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Studies on the effect of *Azospirillum* on the growth of *Vigna mungo*

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ABSTRACT

Bacteria of the genus *Azospirillum* are widely distributed in the rhizosphere of tropical and subtropical grasses and sugarcane. *Azospirillum sp.* is reported to occur in the rhizoplane of plants growing in saline soil. In this study the effect of *Azospirillum* on the growth and biochemical compound of *Vigna mungo* were analyzed *Azospirillum* biofertilizer was obtained from Tamilnadu Agricultural university, Coimbatore. The healthy *Vigna mungo* seed sample was purchased from local agro market at Thanjavur. *Azospirillum* was mixed with soil at different concentration, such as 1, 2, 3 and 4gm. Control was maintained without *Azospirillum*. The *Vigna mungo* seed coated with *Azospirillum slurry* and 10 seeds were sowed in each pot including control pot. After sowing the germinating ability, shoot and root length and leaf length and breath also calculated. After 25th day from the sowing the plant total chlorophyll and protein content was estimated. All the parameters increase in 4gm *Azospirillum* inoculated plant compared to other plants.

KEYWORDS

Azospirillum;
Vigna mungo;
Biofertilizer;
Morphometric;
Biochemical characters.

INTRODUCTION

Introduced strains in promoting the growth of crops due to their superior to their environment. Promoting growth and increased the yield of these cultivators of wheat in different areas of Israel. *Azospirillum spp* are among the most important bacteria involved in N_2 fixation in grasses. It is normal for *Azospirillum spp*. to be isolated from nitrogen free culture media inoculated with soil or roots[1,2]. The free living N_2 fixing rhizobacteria of the genus *Azospirillum* live in close association which plants roots where they exhibit beneficial effects on plant growth and yield of many crops of agronomic importance. Free living N_2 fixing bacteria such as *Azospirillum, Klebsiella* and *Azotobacter*[3,4] *Azosprillum* grown in culture are known to produce growth promoting compounds. Such as gibberellins, cytotoxins like substance and auxins.

Azospirillum was found to improve the growth of maize and rye grasses indicated that the potential of nitrogen fixing bacterium is *Azospirillum brasilense* enhanced the development and increased growth of several gramineae[5]. Inoculation of *Azospirillum brasilense* enhanced the development of plant, thus plant height, leaf length was significantly increased in wheat, sorghum and panicum observed that as microaerophilic

associative symbionts in association with roots of grasses and maize fixing considerable amount nitrogen gave an imptus to several workers to look for this bacterium in tropical soil[6,7]. In India field experiment with *Azospirillum brasilenses* have demonstrated the beneficial effect with without fertilizer nitrogen in different crops[8,9]. *Azospirillum* strains grown in nitrogen free OAB medium plants inoculated with pathogenic bacteria. The involvement of extracellular proteins in the adhension process of *Azospirillum* to biotic surfaces has been provided[10,11]. Based on the above fact the present study has been justifiably the effect of *Azospirillum* on the growth of *Vigna mungo*. Analyze the morphometric and biochemical parameters on the pot culture plant experiments.

MATERIALS AND METHODS

Collection of biofertilizer and seed

In this study the effect of *Azospirillum* on the growth and biochemical compound of *Vigna mungo* were anlaysed *Azospirillum* biofertilizer was obtained from Tamilnadu Agricultural University, Coimbatore. The healthy *Vigna mungo* seed sample was purchased from local agromarket at Thanjavur.

Soil used

In this study red soil sample was used for pot culture experiments which was collected from local area of Thanjavur.

Pot culture experiments

The effect of biofertilizer was analyzed by pot culture experiments. Five pot used for each biofertilizer. 250gm of sterile red sample was taken in each pot and *Azospirillum* was mixed with pot soil in different concentration such as 1, 2, 3 and 4g control also maintain without biofertilizer.

Seed sowing

10 health seeds were used for each pot. *Azospirillum slurry* was prepared with the help of starch solution. The seeds were mixed in the slurry. After the coating the seeds were sown on pots. After the seed sowing gentle flow of water and further irrigation was given frequently. After 10 days initial stage of growth were observed. After germination the morphometric and biochemical characters were analyzed.

Percentage of seed germination

The germinated seeds were counted after 5th, 10th and 15th days from the sowing date. The seed germinating percentage were calculated using this formula.

Root and shoot length

After 15th 20th and 25th days the plants were collected from each pot and washed with distilled water. Then the length of the top root and shoot was measured individually for each plant and expressed in cm.

Leaf length and breadth

The leaf length and breadth ratio were calculated from the well developed leaves of 15th, 20th, 25th days.

