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Roles of *Azospirillum* spp. Inoculation in Two Consecutive Growth of Maize Plants

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Abstract : Two consecutive green house experiments were carried out to examine the effect of *Azospirillum* spp. inoculation on growth, nitrogen and phosphorus accumulation in maize plants grown in pots. There were eight treatments including an uninoculated control and *Azospirillum* strains OAD-3, OAD-9, AZ-22, AZ-8, AZ-9, *Azospirillum brasilense* BR-11001 and *Azospirillum lipoferum* BR-11080. The inoculated plants showed higher values in each of the following measurements; plant height, total dry mass and nitrogen and phosphorus accumulation in shoot when compared to the uninoculated control plants in two consecutive experiments conducted in the same soil. Among *Azospirillum* strains, *Azospirillum* sp. OAD-3 inoculated plants showed higher nitrogen accumulation by 44.5% and 45.1%, total dry mass by 48.6% and 66.9% in two consecutive experiments respectively. The nitrogen concentration in the maize plants was not changed significantly in the first experiment, however it increased significantly in the second experiment due to *Azospirillum* inoculation. In addition, *Azospirillum* sp. OAD-9 and *A. brasilense* BR-11001 also proved to be effective with respect to total dry mass, total nitrogen accumulation and total phosphorus accumulation. The nitrogen concentrations in maize plants were increased in the second experiment due to *Azospirillum* inoculation.

Key words : *Azospirillum*, maize, nitrogen, phosphorus, consecutive growth

INTRODUCTION

Azospirilla are gram negative, free-living nitrogen fixing rhizosphere bacteria. They display a versatile carbon and nitrogen metabolism, which makes them well adapted to establish in the competitive environments of the rhizosphere. Ammonium, nitrite, nitrate, amino acids and molecular nitrogen can serve as nitrogen sources[1]. In unfavorable conditions, such as desiccation and nutrient limitation, azospirilla can convert into enlarged cyst like forms[2,3]. This morphological change is accompanied by the development of an outer coat of polysaccharides and by the accumulation of abundant poly-β-hydroxybutyrate granules, which can serve as carbon and energy sources under conditions of stress and starvation[4,5].

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Recent evidence of significant biological nitrogen fixation in economically important gramineous species, particularly rice[6] and maize[7] generated tremendous interest in N_2 fixation by non-legumes[8-10]. Unlike most studies from the 1960s to 1980s that largely concentrated their attention on rhizosphere bacteria as likely sources of fixed N, much of the recent interest has focused upon the possibility that bacteria within the plant themselves i.e. "endophytic diazotrophs" [11], are responsible for the observed N_2 fixation.

The diazotrophic *Azospirillum* have been isolated from the rhizosphere and roots of a variety of plants including cereals and grasses. These bacteria are N_2 fixing organism living in close association with plants in the rhizosphere. Several reports have described the beneficial role of *Azospirillum* inoculation on plant growth, hence these organisms have attracted interest[12-14].

James and Olivared[15] briefly reviewed the evidence that endophytic diazotrophs may actually fix N_2 in plants and tra-

nsfer the fixed N products to their hosts. Arguably, field studies give the most important evidence for N_2 fixation. Although green house and laboratory studies can provide useful scientific information, significant yield increases in field crops due to N_2 fixation are the ultimate aim of all researchers in this area. Inoculation with *Azospirillum* spp. has been known to increase the yield of many cereals in the field by up to 30%, with often even greater increase under green house conditions. However, these results have been difficult to repeat. The factors responsible for these irregularities have not been identified, primarily because the basic feature of plant *Azospirillum* interactions are not well understood.

With this background, present experiments were conducted to elucidate the *Azospirillum* spp. inoculation effect on growth and nutrient uptake of maize plants in two consecutive experiments.

MATERIALS AND METHODS

The *Azospirillum brasilense* BR-11001 (=SP 7) and *Azospirillum lipoferum* BR-11080 (=SP 59) were obtained from Dr. J. Dobereiner, Embrapa, Brazil. The *Azospirillum* strains OAD-3, OAD-9, AZ-22, AZ-9 and AZ-8 were obtained from the Dep- artment of Microbiology, University of Agricultural Science, Dharwad, India. All the strains were maintained in a N-free semisolid medium.

