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Influence of Soil Application of Growth Promoting Diazotrophs % *Rhizobium* sp., Isolated from Nodules of *Vigna mungo* L. on Certain Paramerters of Carbon and Nitrogen Metabolism and Biomass of Maize

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The present investigation was designed to assess the effect of growth-promoting activities of *Rhizobium* sp- a diazotroph, on plant growth and a number biochemical parameter including *in vivo* nitrogenase activity in the root rhizosphere of *Zea mays* L. The *Rhizobium* in this study was isolated from the root nodules of *Vigna mungo* L. and it was applied in three way, *viz*., seed soaking, seedling soaking, and basal treatment. Plants of *Zea mays* L. were raised in earthenware pots containing soil from paddy field. The results showed significant enhancement in various biochemical parameters (different fractions of leaf pigments (chlorophyll-a and chlorophyll-b, and carotenoids), total soluble sugars, phosphate buffer soluble proteins, total free amino acids, total phenol) and activities of different enzymes, *viz., in vivo* nitrate reductase activity and *in vitro* catalase activity in the leaves and *in vivo* nitrogenase (N2-ase) activity in the roots' rhizosphere; and growth parameters such as lengths of root and coleoptiles and their fresh and dry weights and leaf area also increased. The over all observations revealed that use of *Rhizobium* sp from the root nodules of *Vigna mungo* L. improved the photosynthetic efficiency, nitrogen-metabolizing capacity and plant growth to significant extent and it may be used as a source of potent bio-fertilizer for maize crop.

Key words: Soil, *Rhizobium*, *Vigna mungo,* Biomass, Maize.

Agriculture is being practiced in about 2.5 lakh hectare land in Tripura. It is noticed that in the cultivation process farmers use heavy amount of various kinds of synthetic/chemical fertilizers but yield performance of various crop is not satisfactory. Due to continuous use of chemicals, adverse effect on soil micro-flora and its fertility have been noticed. Excessive supply of nitrogen through synthetic fertilizer has also caused severe environmental hazard and has drastically altered the nature of soil chemistry of various crop fields of Tripura making it either too alkaline or too acidic. These chemicals also contribute to air, soil and water pollution and have adverse effect on fish, wild life and human beings as well. Some times nitrogen is also a deficient nutrient in many

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soils around the world. Diazotrophs with nitrogen fixing capacity may be an alternative source to the synthetic nitrogen fertilizers. Although much of the nitrogen is removed when protein rich grain or hay is harvested, significant amounts remain in the soil for future crops. The diazotrophs are micro-organisms, which can reduce atmospheric molecular nitrogen to ammonia. These organisms include nitrogen fixing free-living bacteria such as *Azotobacter* sp*., Azospirillium* sp*. and Klebsella* sp. etc and symbiotic nitrogen fixers such as a large number of *Rhizobium* species. Martinez-Toledo et al (1985) found that *Azotobacter chroococcum* rapidly grows in maze roots in agricultural soils in Spain and also on a nitrogen-free medium. Acetylene-reducing activity and microbial counts/ populations were on maize root segments were in the range of 0.0053–0.848 n mol $C_2H_2 \cdot g^{-1} \cdot h^{-1}$ and s were 1.4–6.0 × 10^4 micro-organisms · g^{-1}. These results showed that there was an association between *A. chroococcum* strains and roots of maize planted in some Spanish soils.

Maize (*Zea mays* L.) is one of the most economically important quick growing annual cereal crop. It holds a unique position in world agriculture as food, feed and industrial crop. World collection of maize comprises about 12,000 accessions that are represented in 256 races, of which about 30 are in the process of examination (Machado *et al*. 1998).

Although rhizobia do not form nodules on roots of maize plant but they benefit the plant by adhering to the root surface and fix nitrogen even in free-living condition. Taking advantage of this property of rhizobia, the present study was conducted. In this paper we report the effect of a diazotrophic bacterium- *Rhizobium,* isolated from the root nodules of *Vigna mungo* L. on photosynthetic pigments, total soluble sugars, soluble proteins, total free amino acids, total phenol, *in vivo* nitrate reductase activity and *in vitro* catalase activity in the leaves and i*n vivo* nitrogenase (N2-ase) activity in the roots of *Zea mays* L.

MATERIAL AND METHODS

Samples of *Vigna mungo* L., a legume, having intact root nodules were collected from the nearby fields of Botany Department of Tripura University. The rhizobia (bacteroids) were isolated from the nodules under aseptic condition (Vincent, 1970) and then cultured in Yeast Extract Mannitol (YEM) broth. The culture after 96 hours of growth was used for inoculation treatments to maize seeds in various ways. Plants of *Zea mays* L. were raised in earthenware pots (8.5 inch diameter) with one plant in each pot filled with well-sieved 8 Kg paddy field soil having pH 6.5 and the pots were kept in open under natural condition. All parameters were studied on 11th day-old-plants of *Zea mays* L.

