Pak. J. Bot., 47(SI): 325-328, 2015.

THE ROLE OF PLANT GROWTH PROMOTING RHIZOBACTERIA IN STIMULATING GROWTH AND YIELD OF MAIZE

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Abstract

Field experiment was performed to investigate the growth promoting activities of plant growth promoting rhizobacteria (PGPR) on different growth parameters of maize variety 'Pahari'. Three bacterial strains *Azospirillum brasilense* strain R1, *Azospirillum lipoferum* strain RSWT1 and *Pseudomonas* strain Ky1 were used to inoculate maize. Plant growth promotion was observed in all inoculated treatments over un-inoculated control which was evident from increase in plant height, number of grains/ear, thousand grain weight and biological yield. *Azospirillum lipoferum* strain RSWT1 was more effective in plant growth promotion than other strains. The inoculation with *Azospirillum lipoferum* strain RSWT1 evidenced increase in plant height (8.82%), number of ear/plant (35%), ear length (27%), ear weight (19.4%) and thousand grains weight (11.8%) compared to control treatment. Similarly, Inoculation with *Azospirillum brasilense* strain R1 result increased in plant height (6.5%), number of ears/plant (45.9%), ear length (23.7%), ear weight (17.25%) and thousand grain yield (9.68%) as compared to control treatments. Higher biological yield (9210 kg/ha) was produced when treatment was done with *Azospirillum lipoferum* strain RSWT1 followed by treatment with *Azospirillum brasilense* strain R1 (8960 kg/ha). The study revealed that beneficial strains of PGPR can be used as biofertilizer for maize.

Key words: *Azospirillum*, *Pseudomonas*, Inoculation of maize, Effects of inoculation.

Introduction

The world population depends on cereal crops for their food. Maize is the second most important cereal crop after wheat in Khyber Pakhtunkhwa and third most important cereal crop in Pakistan. It is a short duration kharif crop and is grown over an area of 981.8 thousand hectares in Pakistan. The national average yield of maize is 2849 kg/ha with annual production of 2729 thousand tons. In Khyber Pakhtunkhwa, the area under maize cultivation was 498.6 thousand hectares with 855.5 thousand tons production and provincial average yield was 1716 kg/ha. The average production of maize in Pakistan is lower as compared to other developing countries of the world (Anon., 2006). In order to overcome this shortfall, emphasis is given to increase maize productivity which mainly depends upon soil fertility i.e. the availability of soil nutrients like nitrogen and phosphorus etc. Chemical fertilizers were used to meet the nutritional requirement of plant (Khan *et al.*, 2013). However, this leads to environmental pollution and health hazards. The use of chemical fertilizers is also too costly, especially nitrogen, which is one of the common limiting factor of maize production (Ladha & Reddy, 1995; Midrarullah, 2014). The alternative source to fix important nutrients in the soil is the utilization of diastrophic microorganisms. They represent a variety of soil born bacteria associated with plants and results in stimulation of plant growth. They include *Azotobacter*, *Azospirillum*, *Azoarcus*, *Bacillus*, *Enterobacter*, and *Pseudomonas*, which are reported to colonize maize rhizosphere (Egamberdiyeva, 2007). Growth promotion of maize plant by bacterial inoculation has been manifested in several ways. Iswandi*et al*., (1987) reported significant increase in the growth and yield of maize due to inoculation of plant growth promoting rhizobacteria. However, better understanding of the fate of bacterial inoculants is required to fully exploit the potential of PGPRs. The present study was conducted with objectives to test the selected bacterial strains as inoculants for maize grown at Udigram Swat, Pakistan.

