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The Effect of Plant Growth Promoting Rhizobacteria (PGPR) on Germination, Seedling Growth and Yield of Maize

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***Abstract*—**The effect of plant growth-promoting rhizobacteria (PGPR) on seed germination, seedling growth and yield of field grown maize were evaluated in three experiments. In these experiments six bacterial strains include *P.putida* strain R-168, *P.fluorescens* strain R-93, *P.fluorescens* DSM 50090, *P.putida* DSM291, *A.lipoferum* DSM 1691, *A.brasilense* DSM 1690 were used. Results of first study showed seed Inoculation significantly enhanced seed germination and seedling vigour of maize. In second experiment, leaf and shoot dry weight and also leaf surface area significantly were increased by bacterial inoculation in both sterile and non-sterile soil. The results showed that inoculation with bacterial treatments had a more stimulating effect on growth and development of plants in nonsterile than sterile soil. In the third experiment, Inoculation of maize seeds with all bacterial strains significantly increased plant height, 100 seed weight, number of seed per ear and leaf area .The results also showed significant increase in ear and shoot dry weight of maize.

Keywords*—Azospirillum*, biofertilizer, Maize, PGPR, *Pseudomonas*.

I. Introduction

PLANT growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and increase plant growth and yield [1]. The mechanisms by which PGPRs promote plant growth are not fully understood, but are thought to include: - the ability to produce phytohormons [2], [3]. - asymbiotic N_2 fixation [4] , [5]. - against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes and/or fungicidal compounds [6], [7], [8] and also - solubilisation of mineral phosphates and other nutrients [9].

Significant increases in growth and yield of agronomical important crops in response to inoculation with PGPR have been reported [10], [11], and [12]. *Azospirillum*, *Pseudomonas* and *Azotobacter* strains could affect seed germination and seedling growth [13]. Kloepper *et al*. [38] has been shown that wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculation. Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* could increase root and shoot elongation in canola [14] as well as wheat and potato [15], [16]. Inoculation of plants with *Azospirillum* could result in significant changes in various growth parameters, such as increase in plant biomass, nutrient uptake, tissue N content, plant height, leaf size and root length of cereals [11]. Thus it has been shown that *Azospirillum* and *Pseudomonas* had the potential for agricultural exploitation and could use as natural fertilizers [17], [18]. However, the effects of these bacteria on growth and yield of some crop plants studied in previous works. But the effects of PGPR on growth parameter from germination to yield were not evaluated simultaneously. The main objective of this research was to determine if PGPR strains could affects on seed germination, growth parameters of maize seedling in greenhouse and also grain yield of field grown maize.

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II. Material and Methods

Six bacterial strains include *P.putida* strain R-168, *P.fluorescens* strain R-93, *P.fluorescens* DSM 50090, *P.putida* DSM291, *A.lipoferum* DSM 1691, *A.brasilense* DSM 1690 were used in this study that conducted at 2005-2006.

Bacterial strains were used as maize seed treatments. Seeds of maize (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 into the suspension of bacteria (10^8 cfu ml^{-1}) with perlit until uniformly coated.

Germination tests were carried out by the paper towel method. 25 seeds for each treatment with three replications in completely randomized design and incubated in growth chamber at 28°C. After 7 days the number of germinated seeds was counted. Root and shoot length of individual seedling was measured to determine the vigor index with following formula: Vigor index= (mean root length +mean shoot length) × % germination [19].

For the evaluation of maize seedling growth promotion with PGPRs, above bacterial strains were tested in both non-sterile and sterile soils at 2005. The plastic pots had 15cm diameter and capacity to hold 2Kg of soil .For preparation of sterile soil, field soil was autoclaved twice for 20 min at 120°C with a 24 h interval. All treatments (bacterial inoculation × soil condition) arranged in 48 pots i.e., 3 replicates with 14 pots per replication and a double seed per

pot. Treatments were arranged in a factorial experiment based on completely randomized design. Seedlings were watered daily, and no artificial fertilization was used. After 30 days, fresh weight was determined and dry weight calculated by drying plants in an oven at 75°C until the weight remained constant. For leaf area determination, the area of each expanded leaf was calculated as $K \times \text{length} \times \text{width}$, where k =0.75 [20].