A summarized account of various treatments of rhizobia isolated from *Vigna mungo* are given as under:

Control

Un-inoculated seeds and soaked in distilled water for 8 h Petri dishes and raised in earthenware pots.

Seed soaking treatment

Seeds was soaked with rhizobial broth for 8 h in Petri dishes and raised in earthenware pots.

Seedling soaking treatment

3-day-old seedlings were soaked with rhizobial broth for 8 h in Petri dishes and raised in earthenware pots.

Basal treatment

For basal or soil application of *Rhizobium*, its 72-h-old culture broth was adsorbed on rice bran (local name kurah/vushi in Bengali) and it was named *Rhizobium*-biofertilizer. From this 35 gramme was supplied to the soil in each earthenware pot containing single 7-day-old maize plant. To prepare *Rhizobium*-biofertilizer 500 ml from 72-h-old *Rhizobium*-culture broth was mixed with 200 gramme rice bran (oven sterilized at 80 °C for 6 h and then cooled to room temperature before mixing).

The contents of chlorophyll-a (chl-a), chlorophyll-b (chl-b), total chlorophyll and carotenoids were estimated in fresh leaves by following Arnon (1949) method. The level of total soluble sugars was estimated with Anthrone reagent (Yemn and Willis, 1954). The phosphate buffer soluble protein was estimation by following the method of Lowry *et al.* (1951). The level of total free amino acids was estimation by following the method of Yemm and Cocking (1955). The level of total soluble phenol was estimation by following the method of Swain and Hillis (1959). The *in vivo* NRA was measured by following the

method of Hageman and Hucklesby (1971). The catalase activity was estimated by a water-displacement method as described by Meidner (1971). The *in vivo* N_2-ase activity was estimated by following the method of Srivastava *et al*. (1980) which is a modified Conway's micro-diffusion method. The biomass parameters included determination of root and coleoptile length, leaf area index, fresh and dry weight of roots and coleoptiles. Ten replicates (plants) were taken for these studies.

RESULTS AND DISCUSSION

Inoculation and soil application of growth-promoting diazotrophs also increased plant growth and biomass weight as compared to compared with uninoculated control. Root length increased by 16.36% than the control one in seed treatment and in seedling and basal treatment it was also increased by 20.00% and 76.96% in seedling and basal applications respectively. Root fresh weight was increased by 104.21% than the control in seeding treatment followed by 98.24% and 21.05% in basal treatment and seed treatment respectively. Root dry weight was increased by 70.37% than the control in basal treatment followed by 29.62% and 40.74% in seed and seedling treatment respectively. Coleoptiles length increased by 31.52% than the control one in seed treatment followed by 14.28% and 24.63% in seedling and basal treatment respectively. Coleoptiles fresh weight was increased by 62.89% than the control in seedling treatment followed by 32.67% and 43.48% in seed and basal treatment respectively. Coleoptiles dry weight was increased by 48.98% than the control in basal treatment followed by 14.29% and 22.45% in seedling and seed treatment respectively. The leaf area was increased by 72.22% than the control in basal treatment followed by 34.27% and 54.80% in seed and seeding treatment respectively (Table 1).

Application of rhizobia from the root nodules of *Vigna* sp. showed a significant increase in all the biochemical parameters studied in this investigation. Total chlorophyll was 40.19% higher than the control on 11-day-old plant with basal application followed by 19.40% and 10.39% in seedling and seed treatments respectively. The

Table 1. Effect of *Rhizobium* Sp. on biomass (root length, coleoptiles length and their fresh and dry weights and leaf area) of *Zea mays*, inoculated with 72 hours rhizobial broth of *Vigna mungo* L. and control (without application of *Rhizobium* sp. suspension). The plants were grown in the garden soil under natural condition.

Treatments	Root parameter			Coleoptiles parameter			Leaf area
	Length (cm)	Fresh wt (g)	Dry wt (g)	Length (cm)	Fresh wt (g)	Dry wt (g)	(mm2)
Control	16.5± 0.174	0.285±0.15	0.027±0.001	20.3±0.492	0.407±0.011	0.049±0.1	1062± 19.239
Seeds	19.2±0.813 (+16.36)	0.345±0.001 (+21.05)	0.035±0.001 (+29.62)	26.7±0.661 (+31.52)	0.54±0.112 (+32.67)	0.063±0.002 (+22.45)	1426±7.93 (+34.27)
Seedlings	19.8±0.719 (+20.00)**	0.582±0.01 (+104.21)	0.038±0.002 (+40.74)	23.2±0.332 (+14.28)	0.663±0.01 (+62.89)	0.056±0.012 (+14.29)	1644±21.041 (+54.80)
Basal	29.2±0.921 (+76.96)	0.565 ±0.112 (+98.24)	0.046±0.006 (+70.37)	25.3±0.613 (+24.63)	0.584±0.079 (+43.48)	0.073±0.112 (+48.98)	1829±8.832 (+72.22)