Materials and Methods

Roots of maize (*Zea mays* L.) variety 'Pahari' along with the rhizosphere soil of the plant were collected at Udigram, Swat. They were placed in small plastic bags and brought to the Laboratory. The rhizospheric soil was separated and stored at 4^{o}C and used for further studies. Serial dilutions (10X) of the samples were made and 100µL aliquots from 10^{-3}–10^{-5} dilutions were spread on LB plates (Maniatis *et al*., 1982). Semi solid NFM (Okon *et al*., 1977) was incubated with 100µL of these serial dilutions. The inoculated plates and NFM vials were incubated for 24-72 hrs at 30^{o}C.

Morphologically different colonies appearing on the growth medium were selected for further purifications. Isolated colonies were streaked on fresh plates with LB medium to get single-cell colonies. Single colonies were observed under the light microscope (Nikon Japan). Morphological and physiological tests were conducted to characterize bacterial strains. These tests included pigment production on nutrient agar medium, cell morphology and motility and growth at 30^{o}C on NFM and tentatively identified. In addition, three bacterial strains *Azospirillum brasilense* strain R1, *Azospirillum lipoferum* strain RSWT1 and *Pseudomonas* strain Ky1were obtained from the culture collection of Plant Microbiology Division, NIBGE, Faisalabad.

Field experiment was carried out during maize growing season in 2009 at Udigram, Swat. A fertile piece of land that had easy access to the water channel and convenient drainage system was selected for the

experiment. The land was prepared by ploughing with tractor. During seed bed preparation, the fertilizer dose of 120kg N and 60 kg P ha^{-1} was applied. The plot size was kept $15m^2$ each and 4 rows with 75cm distance. Randomized complete block design was used in the current investigation. Four treatments (T1: Control; T2: *Azospirillum lipoferum* RSWT1; T3: *Azospirillum brasilense*; R1; T4: *Pseudomonas* Ky1) were randomly allotted to the plots in four replications. The effect of the inoculated strains on different growth parameters of maize crop was observed. The plant height was recorded by measuring the height of randomly selected 12 plants in each plot with the help of meter rod and then average was taken. The number of ears per plant was calculated by counting the number of ears in 12 randomly selected plants per plot. Twelve ears in each plot were counted and then average was taken to calculate the average number of grains per ear. The number of leaves per plant was calculated by randomly selecting 12 plants from each treatment and then averages were taken. The stem thickness of plant in three central rows of each treatment was measured with the help of Vernier caliper. The thousand grain weight was calculated by randomly selecting 12 ears from each treatment and recording their weight with the help of electric balance. The effect of inoculated strains on grain yield was calculated with a balance after threshing the ears from 3 central rows and then converted into kg/ha. Similarly biological yield were calculated on 3 central rows of each treatment at their maturity and were harvested. They were sun dried, weighed with the help of spring balance and then converted into kg/ha.

Results and Discussion

The present results clearly indicate that plant growth promoting rhizobacteria significantly promoted plant growth and yield when compared to un-treated control. The stimulatory effects of rhizobacteria inoculation have been reported by other studies both in laboratory and field experiments (Kokalis-Burelle *et al*., 2002; Midrarullah, 2014). Okon & Labandera-Gonzalez (1994) reported that local isolates of *Azospirillum* increases maize yield from 15 to 25% with fertilization. All inoculations caused significantly increases in plant height over non-inoculated control. Inoculation with *Azospirillum brasilense* strain R1 increased fresh root weigh (50%) and fresh shoot weight (37.3%) of seedling after one week of inoculation. The increase in the dry root weight and dry shoot weight of seedling was 56% and39% respectively. *Azospirillum lipoferum* strain RSWT1 and *Pseudomonas* strain Ky1 also showed significant increase (30.5% and 24.2% respectively) in the root fresh weight and 42% and 13% in root and shoot dry weight respectively (Table 1). The results are in accordance with the finding of Dobbelaere *et al*., (2002) who reported an increase of 68.4 % in root and 42.6 % in shoot of maize seedling due to PGPR inoculation.

