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Effect of Seed Priming with Growth Promoting Rhizobacteria at Different Rhizosphere Condition on Growth Parameter of Maize

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ABSTRACT

In this experiment the effects of three rhizobacteria named *Azotobacter*, *Azospirillum* and *Pseudomonas* at different rhizosphere condition on growth parameters were evaluated as a factorial experiment.10 straines of rhizobacteria include: P_1=*P.putida* strain R-168, P_2=*P.flurescens* strain R-93, P_3=*flurescens* strain 50090, P_4= *P.putida* DSM291, P_5=*Azotobacter chroococcum* strain 5, P_6=*Azotobacter chroococcum* DSM 2286, P_7=*Azospirillum lipoferum* strain 21, P_8=*Azospirillum lipoferum* DSM 1691, P_9=*Azospirillum brasilense* DSM 1690 and P_{10} = non- inoculation(control) were tested in both sterile and non-sterile soils. The results showed the interaction of two factors on stem and total fresh weight also on total dry weight and leaf area were significant. Results of this study revealed that soil natural condition had the higher effects on growth parameter than soil sterile condition.

Key words: pgpr , maize, growth, rhizosphere

INTRODUCTION

Colonization of plant roots by bacteria has been observed for a long time, but only lately has its importance for plant growth and development become clear. Plant Growth-Promoting Rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and increased plant growth (Glick, 1995). PGPRs are able to enhance plant growth by different mechanisms such as nitrogen fixation, production of phytohormones or status nutritional of plants (Kloepper, 1994; Glick, 1995; Cleyet-Marcel et al., 2001) which can improve the extent or quality of plant growth directly or indirectly. Several of these bacteria have been described as increasing plant water and nutrient uptake (Okon and Labandera-Gonzalez, 1994; Jacoud et al., 1999). PGPR can also enhance the plant competitiveness and responses to external stress factors as well as inhibiting soil-borne plant pathogens through antifungal activity (Sharma and Chahal, 1987) and siderophore production (Neiland, 1981; Suneja et al., 1996).

In last few decades a large array of bacteria including species of *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligens, Arthobacter, Burkholderia, Bacillus* and *Serratia* have reported to enhance plant growth (Kloepper et al., 1989;and Glick, 1995). Several studies clearly showed Inoculation of maize and wheat with *Azotobacter* , *Azospirillum* and *Pseudomonas* increased plant growth, nutrient uptake and yield (Tilak et al., 1982; Dobbelaere et al., 2001; Okon and Labandera- Gonzalez, 1994). Different strains of *Pseudomonas putida* and *Pseudomonas fluorescens* could increase root and shoot elongation in canola, lettuce and tomato (Hall et al., 1996; Glick et al.,

1997). It has also been reported that wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43% with Bacillus inoculation (Kloepper et al., 1991). Inoculation of plants with *Azospirillum* can result in a significant change in various growth parameters, viz. increase in plant biomass, nutrient uptake, tissue N content, plant height, leaf size, tiller numbers, root length and volume in different cereals (Okon,1985;Wani, 1990). Keeping the above information in view, the present experiment was planned to evaluate the effects of *Azotobacter*, *Azospirillum* and *Pseudomonas* inoculants on growth characteristics of Zea mays by means of a pot study.

