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REGULAR ARTICLE

RESPONSE OF PADDY (*ORYZA SATIVA* L.) VARIETIES TO *AZOSPIRILLUM BRASILENSE* INOCULATION

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SUMMARY

An attempt was made to study the effect of *Azospirillum* inoculation on the different paddy varieties to find out the best variety in terms of seedling characters such as seed germination, biomass and phytomass yield under controlled conditions. In the case of seed germination, the percentage of seed germination was higher in *Azospirillum* treated seeds than in control. Similarly, shoot and root lengths and fresh and dry weights of paddy varieties treated with *Azospirillum* inoculation showed better response than the untreated plants due to the secretion of plant growth hormones by *Azospirillum*. The biochemical parameters such as total chlorophyll, carotenoid, soluble protein and sugar and physiological parameters like photosynthetic rate were also increased to varying level in *Azospirillum* treated plants. The overall studies indicated that the growth of *Azospirillum* treated paddy seedlings excelled over the untreated ones due to biofertilizer effect upon nitrogen fixation. Among the several varieties tested for *Azospirillum* treatment, CO43 and AS89044 varieties showed good response.

Key words: Paddy, *Oryza sativa, Azospirillum,* Biofertilizer, Biomass, Phytomass.

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1. Introduction

Rice (*Oryza sativa* L.) is the staple food for half of the world's population especially in oriental countries. In India, about 2500 varieties of rice are being cultivated, from which more than 1500 varieties are in southern India which are preferred over others, owing to their high yield, good quality and quantity of grain, short duration of growth and resistance against pest and diseases. A large number of experiments have been conducted in several countries to investigate the effect of inoculation of various strains of *Azospirillum* spp. on cereals and grasses (Smith *et al*., 1976; Watanabe *et al*., 1981). The aim of the application of *Azospirillum* is to get fast growth, better health of the plant and higher yield. It is known to be a very active nitrogen fixer under laboratory as well as soil conditions. Various kinds of cereals were tested by using a member of nitrogen fixing bacteria *viz*., *Azotobacter, Nitrosomonas* and *Azospirillum* to increase yield under controlled conditions. Balasubramanian and Kuamr, 1987; Wani, 1990; Bashan and Holgain, 1995 investigated that *Azospirillum* treatment showed remarkable increase in the grain and the straw yield in sorghum, wheat, maize, paddy and other food and fodder crops.

The yield responses caused by *Azospirillum* inoculation may be due to biological nitrogen fixation (Hartmann *et al*., 1983). Split application of biofertilizer inoculation through seed, seeding and soil gave the highest grain, straw yield, plant height and number of productive tillers in rice (Gopalswamy and Vidhy asekaran, 1988). The objective of the present study was to investigate the effect of *Azospirillum* inoculation on the different paddy varieties to find out the best variety. The observations were made to note the seedling characters such as seed germination, biomass and phytomass yield of paddy varieties under controlled conditions.

2. Materials and Methods

Healthy, viable paddy seeds of seven varieties viz, CO43, ASD16, ASD18, AS89044, ADT36, ADT37 and IR64 were procured from Tamil Nadu Agricultural University, Coimbatore. The seeds were sterilized with 2% mercuric chloride solution before treatment. After sterilization, the seeds were washed well with sterile distilled water. Twenty five seeds were selected from each variety and dressed well with the paste of 250mg of *Azospirillum brasilense* in water. These seeds were dried under the shade condition and transferred after sowing to a plastic trough containing 2kg of sterilized garden soil. The troughs were watered regularly and being maintained under controlled condition. A control set up was also made by following the same conditions except the addition of biofertilizer. Five seedlings were selected at random from each trough and the following observations were made on the 10th, 20th and 30th day of sowing.

The seedlings were uprooted gently without causing any damage to the root and shoot systems and washed well with water. The shoot and root lengths were measured with a metric scale. The shoot and root fresh weights were determined using an electronic balance. Net photosynthesis rate (Pn rate) was measured at 10th, 20th and 30th day of sowing using infra red gas analyzer (model: ADCLCA-3) fitted with a Parkenson leaf chamber (PLL-3). The same leaf was removed and processed for the estimation of total chlorophyll and carotenoid (Horborne, 1973), protein (Lowry *et al*., 1951) and sugar (Dubois *et al*., 1956).

3. Results and Discussion

The results indicated that the growth of *Azospirillum* treated paddy seedlings excelled over the untreated ones. The seed germination studies revealed that the percentage germination of seeds were higher in *Azospirillum* treated seeds than in control (Table 1). The inoculation of the biofertilizer in the varieties CO43 and AS89044 showed a considerable increase in the seed germination than the other varieties under same experimental conditions. The reason for this may be due to the tremendous pressure developed inside the seeds, which is responsible for breaking of the seed coat quickly (Sifton, 1959). This pressure may be induced by phytohormones especially auxin, indole acetic acid, cytokinin and gibberelic acid like substances secreted by *Azospirillum* (Okon, 1985;1986).