Inoculation with *Azospirillum brasilense* strain R1resulted increase in number of ears/plant (45.9%),ear length (27.7%) and ear weight (17.25%). The increase with *Azospirillum lipoferum* strain RSWT1 was 35%, 27% and 19.4% in number of ears/plant, ear length and ear weight. *Pseudomonas* strain Ky1 also showed significant increased (20.4 % and 10%) in the number of ears/plant and ear length; however the comb weight was not significantly affected by this bacterial strain (Table 1).Similar results were also obtained by Saubident *et al*., (2002), who reported significant increase in dry matter yield of maize due to *Azospirillum* inoculation in the field experiment. Taller plants (150 cm) were measured for treatment with *Azospirillum lipoferum* strain RSWT1 followed by *Azospirillum brasilense* strain R1 (147 cm) and *Pseudomonas* Ky1 (141 cm) while shorter plant height of the plants (138 cm) was measured for non-inoculated control ones. The results showed that plant height was significantly increased up to 8.82% with *Azospirillum lipoferum*strain RSWT1 and with *Azospirillum brasilense* strain R1 up to 6.52% (Fig. 1a). Results of the same nature were also reported by Jacoud *et al*., (1998), who stated that plant height of maize was significantly increased due to inoculation of *Azospirillum lipoferum.*

Table 1. Effect of inoculated bacterial strains on different growth parameters of maize (*Zea mays* L.).

SNo	Variety/ Treatment	n*	After one week of inoculation				No of Ears/plant	Comb dm* (cm)
			FrMwt (g)*	DrMwt (g)*	FSMwt (g)*	DSMwt (g)*		
1	*MZ-P-T1-U*	12	2.211^{c}	1.02^{c}	9.63^{c}	4.98^{c}	1.87^{d}	3.26^{b}
2	*MZ-P -T2-U*	12	3.163^{b}	1.78^{b}	12.21^{b}	6.69^{b}	2.89^{b}	4.23^{a}
3	*MZ-P -T3-U*	12	4.562^{a}	2.35^{a}	15.38^{a}	8.24^{a}	3.46^{a}	4.13^{a}
4	*MZ-P -T4U*	12	2.936^{b}	1.63^{b}	11.67^{b}	5.13^{b}	2.35^{b}	3.97^{a}
SNo	**Variety/ Treatment**	**n***	**RG/comb**	**NG /comb**	**CW Mwt±SD (g)***	**GW/comb Mwt±SD (g)***	**TY Mwt±SD (g)***	**GY*Kg/ha ±SD**
1	*MZ-P-T1-U*	12	12.24^{d}	290^{d}	193.26^{c}	97.32^{d}	328.4^{b}	1795^{d}
2	*MZ-P -T2-U*	12	14.12^{a}	368^{a}	239.73^{a}	133.67^{a}	372.3^{a}	2837^{a}
3	*MZ-P -T3-U*	12	13.47^{b}	345^{b}	233.56^{a}	126.34^{b}	363.3^{b}	2530^{b}
4	*MZ-P -T4U*	12	12.95^{c}	310^{c}	201.47^{b}	103.39^{c}	334.3^{ab}	2150^{c}

*n: sample size; Fr: Fresh root; Dr: Dry root; FS: fresh shoot; DS: dry shoots; Mwt (g): mean weight (g); dm: diameter; RG: rows of grain; NG: number of grains; CW: comb weight; GW: Grain weight; TY: thousand grain yield; GY: grain yield; T1: Control (non-inoculated); T2:*Azospirillum lipoferum* RSWT1; T3:*Azospirillum brasilense* R1; T4:*Pseudomonas* Ky1; U: udigram, Swat; MZ-P:maize variety pahari;data were analyzed by Statistics computer software program and LSD tests; values with different letterssuperscript show significantly different at 5%.

