significant difference was found in nodule number, nodule weight and size in cowpea, mungbean and groundnut in both saline and non-saline soil (Table 5). Nodule production was about double in non-saline soil compared to saline soil. Among the saline locations, legumes grown in Kalapara soils produced lower nodules than the soils of Amtali and Borguna. It was due to presence of more salinity in Kalapara soil (8.41 dS/m) which inhibited infection processes as well as survival and proliferation of *Rhizobium* spp. in the soil and rhizosphere. Among the legumes, cowpea produced heavier nodules than mungbean and groundnut in both saline and non-saline locations. Nodule production of cowpea, mungbean and groundnut was found the highest at flowering stage during February-March. The results are in agreement with Hafeez *et al*. (1988) who reported that the nodulation of *Vigna radiata* was reduced to about half at salinity level of 5.0 dS/m as compared to 1.4 dS/m and nodulation was completely depressed when salinity was raised to 10.0 dS/m regardless of the plant growth stage. The results are also in agreement with Sprint and Zahran, (1988); Elsheikh 1992; Elsheikh and Wood, (1995).

Table 5. Nodulation status of cowpea, mungbean and groundnut in saline areas

Location	Cowpea			Mungbean			Groundnut		
	Nodule /plant	Nodule weight /plant (mg)	Nodule size (mm)	Nodule /plant	Nodule weight /plant (mg)	Nodule size (mm)	Nodule /plant	Nodule weight /plant (mg)	Nodule size (mm)
Kalapara	5.72 c	21.0 d	4.07 b	4.06 b	7.30 c	1.73	49.4 c	71.40 c	1.46 b
Amtali	7.40 bc	27.6 c	4.13 b	5.67 b	10.3 b	1.75	67.0 b	101.4 b	1.51 b
Borguna	9.13 b	39.2 b	4.32 ab	5.60 b	10.4 b	1.81	54.7 c	80.52 c	1.51 b
Babugonj	14.7 a	53.4 a	4.88 a	15.6 a	18.4 a	1.84	86.7 a	167.5 a	1.87 a
CV (%)	10.3	4.97	4.28	18.7	7.47	3.59	4.15	4.18	3.61

Plant characters and yield of legumes in saline soil

Plant height, yield and yield contributing characters of different legume crops are presented in Table 6. It was observed that the plants of non-saline soil performed better in all respects than saline soil. In case of cowpea and mungbean no significant differences were found in plant height, pods/plant, seeds/pod, biomass and seed yield in all the saline locations. Average biomass yield of cowpea and mungbean were 2.06 t/ha and 1.74 t/ha that were 34% and 24% lower than non-saline soil, respectively. Accordingly seed yield of cowpea and mungbean in saline soil were 39% and 40% lower than non-saline soil. In case of groundnut, significant differences were found in biomass and nut yield in saline locations. Among the saline locations, Amtali soil produced the highest biomass (2.82 t/ha) and nut yield (1373 kg/ha). The average biomass and nut yield of groundnut in saline locations were 17% and 28% lower than that of non-saline location. The result resembled with the findings of Bottomley (1991) who reported that salinity stress act as water stress and limits nutrient supplying capacity of the soil which affects plant growth and yield.

Table 6. Plant characters, yield and yield contributing parameters of cowpea, mungbean and groundnut in coastal region

Location		Plant height (cm)	Pods/plant	Seeds/pod	Biomass yield (t/ha)	Seed/nut Yield (kg/ha)
Cowpea						
Kalapara	Saline	56.0 b	8.60 b	11.8 b	2.08 b	745 b
Amtali		54.6 b	9.70 b	10.3 b	2.10 b	700 b
Borguna		38.6 b	8.67 b	10.0 b	1.99 b	804 ab
Average		59.73	8.99	10.7	2.06	749.6
Babugonj	Non-saline	154.3 a	17.7 a	15.7 a	3.07 a	1234 a
CV (%)		9.45	10.2	9.24	5.93	16.96
Mungbean						
Kalapara	Saline	33.7 b	9.30 b	8.33 b	1.69 b	688 b
Amtali		37.7 ab	10.3 b	9.33 b	1.86 b	567 b
Borguna		32.3 b	10.8 b	7.89 b	1.66 b	515 b
Average		34.56	10.13	8.52	1.74	590
Babugonj	Non-saline	42.1 a	18.0 a	10.1 a	2.29 a	984 a
CV (%)		5.63	9.92	13.5	4.69	13.14
Groundnut						
Kalapara	Saline	18.0 b	14.7 b	1.67	2.13 b	1192 b
Amtali		21.3 b	15.7 b	2.00	2.82 a	1373 ab
Borguna		21.7 b	18.0 b	1.67	2.39 ab	1150 b
Average		20.3	16.1	1.78	2.45	1238.33
Babugonj	Non-saline	30.0 a	27.7 a	1.67	2.96 a	1727 a
CV (%)		8.57	13.5	2.94	7.87	11.98

CONCLUSION

Rhizobium populations in coastal saline areas of Bangladesh were lower than non-saline areas. So, to explore the possibilities of increasing potential of coastal saline areas for increased production of legume crops, judicious matching of *Rhizobium* strains with host plants and prudent use of large viable inoculum prepared with coastal native *Rhizobium* is the only way.

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