MATERIALS and METHODS

For the evolution of growth promotion with PGPRs under pot conditions a laboratory study was carried out at research laboratory of Shahrood University of Technology in 2007. 10 levels of rhizobacteria include: P_1=*P.putida* strain R-168, P_2=*P.flurescens* strain R-93, P_3=*flurescens* strain 50090, P_4= *P.putida* DSM291, P_5=*Azotobacter chroococcum* strain 5, P_6=*Azotobacter chroococcum* DSM 2286, P_7=*Azospirillum lipoferum* strain 21, P_8=*Azospirillum lipoferum* DSM 1691, P_9=*Azospirillum brasilense* DSM 1690 and P_{10} = no inoculation were tested in both non-sterile and sterile soils. All treatments (bacterial inoculation× soil type) consisted of 60 plots i.e., 3 replicates with 20 pots per replication and a double seed per pot. Treatments were arranged in a factorial experiments based on completely randomized design. The plastic pots had a size of 15cm in diameter and capacity to hold 2Kg of soil (for sterile and non-sterile factors used autoclaved and natural soil respectively). The soil was silty clay loam in texture, having pH, 7.8; EC, 3.9ds.m-1; 0.75% of organic carbon; 0.04% N, 6.4 and 320 ppm of available P and K respectively. Seeds of maize (*Zea mays*, hybrid SC.647) were surface-sterilized with 0.02% sodium hypochlorite for 2 min, and rinsed thoroughly in sterile distilled water. For inoculation Seeds were coated with 20% gum arabic as an adhesive and rolled in to the suspension of any bacteria (10^8 cfu ml-1) with perlit mixture until uniformly coated. Seeds treated with sterile distilled water amended with gum arabic served as the non treated control. Seedlings were watered daily, and no artificial fertilization was used. After 30 days, fresh weight was determined by weighing the uprooted plants and dry weight by drying plants in an oven at 75̊C until the weight remained constant. The area of each expanded leaf area was calculated as $K \times \text{length} \times \text{width}$, where k =0.75 (Ruget et al., 1996).

All data in the present study were subjected by analysis of variance (ANOVA) using the Statistical Analysis System computer package (SAS system Ver.9). Least significant difference test (LSD) was applied to make comparisons among means at the 0.05 level of significance.

RESULTS and DISCUSSION

Inoculation of seeds with bacterial strains in different soil type did not stimulate leaf fresh weight, leaf and stem dry weight significantly (Table 1). Stem fresh weight and total fresh weight significantly increased with bacterial inoculation in this experiment. The highest stem fresh weight

were recorded from *A.brasilense* DSM1690 in sterile (5.62 g) and non sterile(5.39 g)soil, followed with *A.lipoferum* DSM1691 (5.4g) and *A.lipoferum* strain21(5.34g) in non sterile soil.

A.lipoferum DSM1691 in sterile soil and *A.brasilense* DSM1690 in both soil conditions produced more total fresh weight than other treatments. Stem and total fresh weight in *A.lipoferum* DSM1691 strongly decreased in sterile soil. Total dry weight were significantly ($p<0.05$) enhanced by bacterial inoculation and soil type. Application of *A.brasilense* DSM1690 in sterile soil had the highest effect on total dry weight as compared to control (Table 1).

Application of bacterial strains had significant effect on leaf surface area under both soil types. The results revealed that inoculation of maize seeds with *A.lipoferum* strain21 and *A.lipoferum* DSM 1691 in non sterile soil had the most leaf area (352.5 and 349.9 cm 2, respectively).

Experiment results indicated that the PGPR promote plant growth during the early stages of growth after sowing. This is the period when young seedlings and plants are so vulnerable to environmental stresses. This present investigation confirms the earlier work that showed Bacterial inoculants are able to increase plant growth, seed germination rate, improve seedling emergence, protect plants from disease and external stress factors (Lugtenberg et al., 2002). responses to external stress factors Findings were reported by Dobbelaere *et al.,*(2002), who assessed the inoculation effect of PGPR *A. brasilense* on growth of spring wheat, revealed that inoculated plants resulted in better germination, early development and flowering and increase in dry weight of both the root system and the upper plant parts. The mechanisms by which PGPRs promote plant growth are not fully understood, but one of the main action happend by effects of plant growth regulators such as auxins (Gutierrez Mañero et al., 2003). Kloepper et al.,(1986) reported PGPR synthesize phytohormones can promote plant growth at various stages.

Inoculation of maize seeds with *Azospirillum* strains compared with *Pseudomonas* strains and control under experiment conditions resulted in a more visible increase in shoot development, especially during the establishment of the plant. Kravchenko and Makarova(1993) shwed that PGPR strain of *Pseudomonas fluorescens* was not able to colonize the wheat root in non-sterile soil, and after 6 days only a small number of introduced bacteria were present at the root base, and practically none at the root elongation zone and at the apex. At the same time in sand this strain showed very good colonization of the same wheat genotype. However, *A. brasilense* can induce acidification of the rhizosphere (Carrillo et al., 2002) . *Azospirillum* spp. may change root physiology and patterns of root exudation (Heulin et al., 1987). Woodard and Bly(2000) reported that the corn inoculated with *A. brasilense* increased shoot dry matter.