Field experiment was conducted at research farm of Shahroud University of technology (latitude of 36° 25 'N and longitude of 54° 57' E with an elevation of 1345 m) in the period of May-September, 2006. The field 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 were washed with distilled water then inoculation was performed by a suspension of any bacteria (10^8 cfu ml^{-1}) with perlit mixture. Treatments were arranged as randomized complete block design with three replications. There were four rows in each plot. Which the row width and length was 0.7 and 9 meter, respectively. Before sowing, the soil was fertilized with N, P and K at rate of 300,150 and 50kg ha^{-1} as urea, single super phosphate and potassium sulphate, respectively. Half of nitrogen was applied at sowing time and residue at the start of reproductive stage. Seeds were placed at 5 cm depth. At the third leaf stage, plants were thinned to one plant per hill for the appropriate final stand of 75000 plants ha^{-1}. Ten mature maize plants were sampled from each treatment for final measurements in October (120 days after sowing). Leaf surface area was measured as describe above. In laboratory, samples were separated into different components and oven-dried at 75°C until reached to constant weight.

Data were statistically treated by ANOVA, Least Significant Difference (LSD) test at probability level 0.05 was used to separate the means when the ANOVA F-test indicated a significant effect of the treatments.

III. Results

Seed Inoculation significantly enhanced seed germination and seedling vigour of maize. However, the rate of enhancement varied with bacterial strains. All bacteria except *A.lipoferum* DSM1691, increased seed germination up to 18.5% over nontreated control (Fig.1). The highest enhancement of vigor indexes were obtained from *A.brasilense* DSM 1690 and *P.putida* strain R-168, which recorded 975 and 873 vigor index respectively.

The results of pot study showed that inoculation of maize seeds with bacterial strains did not affect leaf fresh weight and stem dry weight (Table I). In contrast, stem and total fresh weight significantly increased by inoculation in sterile and non-sterile soil. The highest stem and total fresh weight were recorded from *A.lipoferum* DSM 1691 in non sterile soil and from *A.brasilense* DSM 1690 in sterile soil. Leaf and shoot dry weight were significantly enhanced by bacterial inoculation and soil type ($p<0.05$). Application of *A.lipoferum* DSM 1691 in non sterile soil and *A.brasilense* DSM 1690 in sterile soil respectively had the best effect on leaf dry weight (0.52 gr.$plant^{-1}$) and shoot dry weight (1.30 gr. $plant^{-1}$). Furthermore, inoculation with bacterial strains had significant effect on leaf surface area under both soil conditions. The results revealed that in non sterile soil, *A.lipoferum* DSM 1691 caused an increase of 78.3% in leaf area while in sterile soil , leaf area was increased up to 65% in response to inoculation with A.*brasilense* DSM 1690 compared to control.

The results showed that inoculation with bacterial treatments had a more stimulating effect on growth and development of plants in nonsterile soil than sterile condition. *P.putida* strain R-168 and *A.lipoferum* DSM 1691 performed better than other strains in stimulating plant growth in pot experiment especially at nonsterile soil. Inoculation of maize seeds with all bacteria strains significantly increased the plant height (14.3-21.7%) and leaf area (Table2). However, seed dry weight increased with bacterial inoculation, but there were no significant differences between all treatments and control. 100 seed weight was significantly affected due to treatments with *Azospirillum* and *Pseudomonas* strains over the control. The most effective strain was *P.fluorescens* DSM50090 which increased 100 seed weight up to 44% over control. Effect of inoculation on number of seed per ear was also significant. Application of *P.fluorescens* strain R-93 and *A.lipoferum* DSM1691 gave the maximum number of seed per ear.

The results showed a clear and significant increase in ear and shoot dry weight of maize ($p<0.05$). The increase in these parameters, with different inoculation ranged from *P.putida* DSM291 (up to 70%) to *A.lipoferum* DSM1691 (up to 100 %) when compared with control (Table II).

IV. Discussion

Plant growth promoting effects of PGPR strains in different crops were clearly demonstrated [1]. Bacterial inoculants are able to increase plant growth and germination rate, improve seedling emergence, responses to external stress factors and protect plants from disease [21].

This present investigation confirms the earlier works. It revealed that under *in vitro* conditions, seed treatment with PGPR strains improved seed germination, seedling vigor, seedling emergence and seedling stand over the control. Similar improvement of seed germination parameters by rhizobacteria has been reported in other cereals such as sorghum [22] and pearl millet [23], [24]. The improvement in seed germination by PGPR was also found in work with wheat and sunflower[13], [25], where it was found that some PGPR induced increases in seed emergence, in some cases achieving increases up to 100% greater than controls. These findings may be due to the increased synthesis of hormones like gibberellins, which would have triggered the activity of specific enzymes that promoted early germination, such as a-amylase, which have brought an increase in availability of starch assimilation. Beside, significant increase in seedling vigor would have occurred by better synthesis of auxins [7].