Table 1. Seed germination studies between control and *Azospirillum* inoculated seedling of different varieties of paddy

Paddy variety	Control plants (%)	*Azospirillum* inoculated plants (%)
CO 43	78.3	93.3
ASD 16	66.0	78.3
ASD 18	72.5	88.5
AS 89044	74.5	90.5
ADT 36	63.0	76.5
ADT 37	65.3	76.3
IR 64	60.3	72.0

Mean of three replicates

The observations made on 10th, 20th and 30th days of sowing revealed that *Azospirillum* treated seeds had higher productivity than control. The seedlings from this particular biofertilizer treated seeds had longer shoot and root lengths than the untreated ones. Similar results were observed in other plant species. The seed

dressing by this biofertilizer induces the production of plant growth promoting substances and leads to the increase of shoot and root length (Table 2). Secretion of plant growth hormones by *Azospirillum* was reported in several cereals and grasses Balasubramanian and Kuamr, 1987: Bashan and Holgain, 1995). This also reflects a specific capability of the host plant to attract the bacteria and modify the rhizosphere and/or to respond to some bacterial activity and benefit from it (Bottini *et al*., 1989).

Table 2. Shoot and root lengths between control and *Azospirillum* inoculated seedlings of different varieties of paddy

Paddy variety	Control plants						*Azospirillum* inoculated plants					
	10th		20th		30th		10th		20th		30th	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
CO 43	15.3	6.8	19.3	14.1	26.8	16.5	18.4	8.4	21.5	14.8	28.2	19.4
ASD 16	12.4	5.3	15.5	11.1	20.5	14.7	17.5	7.9	19.6	13.1	26.1	15.7
ASD 18	14.1	5.9	17.4	12.3	20.3	15.8	17.1	9.2	20.2	12.7	25.4	17.7
AS 89044	12.4	6.3	16.7	10.8	20.1	16.0	17.0	7.0	18.1	13.1	22.0	17.3
ADT 36	14.3	6.1	16.2	11.2	19.4	14.3	15.3	7.6	19.2	13.5	23.1	16.9
ADT 37	14.7	7.2	18.1	13.1	20.9	16.1	17.0	8.8	18.7	14.2	22.0	18.1
IR 64	10.5	5.8	16.5	12.0	19.6	15.7	14.8	6.3	16.9	12.8	20.9	18.0

Values are the Mean $\pm$ standard deviation of five replicates

The fresh and dry weights of root and shoot system of paddy varieties were also found to be increased to a considerable extent in *Azospirillum* treated seedlings (Table 3 and 4). This may be due to the formation and development of numerous root branching, root hairs and primary and secondary lateral roots which increases the nutrient uptake capacity of roots (Gopalswamy and Vidhy asekaran, 1988; Hartmann *et al*., 1983). This effect on the root system as well as more root colonization and root proliferation are probably due to the growth hormones secreted by the bacteria and also nitrogen fixation by it. The increased nitrogen uptake from the soil might have correspondingly increased the biomass to some extent. The changes in root functions due to *Azospirillum* treatment in different wheat cultivars were also reported (Kapulnik *et al*., 1981). These growth enhancing effects are of interest because of their potential significance for yield increases in agronomic systems in which the use of fertilizers is the limiting factors for their development (Sarig *et al*., 1984).

Table 3. Shoot and root fresh weights between control and *Azospirillum* inoculated seedling of different varieties of paddy

Paddy variety	Control plants						*Azospirillum* inoculated plants					
	Days of sowing						Days of sowing					
	10th		20th		30th		10th		20th		30th	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
CO 43	240	22.0	425	42.1	717	71.7	272	31.3	560	51.5	928	101.1
ASD 16	182	25.5	374	34.7	557	70.6	194	30.8	488	45.7	618	98.8
ASD 18	232	24.3	417	40.1	725	69.6	243	31.4	467	48.4	768	96.7
AS 89044	195	18.8	398	40.4	641	77.0	206	25.8	424	50.0	691	78.3
ADT 36	185	20.1	432	39.9	684	70.1	196	29.9	435	46.5	721	97.0

ADT 37	210	20.4	426	40.7	710	76.0	280	24.6	427	41.6	812	103.1
IR 64	214	23.1	362	39.8	691	78.1	264	31.2	407	48.2	694	78.7

Values are the Mean ± standard deviation of five replicates

Table 4. Shoot and root dry weights between control and *Azospirillum* inoculated seedling of different varieties of paddy

Paddy variety	Control						*Azospirillum* inoculated					
	Days of sowing						Days of sowing					
	10th		20th		30th		10th		20th		30th	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
CO 43	21.0	6.4	42.9	10.2	67.1	17.4	28.5	7.2	62.2	11.5	99.8	24.7
ASD 16	20.2	5.1	40.7	9.4	75.7	17.6	24.3	6.7	54.3	10.9	97.3	18.5
ASD 18	20.3	5.4	43.7	10.6	69.7	18.3	23.4	5.8	59.7	11.0	97.2	19.4
AS 89044	20.6	5.7	35.5	7.6	83.4	16.1	20.9	5.8	51.3	10.4	90.9	19.4
ADT 36	19.7	4.7	39.9	10.0	81.3	16.4	20.9	6.0	49.7	10.8	88.3	18.6
ADT 37	18.8	4.2	28.7	9.9	80.6	16.2	25.3	5.1	42.6	10.1	80.6	20.2
IR 64	21.2	3.8	40.1	9.6	78.7	15.7	23.1	5.4	50.8	10.1	92.1	16.6