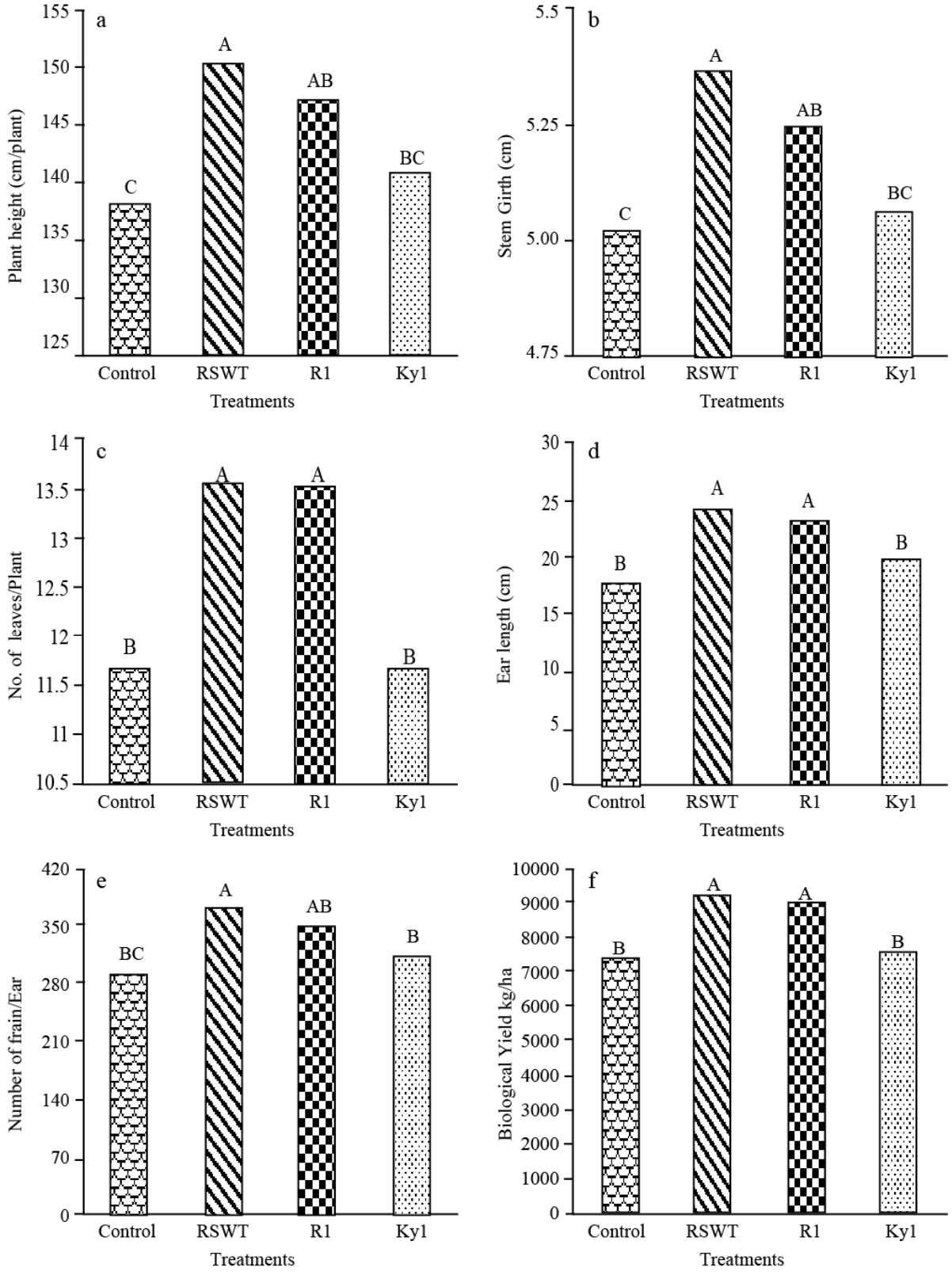

Fig. 1. The effect of PGPRs on different growth parameters of Maize; a: plant height; b: stem girth; c: number of leaves/plant; d: ear length; e: number of grains/ear; f: biological yield; control: non-inoculated; RSWT: *Azospirillum lipoferum*; R1:*Azospirillum brasilense*;Ky1:*Pseudomonas*; the values are an average of 4 replicates; data were analyzed by Statistics computer software program and LSD tests; different letters given above the bars in the graphs show that value are different at 5 % level of significance.