Results indicate that application of *A. chroococcum* strain 5 sterile soil had more effect on growth parameters in sterile soil compared to non sterile soil. Martinez-Toledo (1988) showed that the numbers of *Azotobacter* decreased as plant growth continued in non-sterile agricultural soils, while the numbers of *Azotobacter* associated with maize roots grown in sterile agricultural soils remained similar to those of the original inoculums. In contrast for *A.chroococcum* DSM 2286 higher

enhancement observed in non sterile soil. This may imply that this strain had more competitive ability to survive and affect the growth of inoculated plants in the presence of indigenous micro flora.

It is concluded that in *Zea mays* different bacterial strains stimulated significantly growth parameters at both soil conditions (especially in non-sterile soil), when compared with that of control. Increase in growth parameters could associated with the ability to produce phytohormons, asymbiotic N2 fixation, antagonism against phytopathogenic microorganisms and solubilisation of mineral phosphates and other nutrients. Hence the strains used in the present study can be used as biofertilizer for the improvement of growth parameters of commercially cash crop i.e. maize.

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Table1.Effect of bacterial inoculation on growth characteristics of maize seedlings at 30 days after sowing in two different soils

Treatments	Fresh weight (g)						Dry weight (g)						Leaf area (cm^2)	
	Leaf		Stem		Total		Leaf		Stem		Total			
	non sterile	Sterile	non sterile	Sterile	non sterile	Sterile	non sterile	Sterile	non sterile	Sterile	non sterile	Sterile	non sterile	Sterile
P.putida strain R-168	5.28	3.58	4.55ab	3.20bcde	9.84abc	6.77cdefg	0.28	0.27	0.62	0.57	0.90abcde	0.84abcde	250.6abcd	173.1def
P.fluorescens strain R-93	5.04	1.42	4.48ab	1.15 e	9.53abc	2.56 g	0.33	0.11	0.61	0.38	0.94abcde	0.49efg	274.6abcd	109.3ef
P.fluorescens DSM50090	5.09	4.32	4.31ab	3.80abc	9.41abc	8.12abcde	0.27	0.26	0.59	0.55	0.86abcde	0.81bcde	244.1abcd	169.2def
P.putida DSM29*1*	5.28	3.56	4.55ab	2.89bcde	9.83abc	6.45cdefg	0.34	0.17	0.65	0.43	0.99abcd	0.60defg	252.4bcde	166.7def
A.chroococcum strain 5	4.26	4.85	3.63abcd	4.15abc	7.9abcde	8.99abcd	0.41	0.26	0.52	0.67	0.92abcde	0.93abcde	221.6abcd	247.8abcd
A.chroococcum DSM 2286	5.11	5.09	4.19abc	3.9abc	9.29abc	9.01abcd	0.28	0.27	0.70	0.61	0.99abcd	0.88abcde	255.0cdef	227.2abcde
A. lipoferum Strain 21	5.82	4.01	5.34a	3.2bcde	11.17ab	7.22bcdef	0.50	0.20	0.71	0.47	1.22ab	0.67cdef	352.5a	148.8def
A.lipoferum DSM 1691	6.13	1.50	5.40 a	1.56 de	12.20 a	3.07 fg	0.51	0.07	0.61	0.20	1.13abc	0.28 fg	349.9ab	87.50 f
A.brasilense DSM 1690	6.32	6.64	5.39a	5.62 a	11.72a	12.27 a	0.36	0.37	0.72	0.92	1.08abc	1.30 a	267.3abcd	325.4 a
Control	2.47	2.45	1.41 e	2.13cde	3.88efg	4.58defg	0.04	0.18	0.12	0.36	0.17g	0.54defg	196.3cdef	170.0def