The values are average of ten replicates. * Standard errors of the mean (N=10). **Values in parentheses are per cent inhibition (-) or stimulation (+) with respect to control.

ratio of Chl-a / Chl-b was maximum in seedling treatment (11.54) followed by 7.11 and 5.83 with seed and basal treatment respectively. The level of carotenoids was 64.81% higher than the control with basal treatment on 11-day-old in basal application treatment followed by 20.37% and 12.96% in seed and seedling treatment respectively (Table 2). The total soluble sugar was 141.27% higher than the control in basal treatment followed by 55.42% and 51.83% in seed and seedling treatments respectively. The potassium phosphate buffer soluble protein was 44.13% higher than the control in basal treatment followed by 28.90% and 25.40% in seed and seedling treatments respectively. In the case of total free amino acids maximum level was notice in basal treatment (106.20%) followed by 21.25% and 10.96% in seedling and seed treatment respectively. The total phenol content decreased with respect to control and the maximum decrease was noticed with seed treatment (53.91%) followed by basal (31.91%) and seedling treatment (11.89%) as shown in Table 3.

The increase in the level of soluble protein in the leaves may be due to availability of the fixed nitrogen from the bacterial source supplied externally in the soil through roots in the rhizosphere of the plant. The level of *in vivo* nitrate reductase activity in the leaves was 62.01% higher than the control in basal treatment followed by 52.42% and 35.06% in seed and seedling treatment respectively. The levels of *in vivo* nitrogenase (N2-ase) activity in the roots of *Zea mays* was 63.83% higher than the control in basal treatment followed

Table 2. Effect of *Rhizobium* Sp. on level of chlorophyll fractions, the ratio of Chl-a/ Chl-b and carotenoids, in the leaves of *Zea mays,* inoculated with 72 hours rhizobial broth of *Vigna mungo* L. and control (without application of *Rhizobium* Sp suspension). The plants were grown in the garden soil under natural condition.

Treatments	Level of chlorophyll (mg/g leaf fr. wt.)				Carotenoids
	Chl-a	Chl-b	Total chlorophyll	Chl-a/ Chl-b	
Control	0.638 ± 0.004*	0.372 ± 0.006	1.010	1.715	0.054 ± 0.000*
Seed	0.722 ± 0.006	0.393 ± 0.003	1.115(+ 10.39)	1.837(+ 7.11)	0.065 ± 0.000(+ 20.37)
Seedling	0.727 ± 0.004	0.479 ± 0.011	1.206(+ 19.40)	1.517(- 11.54)	0.061 ± 0.000(+ 12.96)**
Basal	0.913 ± 0.007	0.503 ± 0.005	1.416(+ 40.19)	1.815(+ 5.83)	0.089 ± 0.666(+ 64.81)

The values are average of ten replicates. * Standard errors of the mean (N=10).
**Values in parentheses are per cent inhibition (-) or stimulation (+) with respect to control

Table 3. Effect of *Rhizobium* Sp. on level of total soluble sugars, potassium phosphate buffer soluble proteins, total free amino acids and total phenol in the leaves of *Zea mays,* inoculated with 72 hours rhizobial broth of *Vigna mungo* L. and control (without application of *Rhizobium* sp suspension). The plants were grown in the garden soil under natural conditions

Treatments	Level of total soluble sugars (mg/g leaf fr wt)	Potassium phosphate buffer soluble proteins (mg/g leaf fr wt)	Total free amino acids (mg/g leaf fr wt)	Total soluble leaf fr wt) phenol(mg/g
Control	2.120± 0.092	5.377± 0.048	4.450± 0.154	2.188± 0.057
Seed	3.295± 0.161(+ 55.42)	6.931± 0.310(+ 28.90)	4.938± 0.466(+ 10.96)	0.976± 0.027(-53.91)
Seedling	3.219± 0.015(+ 51.83)	6.743± 0.232(+ 25.40)	5.396± 0.051(+21.25)	1.864± 1.767(-11.89)
Basal	5.115± 0.369(+ 141.27)	7.750± 0.253(+ 44.13)	9.176± 0.378(+ 106.20)	1.442± 0.067(-31.91)

The values are average of ten replicates. * Standard errors of the mean (N=10).
**Values in parentheses are per cent inhibition (-) or stimulation (+) with respect to control.

by 39.84% and 11.769% in seed and seedling treatment respectively on weight basis. In terms of per plant basis *in vivo* N2-ase activity was 123.66% higher than the control in seedling treatment followed by 92.27% and 28.58% in seed and basal treatment respectively. *In vitro* catalase activity decreased with respect to control and maximum decrease was notice with seed treatment (55.40%) followed by 35.13% in seedling and basal treatment (Table 4).