In pot experiment, it was observed that inoculation with PGPR strains significantly promoted growth of seedling maize under different soil conditions. In general, inoculation resulted in early seedling growth and development. These results are similar with the findings of Dobbelaere *et al.* [36], [37]who assessed the inoculation effect of PGPR *Azospirillum brasilense* on growth of spring wheat. They observed that inoculated plants resulted in better germination, early development and flowering and also increase in dry weight of both the root system and the upper plant parts. Similarly, promotion in growth parameters and yields of various crop plants in response to inoculation with PGPR were reported by other workers [26], [27], [3]. Inoculation of maize seeds with *Azospirillum* strains compared with *Pseudomonas* strains under experiment conditions resulted in a more visible increase in shoot development, especially during the establishment of the plant. Khalid *et al*., [29]showed that responses of wheat growth to inoculation with rhizobacteria depend on plant genotype and PGPR strains as well as environmental conditions.

Soil condition influenced growth promotion by bacterial strains. *A.brasilense* DSM 1690 had more effect on growth parameters in sterile soil compared with non sterile soil. Martinez-Toledo *et al.* [31]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, inoculation with other bacterial treatments had a more stimulating effect on growth of plants in non-sterile soil than sterile condition. Abbass and Okon [34] hypothesized that IAA and other plant hormones were responsible for increased growth of canola, tomato (*Lycopersicon esculentum* Mill.), and wheat (*Triticum turgidum* L.) in non-sterile soil inoculated with *Azotobacter paspali*. Auxins produced by rhizobacteria can influence plants growth , including root development which improve uptake of essential nutrients thus increasing plant growth[28].This may imply rhizobacteria had more competitive ability to survive and affect the growth of inoculated plants in the presence of indigenous micro flora[29].In the other hand, as suggested by Roesti *et al.* [39] this result mean that inoculums of the PGPR strains on the seeds may have shifted the bacterial community equilibrium at early stages of plant growth and favoured for growth of beneficial populations.

In this study, inoculation of PGPR strains increased all parameters determined in field experiment. The positive effects of PGPRs on the yield and growth of crops such as wheat [30], [5] maize [2] soybean [9] and sugar beet [18] were explained by N_2 fixation ability, phosphate solubilizing capacity and phytohormons production.

The present experiment revealed that seed inoculation with all bacteria resulted in an increased plant height and leaf area (Table II).Similar increases in plant height and leaf area were observed in different crops inoculated with *Pseudomonas*, *Azospirillum* and *Azotobacter* strains [31], [13], [25], and [32]. Burd *et al.* [35] reported that plant growth promoting rhizobacteria might enhance plant height and productivity by synthesizing phytohormones, increasing the local availability of nutrients, facilitating the uptake of nutrients by the plants decreasing heavy metal toxicity in the plants antagonizing plant pathogens.Results have also showed that plants inoculated with PGPRs generally have higher seed dry weight than un-inoculated plants. The increases in seed dry weight were derived mainly from increase in 100 seed weight and number of seed per ear. This finding was supported by Yasari and Patwardhan [40] reported that application of *Azotobacter* and *Azospirillum* strains increased canola yield (21.17%), pod per plant (16.05%), number of branches (11.78%) and weight of 1000grain (2.92%).

The higher ear and shoot dry weight response to all inoculants compared to control clearly showed the beneficial role of these rhizobacteria. The enhancing effect of seed inoculation with rhizobacteria on shoot dry weight and yield of maize were reported by many researchers [33], [3]. Such an improvement might be attributed to N_2-fixing and phosphate solubilizing capacity of bacteria as well as the ability of these microorganisms to produce growth promoting substances [5]. In conclusion the results of this study suggest that simultaneous screening of rhizobacteria for growth and yield promotion under pot and field experiment is a good tool to select effective PGPR for biofertilizer development biotechnology.