The results showed that numbers of leaves per plant were significantly affected by bacterial inoculation. Maximum numbers (13.56) of leaves per plant were recorded in *Azospirillum lipoferum* strain RSWT1. This was followed by *Azospirillum brasilense* strain R1 with a number of leaves per plant of 13.52. Minimum number of leaves per plant of 11.67 and 11.66 were recorded in treatment with *Pseudomonas* Ky1 and non-inoculated control treatment respectively (Fig. 1c). Positive response of bacterial inoculation on stem thickness was also recorded. Maximum stem girth (5.36 cm) and ear length (24.24 cm) was noted in case of *Azospirillum lipoferum* strain RSWT1. This is followed by *Azospirillum brasilense* strain R1 with stem girth of 5.24 cm and ear length of 23.16 cm. Minimum stem thickness of 5.06cm and 5.02 cm and ear length of 19.65 cm and 17.67 cm was recorded in treatment with *Pseudomonas* Ky1 and non-inoculated control treatment respectively (Fig. 1b and 1d). Kokalis-Burelle *et al*., (2002) reported significant increases in stem diameter stem area, number of leaves and yield of maize due to bacterial inoculation.

The inoculation caused the highest significant increase in number of grains/ear over all treatments except non inoculated control ones. Maximum numbers (368) of grains per ear were noted in case of Azospirillum *lipoferum* strain RSWT1. This is followed by *Azospirillum brasilense* strain R1 and *Pseudomonas* strain Ky1 with 345 and 310 numbers of grains per ear, respectively. Minimum number of grains (290) per ear was recorded for non-inoculated control treatment(Fig. 1e).Similar results were also obtained by Boddey & Dobereiner (1988) who pointed out that *Azospirillum brasilense* strain Sp-245 significantly increases the grains number and yield.

Biological yield data collected from whole plots indicated that the effect of inoculations was significant. Higher biological yield (9210 kg/ha) was produced when treatment was done with *Azospirillum lipoferum* strain RSWT1 followed by treatment with *Azospirillum brasilense* strain R1 (8960 kg/ha). The treatment with *Pseudomonas* Ky1 showed biological yield of 7496 kg/ha while lower biological yield (7360 kg/ha) was noted for non-inoculated control treatments (Fig. 1f). These results are in agreement with that of Okon & Labandera-Gonzalez (1994), who studied the effect of local isolates of *Azospirillum* on wheat crop in the field and reported 15-30 % increase in the yield and an increase of 50- 60 % in the yield when fertilized.

The results showed that all the 3 selected PGPRs (*Azospirillum brasilense* strain R1, *Azospirillum lipoferum* strain RSWT1, *Pseudomonas*Ky1 had promising positive effects on different growth parameters of maize grown under natural condition. In the light of the above results it is recommended that new techniques may be developed for the utilization of PGPRs (especially *Azospirillum*) as biofertilizers in order to reduce utilization of chemical fertilizers in agriculture practices for preventing environmental pollution and for maximum crop yield.

Conclusion

The present study showed positive impact of Plant Growth Promoting Rhizobacteria (PGPR) on the growth and yield of maize when used as inoculants. The beneficial strains of PGPR like *Azospirillum brasilense* strain R1, *Azospirillum lipoferum* strain RSWT1 and *Pseudomonas* strain Ky1 can be used as biofertilizer for cereal crops. It is also recommended that new techniques may be developed for the utilization of PGPRs (especially *Azospirillum*) in order to reduce utilization of chemical fertilizers in agriculture practices for preventing environmental pollution and for maximum crop yield.

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(Received for publication 26 August 2014)