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Biodiversity of Rhizospheric Soil Bacteria and ArbuscularMycorrhizal (AM) Fungi in Some of the Wild Medicinal Legumes of Barak Valley

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ABSTRACT

Present investigation was aimed to isolate and study the rhizobacteria and AM fungi from rhizosphere of wild legumes: *Mimosa pudica*(sensitive plant), *Crotolariapallida* (Sunhemp), *Cassiatora*(Sickle pod) and*Desmodium*.The molecular characterization of four bacterial isolates were done. Four bacterial species*Bacillus megaterium, Bacillusaerophilus, Microbacterium laevaniformans* and *Staphylococcus xylosus*were isolated from strains M1, RT,D5 and D7 respectively.Also,the distribution of AM fungi population was studied from rhizosphere soils of these legumes.Among the AM fungi,*Glomus*species was dominant and bacterial genus *Bacillus*was found to be dominant.Maximum number of VAM infection was found in the rhizosphere soil of *Mimosa pudica*of Srikona.

Key words: ArbuscularMycorrhizal Fungi, *Glomus*, Spore population, Diversity.

INTRODUCTION

Leguminous plants are abundant in Barak Valley , where they grow in barren soils and drysites that are unsuited for most crops.Medicinal plants are the rich heritage of country serving the age old medicinal system i.e.Ayurveda.Despite being so important these plants have been totally neglected as far as biofertilizers are concerned. For their utilization,medicinal plants are indiscriminately taken from wild habitats causing their depletion andextinction.Pertaining to the negligence toward the rhizobialandVAM biodiversity,we took the initiative to characterize the microbial diversity associated with the medicinal legumes.Rhizobia are of particular interest due to their symbiotic nitrogen fixing ability with members of Leguminosaewhich is the second largestfamily of flowering plants and includes more important drugs than any other family. Rhizobia are genetically diverse and physiologically heterogeneous group of symbiotic nitrogen fixing bacteria that form nodules on the roots or rarely on the stem of legume hosts, within which thebacteria fix atmospheric nitrogen into ammonia.Leguminous plants are said to behighly specific to nodulatingorganisms[1] . (SubbaRao,1999). The root nodule formation and fixationof nitrogen from the atmosphere in the rootsof leguminous plants occur only if the specificcross-reacting species of Rhizobia is present inthe soil. The productionof specific flavonoidsby the plants may also attract specific*Rhizobium* strains and facilitate their entry intothe host plant and nodule formation[2]. (SubbaRao,1993). Arbuscularmy corrhizae (AMs) are characterized by the formation of uniquestructures such asarbuscules andvesicles by fungi of the phylumGlomeromycota (AM fungi). Oftheseven typesof*mycorrhizae*described in currentscientific literature (arbuscular, ecto, ectendo,arbutoid, monotropoid, ericoid and orchidaceousmycorrhizae), the arbuscularand ectomycorrhizaeare the most abundant and widespread[3] . (SiddiquiandPichtel,2008). The

Vesicular ArbuscularMycorrhiza(VAM) fungi, grouped in the phylumGlomeromycota,are the commonestmycorrhizal type involved in agricultural systems[4].(Bethlenfalvay, 1992).AM fungi (AMF) help plants to capturenutrientssuch as phosphorus and micronutrients from the soil. It is believed that the development ofthe arbuscular mycorrhizalsymbiosis played a crucial role in the initial colonisation of landby plants andin the evolution of the vascular plants. Our present investigation was aimed to isolate and study therhizobacteria and AM fungi from rhizosphereof wild legumes :*Mimosapudica*(sensitive plant), *Crotolariapallida* (Sunhemp),*Cassia tora*(Sickle pod) and *Desmodium.*collected from Assam university, Rongpur,Irongmara andDoluof Barak Valley. Also,the distribution of AM fungi population was studied fromrhizospheresoils of these legumes.Among theAM fungi,*Glomus*species was dominant.

MATERIALS AND METHODS

Experimental sites

Four regions of Barak Valley(Assam University, Rongpur, Irongmara and Dolu) were selected. The vegetation in the valley is mostly Tropical evergreenand there are large tracts of Rain forests in the northern and southern – eastern parts of the valley.