Estimation of chlorophyll content

The chlorophyll content of each pot culture plant was estimated by Arnon[12]. One gram of leaf material was collected and grinds it with 20ml of 80% of acetone. The extract was centrifuged at 5000rpm for 5 minutes. The supernatant was saved and the pellet was re-extracted repeatedly with the same solvent. Until the residues become colorless. Then the supernatant were collected and it was made up to 100ml with 80% acetone. Finally the optimal density was measured against acetone as blank at 645nm and 663nm. Chlorophyll-a, b and total chlorophyll were computed using Arnon's formula.

$$\%\ \text{of seed germination} = \frac{\text{No. of seed germinated}}{\text{No. of seed sowed}} \times 100$$

$$\text{mg chlorophyll a / g tissue} = 12.7(A_{663}) - 2.69(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{mg chlorophyll b / g tissue} = 2.29(A_{645}) - 468(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{mg total Chlorophyll / g tissue} = 20.2(A_{645}) + 8.02(A_{663}) \times \frac{V}{1000 \times W}$$

Where A = absorbance at specific wavelengths,
V = final volume of chlorophyll extract in 80% acetone,
W = fresh weight of tissue extracted.

Estimation of protein

The total protein was measured by the method of Lowry et al.[13] with bovine serum albumin as the standard. 0.2ml to 1ml of working standard and test samples were taken into a series of test tubes and made up to 1ml with phosphate buffer. A tube with 1ml of water served as a blank. To all the tubes 5ml of alkaline copper reagent was added and kept at room temperature for 10minutes. After 10minutes 0.5ml of Folin's phenol reagent was added and incubated a room temperature in the dark for 30minutes. After 30 minutes intervals blue colour was developed and it

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was read at 660nm. Standard graph was prepared by plotting the OD values obtained against the concentration of the standard.

RESULTS AND DISCUSSION

Percentage of seed germination

The seed germination effect was estimated on 5th, 10th and 15th day of each experimental pot, inoculated with *Azospirillum* and control. Highest seed germination (90%) was observed in the 15th day inoculated with 4gm of *Azospirillum*. At the same time lowest seed germination was noted control pot (60%), the 70, 70 and 80% of seed germination were noted in 1, 2 and 3gm *Azospirillum* inoculated pot. The results were presented TABLE 1. Previously studied *Azospirillum spp.* is frequent inhabitants of the rhizosphere of a wide variety of plants with three-carbon, four carbon, and crassulacean acid metabolism-type photosynthesis in diverse climatic regions of the world[14,15].

Estimation of root and shoot length

The root and shoot length of *Vigna mungo* was analysed both *Azospirillum* inoculated and control pot. Both root and shoot length were increased (9.3, 9.4cm) with application of *Azosprillum* when compared to control (3.9, 6.1) potted plant. The shoot and root length was estimated at 15th, 20th and 25th day, the results were presented in TABLE 2 and 3. Curl and Truelove[7], reviewed root exudates, which primarily contain organic acids, sugars, and amino acids, are a major source of nutrients for the microflora in the rhizosphere. Rosalind Padma[6], reported increase in root length in Papaya due to *Azospirillum*. Panwar et al.[16] reported that there was remarkable increase in the total biomass and overall growth of wheat due to *Azospirillum* treatment. This is accordance with the work showing increased germination, shoot length, root length obtained in the present study.

Leaf length and breadth

The leaf length and breadth were calculated on 15th, 20th and 25th day. Maximum leaf length and breadth (7.3cm and 3.9cm respectively) were noted in 4gm *Azospirillum* inoculated *Vigna mungo* plant compared to control plants. The results were presented in TABLE 4 and V.Jeeva[17], previously reported the pseudostem and leaf area in the pooven banana was also found to be increased by the application of *Azospirillum*.

Estimation of chlorophyll content

Chlorophyll content was estimated in the plants treated with *Azospirillum* and control. The results were presented in the TABLE 6. In this study highest chlorophyll content were noted in 3 and 4g *Azospirillum* inoculated plants when compared to other treatment plant. Lowest level of total chlorophyll content was noted in the control plant (0.034mg/g/FW). The total chlorophyll content of 0.035, 0.039mg/g/FW observed in the 2 and 3g *Azospirillum* inoculated plants respectively. At the same time lowest chlorophyll b content was noted

TABLE 1 : Percentage of seed germination

S.No	Concentration of *Azospirillum*	Days after sowed			% of germination
		5th	10th	15th	
1	Control	3	4	6	60
2	1g	6	6	7	70
3	2g	6	6	7	70
4	3g	7	7	8	80
5	4g	6	8	9	90

TABLE 2 : Root length of treated plant

S.No	Concentration of *Azospirillum*	Root length (cm)		
		15th day	20th day	25th day
1	Control	2.1	3.5	3.9
2	1g	4.6	5.1	5.8
3	2g	4.9	6.2	7.1
4	3g	4.7	7.2	8.2
5	4g	6.2	8.3	9.3

TABLE 3 : Shoot length of treated plant

S.No	Concentration of *Azospirillum*	Shoot length (cm)		
		15th day	20th day	25th day
1	Control	5.5	5.9	6.1
2	1g	6.7	6.2	7.4
3	2g	7.1	7.3	7.9
4	3g	6.3	8.2	8.1
5	4g	7.8	8.9	9.4

TABLE 4 : Leaf breadth of treated plant

S.No	Concentration of *Azospirillum*	Leaf breadth (cm)		
		15th day	20th day	25th day
1	Control	1.3	2.4	3.1
2	1g	1.5	1.9	2.3
3	2g	1.4	2.1	2.5
4	3g	1.5	2.2	2.7
5	4g	1.1	3.1	3.9