Soil collected from the agricultural farm of Chungbuk National University was autoclaved twice with 7 days interval, and the pots (10x10 cm) were filled with 500 g of autoclaved soil. After completion of the first experiment, the same soil of each treatment and replication was retained and it was used for the second experiment.

Corn (*Zea Mays*) seeds were purchased from a market, and seeds were presoaked in water for one day before sowing. Three seeds per pot were dibbled into the soil. Thinning was done to maintain one seedling per pot 15 days after sowing.

The carrier-based inoculum of each bacterium was prepared separately by transferring a loopful of culture from 48 h old culture to 50 mL NFb broth. After five days of incubation at 28℃, the entire broth culture was transferred to a 1-L Erlenmeyer flask containing 500 mL NFb broth. The flasks were incubated at 28℃ for five days. The standard population of each *Azospirillum* was 10^8 CFU/mL which was made after serial dilution plate count method and inoculated to soil.

The pots were watered periodically to maintain the moisture to field capacity. Plant height, dry matter and N and P concentration in shoot were all measured at 45 days after sowing by following standard methods. The plant samples were dried and ground to powder using Wiley mill in order to estimate the N and P concentration.

Concentration of N and P was measured using the standard Kjeldahl method[16] and ammonium molybdate-ascorbic acid method[17] respectively. N and P accumulation was calculated by multiplying the dry mass and concentration of N and P respectively.

Results of the present experiment were statistically analyzed as Completely Randomized Design using the software package DYRSOFT DESIGN.

RESULTS AND DISCUSSION

The inoculation of plants with associative diazotrophicus *Azospirillum* was proposed as a new approach to supply the fixed nitrogen and substitute the nitrogen fertilizer application or to increase yield or both[18]. The nitrogen fixation and plant growth promoting abilities of *Azospirillum* have increased interest in its use as bacterial fertilizer. One of the principal mechanisms of its promotion of plant growth is related to the capability of *Azospirillum* to produce plant growth promoting substances[19].

The recorded plant height increase was not significant in the first experiment, however, it was significant in the second experiments due to *Azospirillum* inoculation (Table 1). In the first experiment, *Azospirillum* strains OAD-9, OAD-3, *A. brasilense* BR-11001 and *A. lipoferum* BR-11080 increased the plant height in descending order, however, all other strains were statistically on par with the uninoculated control. In the second experiment, *Azospirillum* sp. OAD-3 recorded the highest plant height, which is significantly superior over all other strains including standard *A. brasilense* BR-11001 and *A. lipoferum* BR-11080. In pooled analysis from both experiments, *Azospirillum* sp. OAD-3 showed the highest plant height increase closely followed by *Azospirillum* sp. OAD-9. Both of these were on par with each other, but significantly superior over other *Azospirillum* strains. The uninoculated plants showed the least plant height increase when compared to plants inoculated with *Azospirillum*. Increase in height of plants due to inoculation of *Azospirillum* has been reported by several workers[20-22]. Increased cell elongation and multiplication due to enhanced nutrient uptake by plants[23] following inoculation of

Table 1. Effect of *Azospirillum* spp. inoculation on plant height and total dry mass of maize plants in two consecutive growth

Inoculation	Plant height			Total dry mass		
	1st Expt	2nd Expt	Mean	1st Expt	2nd Expt	Mean
	cm			g/plant		
A. brasilense BR-11001	84.30	84.38	84.34	2.99	2.60	2.80
A. lipoferum BR-11080	84.50	86.38	85.44	3.44	2.38	2.91
Azospirillum sp. OAD-9	97.12	79.88	88.50	3.33	2.25	2.79
Azospirillum sp. OAD-3	91.75	88.88	90.31	3.50	3.10	3.30
Azospirillum sp. AZ-22	71.62	80.38	76.00	2.48	2.28	2.38
Azospirillum sp. AZ-9	82.50	83.25	82.87	3.67	2.06	2.86
Azospirillum sp. AZ-8	92.75	88.50	90.62	2.90	2.01	2.82
Uninoculated control	85.00	79.00	82.00	3.18	1.76	2.39
$LSD_{0.05}$	NS	4.87	3.02	0.42	0.58	0.28