Values are the Mean ± standard deviation of five replicates

Table 5. Photosynthetic rate between control and *Azospirillum* inoculated seedlings of different varieties of paddy

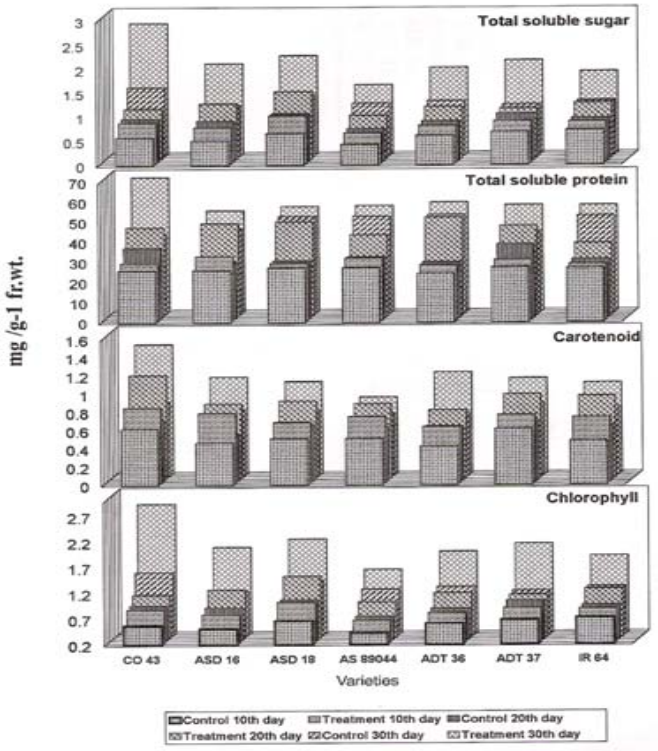
Paddy variety	Control plants			*Azospirillum* inoculated plants		
	Days of sowing			Days of sowing		
	10th	20th	30th	10th	20th	30th
CO 43	5.83	7.43	7.99	6.83	8.93	9.48
ASD 16	5.13	6.35	7.23	6.33	7.88	9.14
ASD 18	4.55	5.36	6.69	5.36	6.98	8.78
AS 89044	4.40	6.31	7.00	5.11	6.53	7.88
ADT 36	5.10	6.11	7.19	5.99	7.77	8.98
ADT 37	4.88	6.23	7.06	5.99	7.36	9.14
IR 64	4.69	5.81	7.23	5.63	6.98	8.68

*μ mole/m^2/S
Values are the Mean ± standard deviation of five replicates

Net photosynthetic rate was higher in *Azospirillum* inoculated plants than in control plants (Table 5). It may be due to the absorption of nutrients from the soil and stimulate the metabolism of photosynthesis. Photosynthetic activity plays an important role in the increase of leaf area leading to more biomass accumulation. The leaf area

was also increased to some extent in *Azospirillum* treated seedlings than untreated ones. The plants like *Digitaria decumbens*, *Panicum maximum and Pennisetum americanum* were subjected to *Azospirillum* inoculation and observed that the photosynthetic rate and dry matter contents were increased to a limited extent (Smith *et al*., 1976; Sarig *et al*., 1984;). The biochemical parameters such as total chlorophyll, carotenoid, soluble protein and sugar were increased to varying level in *Azospirillum* treated plants when compared to control ones (Fig.1). Very high contents of biochemical constituents in CO43 and very less in AS89044 were observed. The increased chlorophyll content could be correlated with the high level of photosynthesis this might be due to uptake of more nitrogen from the soil, which is fixed by this bacteria. The increased protein content may be due to the presence of kinetin which promotes the amino acid content which in turn helps in active protein synthesis (Tien *et al*., 1979). Similarly, the increased sugar content in the leaves might also be due to active role of *Azospirillum* in sugar metabolism (Watanabe *et al*., 1981).

Fig. 1: Certain biochemical parameters recorded between control and *Azospirillum* treated seedlings of different varieties of paddy



The results clearly showed that paddy variety, CO43 accounted well followed by ADT36 and ADT37. Other varieties showed poorer response. From these observations, it can be concluded that among the paddy varieties tested in response to *Azospirillum* inoculation, CO43 had high phytomass and biomass accumulation, physiological and biochemical parameters. It may be due to nitrogen uptake from the soil and proper utilization. The beneficial effect of *Azospirillum brasilense* varies itself which depending upon the plant varieties, microbial strains, method of inoculation and environmental factors particularly soil temperature, pH, Ec, moisture content and water holding capacity.

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