TABLE 5 : Leaf length of treated plant

S.No	Concentration of *Azospirillum*	Leaf length (cm)		
		15th day	20th day	25th day
1	Control	3.1	3.9	4.2
2	1g	3.7	4.1	5.5
3	2g	3.9	4.6	5.9
4	3g	3.6	4.9	6.1
5	4g	4.1	5.2	7.3

TABLE 7 : Estimation of protein in *Azospirillum* treated plant

S.No	Concentration of *Azospirillum*	% of total protein
1	Control	12.11
2	1g	13.31
3	2g	15.22
4	3g	16.18
5	4g	18.41

at 1g *Azospirillum* treated plant (0.013) compared to other treatments. Rosalind padma[6] reported that chlorophyll a, chlorophyll b and total chlorophyll was found to be increased over control, by the application of *Azospirillum* in papaya and tobacco. In the present study also there was an increase in chlorophyll a in 4gms of *Azospirillum* and a general increase in chlorophyll b with the biofertilizer treatment regarding total chlorophyll content at 4gm.

Total protein

Total protein content was estimated in all treated plant leaves samples. The investigation results were presented in the TABLE 7. In this present study the protein content was significantly increased by the addition of biofertilizer at the dosage of 4gm. The seed treated with 4gm of *Azospirillum* the protein 18.41% was observed. The control plant leaves protein content was low (12.11%) when compared to *Azospirillum* treated plants. The total protein content was 13.31, 15.22 and 16.18 was noted in 1, 2 and 3g *Azospirillum* inoculated plants. Kapulnik et al.[18] reported that inoculation of *Azospirillum* brasilense enhanced the development of plants there by plant height; leaf length was significantly increased in wheat, sorghum and panicum. Root branching, root uptake capacity was also found to be enhanced by *Azospirillum brasilense* and this effect on the root system is probably due to the growth hormone secreted by bacteria. He also reported that the potential *Azospirillum* also enhanced the development of increased growth of several gramineae.

TABLE 6 : Estimation of Chlorophyll of treated plant

S.No.	Concentration of *Azospirillum*	Chlorophyll 'a' (mg/g fw^{-1})	Chlorophyll 'b' (mg/g fw^{-1})	Total Chlorophyll (mg/g fw^{-1})
1	Control	0.018	0.016	0.034
2	1g	0.022	0.013	0.035
3	2g	0.022	0.017	0.039
4	3g	0.023	0.018	0.041
5	4g	0.024	0.018	0.042

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REFERENCES

[1] K.C.Marshall; 'Interfaces in Microbial.Ecology', Cambridge, MA: Harvard University Press, **(1976)**.
[2] N.Tejera; Plant and Soil, **270**, 223-232 **(1996)**.
[3] I.Nur; J.Cern.Microbiol., **123**, 27-32 **(1981)**.
[4] Y.Bashan, G.Holguin; Fla., 331-345 **(1993)**.
[5] E.Murgenstern, Y.Okan; Arid Soil.Res.Rehabil., **1**, 115-127 **(1987)**.
[6] T.M.Rosalind padma; 'Studies on the Response of Papaya and Tobacco to the Inoculation of *Azospirillum* and Phosphobacteria', M.Sc., (Agri.) Thesis. Tamilnadu Agricultural University, **(1988)**.
[7] E.A.Curl, B.Truelove; 'The Rhizosphere', Springer Verlag, Berlin, 55-92 **(1986)**.
[8] N.S.Subba Rao; Soil Biol.Biochem., **17**, 119-121 **(1985)**.
[9] P.J.Dart; Plant Soil., **90**, 303-334 **(1986)**.
[10] V.F.Dufrene, C.J.P.Boonaert, P.C.Rourhet; Colloids Surf.Biointerfase, **7**, 113-128 **(1996)**.
[11] B.R.Glick; 'Biochemical and Genetic Mechanisms Used by Plant Growth Promoting Bacteria', Imperial College Press, London, **(1999)**.
[12] D.I.Arnon; Plant Physiol., **24(1)**, 1-15 **(1949)**.
[13] O.H.Lowry, N.H.Rosebrough, A.L.Farr, R.J.Randall; J.Biol.Chem., **193(1)**, 265-275 **(1951)**.
[14] N.R.Krieg, J.Dobereiner; Bergey's Manual of Systematic Bacteriology, **1**, 94-104 **(1984)**.
[15] J.M.Harris; Soil Biol.Biochem., **21**, 59-64 **(1989)**.
[16] J.D.Panwar, S.M.Panday, Y.P.Abosi; Indian J.Plant Physiol., **33**, 185-189 **(1990)**.
[17] M.Jeeva; 'The Effect of *Azospirillum* on the Growth and Development of Banana', M.Sc (Hort) Thesis Tamilnadu Agricultural University, Coimbatore, **(1987)**.
[18] Y.S.Kapulnik, I.Soring, Y.Nur, J.Okon, Kigel, Hensis; Exp.Agri., **17**, 170-178 **(1981)**.