Table 2. Effect of *Azospirillum* spp. inoculation on nitrogen and phosphorus accumulation in shoot of maize plants in two consecutive growth

Inoculation	N-Accumulation			P-Accumulation		
	1st Expt	2nd Expt	Mean	1st Expt	2nd Expt	Mean
	mg/plant			mg/plant		
A. brasilense BR-11001	39.00	32.72	35.86	1.88	0.74	1.31
A. lipoferum BR-11080	39.89	33.45	36.67	1.91	0.73	1.31
Azospirillum sp. OAD-9	35.74	32.99	34.36	1.83	0.70	1.26
Azospirillum sp. OAD-3	40.43	40.39	40.41	2.09	0.86	1.47
Azospirillum sp. AZ-22	34.20	31.34	32.77	1.49	0.63	1.06
Azospirillum sp. AZ-9	37.35	28.21	32.78	1.60	0.63	1.12
Azospirillum sp. AZ-8	31.70	30.34	31.02	1.37	0.81	1.09
Uninoculated control	23.17	27.84	25.50	1.46	0.66	1.03
$LSD_{0.05}$	5.59	5.46	4.26	0.44	0.07	0.23

Azospirillum probably caused the increased plant height. Plant growth promoting substances also play an important role in root elongation and shoot growth[24].

The plant dry mass also increased significantly due to *Azospirillum* spp. OAD-3, OAD-9, AZ-9 and *A. lipoferum* BR-11080 in both experiments. The pooled analysis showed higher dry mass in the *Azospirillum* inoculated plants, which were significantly superior over the uninoculated control plants. The highest dry mass observed occurred in plants inoculated with *Azospirillum* sp. OAD-3, which is significantly higher than all other *Azospirillum* inoculated plants. The least plant dry mass observed occurred in the plants without any inocu- lation (Table 1). Similar increases in dry mass and yield of other crops[25,26] due to *Azospirillum* inoculations have been reported earlier. *Azospirillum* attributed the increased production of growth promoting substances and N_2-fixation.

The nitrogen accumulation also increased in inoculated plants with *Azospirillum* in both consecutive experiments (Table 2). In the first experiment, *Azospirillum* sp. OAD-3 inoculated plants recorded the highest nitrogen accumulation which is followed by *A. lipoferum* BR-11001, *A. brasilense* BR-11080, *Azospirillum strains* AZ-9, OAD-9, AZ-22 and AZ-8 in the descending order, and all of which were sign- ificantly higher when compared to the uninoculated plants. In the second experiment, similar results were obtained. The ma- ximum nitrogen accumulation occurred in plants inoculated with *Azospirillum sp.* OAD-3, and least nitrogen accumulation occurred in plants without any inoculation. The pooled analysis also revealed similar results. These findings are in line with the results of Subba Rao[21] and Gadagi[22] who observed the increased nitrogen uptake in crops due to inoculation with *Azospirillum.*

The plants inoculated with *Azospirillum* also increased in phosphorus accumulation when compared to the uninoculated control plants (Table 2). The highest phosphorus accumulation occurred in plants inoculated with *Azospirillum* sp. OAD-3 in both experiments as well as pooled analysis. The least phosphorus accumulation occurred in plants devoid of inoculation. Nitrogen is known to increase the root growth and proliferation, which creates more absorptive area for the uptake of other nutrients like NO_3 and PO_4, K, Rb and Fe[27].

Nitrogen concentration in maize plants was not changed significantly in the first experiment due to *Azospirillum* inoculation, however it increased significantly in the second experiment when compared to the uninoculated control (Fig. 1-A). These results support the hypothesis of N_2 fixation by *Azospirillum*[28] in the present study. However, there are several reports which claim that the nitrogen contribution of *Azospirillum* is much less and the attributed plant growth is due to phytohormone production by the *Azospirillum*[29]. The Azo-