Collection of root nodules

Root nodules offour commonly growing wild legumes *Mimosa pudica* (sensitive plant), *Crotolariapallida* (Sunhemp),*Cassiatora* (Sickle pod) and *Desmodium*were collected and transported to the laboratory in plastic bags along with seedlings.

Isolation of rhizobia

Nodules were separated from the roots and washed in sterilized distilled water for several times. Following serial dilution agar plate techniquebacterial isolation was carried out[5,6].After that these plates were incubated at 28±1ºC and observed daily. Bacterial colonies appeared after 2-3 days were picked up and streaked on YEMA plates. Pure cultures were obtained with one or more further sub – culturing steps.

Isolation ofVAM andestimatonof AM fungal colonization and AM fungal spores

Staining of mycorrhizal roots were done[7].VAM isolation was done using wet sieving and decantation method[8].

RESULTS

Agood number of isolates wereobtained from root nodules of*Mimosa pudica*(sensitive plant),*Crotalaria pallida*Sunhemp),*Cassia tora*(Sickle pod) and *Desmodium*.Out of the total 20 isolates,only four isolates(M1,RT1,D5and D7)were subjected to molecular characterization test.The isolates were round in shape,gummy white colour,smooth margin and superficial in position.Four bacterial species*Bacillus megaterium, Bacillus aerophilus, Microbacterium laevaniformans* and *Staphylococcus xylosus* were isolated from strains M1,RT1,D5 and D7 respectively as shown below:

Further studies on vesiculararbuscular fungal spore population were studied.A total of 17 fungal taxa were isolated from the collected soil samples.The isolated spores belonged to the genus*Gigaspora,Ambispora*, *Acaulospora*and

Table 1: Assam bacteria: 16SrRNA gene – based identification

Strain number	Taxonomy	Gene homology(%)	16S rRNA(bp)
M1	*Bacillus megaterium*	96	1471
RT1	*Bacillus aerophilus*	93	1000
D5	*Microbacteriumlaevaniformans*	96	1470
D7	*Staphylococcus xylosus*	98	132

Table 2: The abundance of spore population at three sites of Barak Valley

AM fungi	Rongpur				Irongmara				Dorgakuna				Total of three sites			
	Aug	Sept	Oct	Nov	Aug	Sept	Oct	Nov	Aug	Sept	Oct	Nov	Aug	Sept	Oct	Nov
Gigaspora	2	4	11	12	5	7	911	689	13			13	1929	36		
Ambispora	1	3	3	4	2	2	3	4	0	1	2	2	3	6	8	10
Acaulospora	0	012			0	0	0	0	00	0	0		0	0	12	
Glomus	6	9	11	12	5	7	9	11	3	4	7	9	14	20	27	32
Total AM spore population													30 + 45 + 65 + 80 =220			

Table 3: Percentage of mycorrhiza infection in *Mimosa pudica* at different sites of Cachar district

Site	Total no. of root segments	No. of segments infected	Percentage infection
Rongpur	11	09	81.8%
University campus	15	12	80%
Dorgakona	12	08	66.6%
Dolu	11	04	36.36%

*Glomus.*The number of *Glomus*species were found to be dominantamong all.The following table shows the spore density from some wild legumerhizosphere soil.

DISCUSSION

The results of present investigation indicated that root nodules of leguminous plants are the habitat of many species of bacteria like *Bacillus megaterium, Bacillus aerophilus, Microbacterium laevaniformans* and *Staphylococcus xylosus.*The abundance of root nodules were also studied in the selected plant species.Nodules were highest in*Mimosa pudica , Crotolariapallid*and*Desmodium* while totally absent in *Cassia tora*.The absence of nodulation may be due tothe absence of specific no dulating *Rhizobium*strain in therhizospheresoil (Sundar et al).Also, the rhizosphere soils are the habitat of many AM fungal taxa like *Gigaspora,Ambispora, Acaulospora*and*Glomus*.The genus *Glomus*was found to be the most dominant,second dominant genus was*Gigaspora*followed by *Ambispora*and *Acaulospora*.Further,the number of spores were less in number during Augustand September and gradually increased towards November. Percentage of mycorrhizal infection was studied at five different sites of Cachar district. The percentage of infection was highest in Srikonaand lowest in Dolu area. The variation of percentage infection may be due to the soil characteristics.

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