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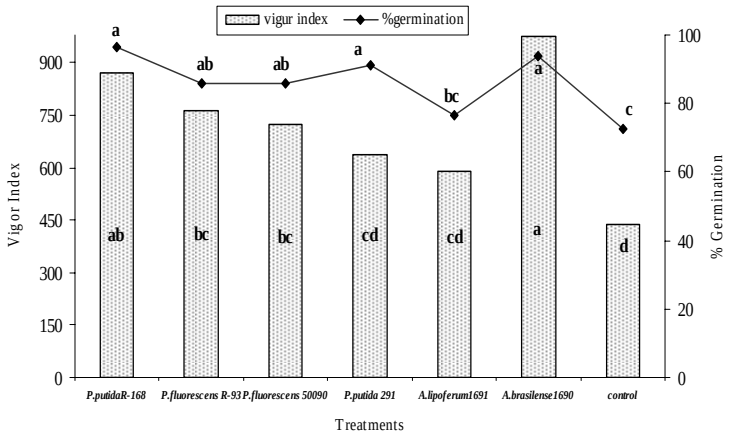

Fig. 1 Effects of bacterial inoculations on seed germination and vigor index of maize 7 days after germination in vitro conditions

TABLE I
EFFECT OF BACTERIAL INOCULATION ON GROWTH CHARACTERISTICS OF MAIZE SEEDLINGS AT 30 DAYS AFTER SOWING IN DIFFERENT SOIL CONDITION

Treatments		Fresh weight (g)			Dry weight (g)			Leaf area (cm^2)
		Leaf	Stem	Total	Leaf	Stem	Shoot	
P.putida strain R-168	non sterile	5.28*	4.55abc	9.84abc	0.29ab	0.62	0.90abc	250.6ab
	sterile	3.57	3.20abc	6.78abc	0.27ab	0.57	0.84abc	173.1bc
P.fluorescens strain R-93	non sterile	5.04	4.49abc	9.53abc	0.33ab	0.61	0.94abc	274.6ab
	sterile	1.42	1.15 c	2.56 c	0.11 b	0.38	0.49abc	109.3 c
P.fluorescens DSM50090	non sterile	5.09	4.31abc	9.31abc	0.27ab	0.59	0.86abc	244.1ab
	sterile	4.32	3.80abc	8.12abc	0.26ab	0.55	0.81abc	169.2bc
P.putida DSM29*1*	non sterile	5.28	4.55abc	9.83abc	0.34ab	0.65	0.99abc	252.4ab
	sterile	3.56	2.89abc	6.45abc	0.17ab	0.43	0.60abc	166.7bc
A.lipoferum DSM 1691	non sterile	6.13	6.07 a	12.20 a	0.52 a	0.61	1.13ab	350.0 a
	sterile	1.50	1.57 bc	3.07 c	0.08 b	0.20	0.28 bc	87.50 c
A.brasilense DSM 1690	non sterile	6.32	5.39ab	11.72ab	0.36ab	0.72	1.08ab	267.3ab
	sterile	6.65	5.62 a	12.27 a	0.37ab	0.93	1.30 a	325.4 a
Control	non sterile	2.47	1.08 c	3.88 c	0.05b	0.12	0.17c	196.3bc
	sterile	2.45	2.13abc	4.58 bc	0.18ab	0.36	0.54abc	170.0bc
LSD (0.05)		-	4.002	7.607	0.385	-	0.857	112.2

*In a column, means followed by a common letter are not significantly different at 5 % level by LSD.

TABLE II
EFFECT OF BACTERIAL INOCULATION ON GROWTH CHARACTERISTICS OF MAIZE SEEDLINGS AT 30 DAYS AFTER SOWING IN DIFFERENT SOIL CONDITION

Treatments	Plant height (cm)	Leaf area ($cm^2.plant^{-1}$)	Seed dry weight ($g.m^{-2}$)	100-seed weight (g)	No. of ($seed.ear^{-1}$)	Ear dry weight ($g.plant^{-1}$)	Shoot dry weight ($g.plant^{-1}$)
P.putida strain R-168	183.00 a*	5838.0 a	1394.36	25.84 bcd	761.36 ab	241.37 a	340.66 a
P.fluorescens strain R-93	178.33 a	5762.5 a	1617.21	24.35 cd	867.36 a	267.16 a	351.90 a
P.fluorescens DSM50090	185.00 a	6137.7 a	1454.64	31.00 a	736.23 ab	258.26 a	355.45 a
P.putida DSM291	176.58 a	6322.7 a	1361.64	30.31 ab	616.88 bc	235.16 a	331.20 a
A.lipoferum DSM 1691	187.75 a	5968.0 a	1653.35	28.41 abc	814.79 a	280.95 a	388.98 a
A.brasilense DSM 1690	176.33 a	5628.0 a	1464.42	29.98 ab	685.83 abc	259.63 a	355.97 a
Control	154.25 b	2176.0 b	778.57	21.41 d	509.56 c	133.72 b	192.97 b
LSD (0.05)	19.614	1743.4	560	4.509	193.48	84.306	102.69

*In a column, means followed by a common letter are not significantly different at 5 % level by LSD.