Results of present study showed that application of rhizobia from the root nodules of *Vigna* sp was more effective in increasing the chlorophyll, carotenoids content, soluble sugar, soluble protein, amino acids, NR activity and plant dry weight. Some of these observations are in agreement with the findings of Dey and Srivastava (2007). Nitrogen fixing bacteria (rhizobia) promote growth, yield and nutrient uptake of lowland rice (Biswas, 1998; Biswas *et al*. 2000a and 2000b; Vasudevan *et al*. 2002). Wilson (2004) postulated that the benefits of rhizobial-strain-cereals association, leading to greater production of vegetative and reproductive biomass, more likely involve rhizobial modulation of the plant's root architecture for more efficient acquisition of certain soil nutrients (P, K, Mg, Ca, Zn, Na and Mo), rather than biological N_2 fixation alone and selected rhizobial strain produced two major classes of plant growth hormones, *viz*., auxin and gibberellin. Srivastava *et al*. (2004) observed that application of *Rhizobium* sp. from different legumes increases *in vivo* nitrate reductase activity, soluble protein, total nitrogen and biomass in maize. Use of broth of bio-fertilizers (*Azospirillum brasilense* and *Bacillus subtilis*) enhanced leaf area, chlorophyll concentration nitrate reductase activity, total biomass production and grain yield in wheat under field conditions (Panwar and Singh 2000).

Naidu *et al*. (2002) noticed induction of nodule like structure on roots of rice cv. Pusa-834 upon treatment with 2, 4-D and *Azospirillum brasilense*. Similar observations have been reported in wheat, sorghum and pearl millet also (Panwar 1993, Nayak *et al*. 1985). Application of biofertilizers (*Azospirillum brasilense*) enhanced the rate of flow of different amino compounds (Dhar *et al*., 1994). A number of reports have suggested that symbiotic nitrogen

Table 4. Effect of *Rhizobium* Sp. on *in vivo* nitrate reductase activity and *In vitro* catalase activity in the leaves and *in vivo* nitrogenase (N_2-ase) activity in the roots of *Zea mays,* inoculated with 72 hours rhizobial broth of *Vigna mungo* L. and control (without application of *Rhizobium* Sp suspension). The plants were grown in the garden soil under natural condition

Treatments	*In vivo* nitrate reductase activity (m mol NO_2 $^{-}h_{-1}g^{-1}$ leaf fr. wt.)	*In vivo* nitrogenase (N_2-ase) activity (m mol NH_3 produced $h^{-1}g^{-1}$ root fr. wt.) Weightper g	Weight per plant	*In vitro* catalase activity (ml O_2 produced/ min/ g leaf fr. wt.)
Control	3.120± 0.357*	52.270 ± 1.770	8.591 ± 0.399	74.00 ± 0.000
Seed	4.034 ± 0.080(+ 52.42)	73.098 ± 0.164(+ 39.846)	16.518 ± 0.719(+ 92.270)	33.00 ± 0.000(- 55.40)
Seedling	4.214 ± 0.055(+ 35.06)**	58.422 ± 0.471(+ 11.769)	19.215 ± 2.760(+ 123.664)	48.00 ± 0.000(-35.13)
Basal	5.055 ± 0.052(+ 62.01)	85.634 ± 1.869(+ 63.83)	11.047 ± 1.597(+ 28.588)	48.00 ± 0.000(- 35.13)

The values are average of ten replicates. * Standard errors of the mean (N=10).
**Values in parentheses are per cent inhibition (-) or stimulation (+) with respect to control.

fixers (*Azotobactor*, *Azospirillum*, *Bacillus*, *Clostridium* and *Beijerinekia* etc.) associated with root of certain cereals and grasses have considerable potential to fix nitrogen utilized by associated plants (Dobereiner *et al*. 1972, Boddey and Dobereiner 1982, Patriquin *et al*. 1983). Suman *et al*. (2003) observed that bacterial inoculants (*Pseudomonas fluorescence* isolate PGr6 and *Azotobactor chrococcum* isolate Ac3) differentially influenced plant weight, shoot/root ratio, leaf lamina/leaf sheath ratio and partitioning of dry matter to various plant parts in *Saccharum* spp.

From the above results it may be concluded that maize responses well to the application of growth-promoting diazotrophs-*Rhizobium*, collected from *Vigna mungo* L. Now-a-days the use of growth-promoting and chemical fertilizers became essential for maximizing and stabilizing the crop productivity in the field of Tripura. In the present investigation, results on the effect of growth-promoting diazotrophs showed significant improvement in all parameters. Therefore, use of growth-promoting diazotrophs may reduce the amount of synthetic fertilizer.

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