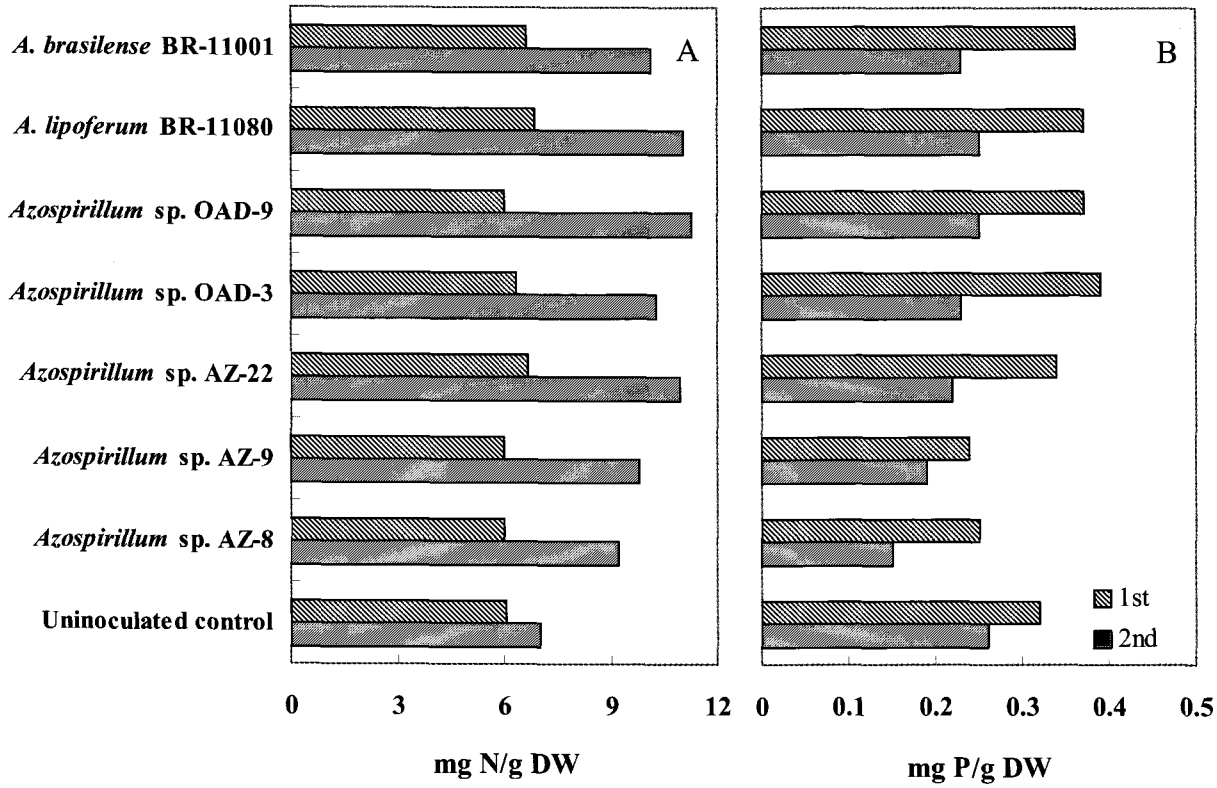

Fig. 1. Effct of *Azospirillum* spp. inoculation on nitrogen(A) and phosphorus(B) concentration in shoot of maize plants in two consecutive growth

pirillum sp. OAD-3 inoculation showed the maximum nitrogen accumulation and dry mass, however nitrogen concentration in the plant was less compared to *Azospirillum strains* OAD-9, BR-80 and AZ-22 inoculation, probably due to IAA and GA production of this organism[22] helps to enhance dry mass and N-uptake in the maize plants. These results indicate that apart from N_2 fixation, IAA and GA production also play an important role in improving plant growth and yield parameters.

Phosphorus concentration in the maize plant was not changed significantly in both experiments (Fig. 1-B). The phosphorus concentration in the maize plants reduced in the second experiment. This reduction is mainly because of experiments conducted without application of phosphorus fertilizer.

In two separate consecutive studies of *Azospirillum* inoculation in maize plants, the first experiment plants showed higher plant height, dry mass and nitrogen and phosphorus accumulation when compared to the second experiment plants. These results are mainly due to the experiments conducted without application of nitrogen fertilizer to know the nitrogen fixing capacity of *Azospirillum* strains. The reduction of these parameters might indicate that the application of nitrogen fertilizer is necessary, which speaks about the integrated nutrients management. *Azospirillum* inoculation alone would not help in augmenting maximum crop productivity. Hence, judicious application of nitrogen fertilizer with *Azospirillum* inoculation would have to be done for maximum plant growth and crop yield.

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