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EFFECT OF PLANT GROWTH PROMOTING BACTERIA AND DROUGHT ON SPRING MAIZE (*ZEA MAYS* L.)

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Abstract

The aim of this study was to determine the affectivity of plant growth promoting rhizobacteria (PGPR) on some biochemical and agronomic parameters of maize exposed to drought stress. Two PGPR species *viz. Bacillus cereus* and *Pseudomonas putida* were applied as bioinoculant. Different methods of application of PGPR were used at different developmental stages of maize growth namely: M_1: Untreated seeds (no treatment with PGPR); M_2: Seeds soaked in the broth culture (7 day old) of the PGPR for 2-3 hours at room temperature prior to sowing; M_3: Foliar spray of PGPR at 3-4 leaf stage of plants; M_4: Broth culture (7 day old) incorporated to the soil in rhizosphere at 40 days after sowing (DAS). Results revealed that *P. putida* was more effective for the increase of chlorophyll content and showed linear increase in TDM (total day matter), higher CGR (crop growth rate), grain yield and water use efficiency (WUE) for TDM than *B. cereus.* PGPR application in the rhizosphere and foliar spray were more efficient for grain yield and WUE for TDM. However, foliar spray of PGPR showed maximum harvest index. So, it is recommended that foliar spray of PGPR at 3-4 leaf stage is a good strategy for getting higher maize yields. Water stress at blister was found to be more detrimental for spring maize crop. In our study, the effect of PGPR was maximized at 60 DAS.

Key words: Agronomy, Drought, PGPR, *Poaceae*.

Introduction

Maize (*Zea mays* L.) is an important cereal crop after wheat and rice. According to previous reports, reduction in yield occurs when maize suffers from water stress, especially at the time of critical growth periods (Panitnok *et al*., 2005; Gerpacio & Pingali, 2007; Mubeen *et al*., 2013a, b, c). Regulated deficit irrigation provides a way of reducing water costs without detrimental effects on yield. Recognizing drought sensitive stages in promising maize cultivars (under local situations of climate and soil fertility) permits irrigation scheduling; this may help us to get the maximum yield and efficient use of inadequate resources of water (Pandey *et al.*, 2000; Mubeen *et al.*, 2016).

Beneficial microbes are applied to the soil and to plant tissues directly or through seed inoculation, whereas soil application is preferred when there is risk of inhibitors or antagonistic microbes on the plant tissues (Mahmood *et al.*, 2016). According to Herman *et al.*, (2008) plant growth promoting rhizobacteria (PGPR) could be used for maximizing maize growth, development and yield. Kloepper *et al.*, (2004) reported the positive effects of PGPR on various plant attributes such as rate of germination, drought tolerance, shoots and roots dry weight, yield and yield components. PGPR are thought to enhance the availability of key nutrients for the host plant (Wu *et al*., 2005) by stimulating the synthesis of enzymes, fungicidal compounds and antibiotics (Asadullah & Bano, 2018). Research is needed to clearly define which bacterial strains are beneficial and essential for various environmental conditions and plants, therefore, the most favorable strains of bacteria can either be adopted or proliferated (Figueiredo *et al.*, 2010). Moreover, PGPRs are the potential tools in sustainable agriculture as well as trend for the future (Nasim & Bano, 2012).

Plants tolerance to drought can be encouraged by PGPR inoculations (which are adapted to soil water stress) (Marulanda *et al.*, 2008; Yasmin *et al.*, 2013). PGPR obtained from stress areas can help host plant to adapt to stresses (Marulanda *et al*., 2008; Sandhya *et al*., 2010). In a study published by Adjanohoun *et al.*, (2011), PGPRs showed specific behavior to specific crops, for example, *Pseudomonas* spp. including *Pseudomonas putida* (Trevisan) was found to be the best PGPR candidate for maize crop improvement. *Pseudomonas* spp. (with ACC-deaminase activity) in combination with optimal nitrogenous fertilizer concentration showed considerable impact on corn yield (Shaharoona *et al*., 2006). *Pseudomonas* spp. have the potential to survive in stress conditions because of exopolysaccharides (EPS) production, which guards microorganisms from water stress and variations in water potential by improving

water retention and regulation of carbon sources diffusion in bacterial environment (Ansary *et al.*, 2012). Similarly, some of the tested strains of *Bacillus cereus* (Grace and Percy) showed tolerance to Cd (1.78-4.45 mmol L^{-1}) and were positive for catalase, oxidase, phosphate solubilization, exopolysaccharide (EPS), and auxin production in maize (Ahmad *et al*., 2016).

In the present study we investigated the growth and yield of spring maize under drought stress and application of indigenous PGPR strains used as bioinoculant. Moreover, the purpose of the study was to optimize the most efficient method of application of these strains for managing water stress in maize.

Materials and Methods

Experimental place: This research was conducted in the greenhouse of Plant Sciences Department, Quaid-i-Azam University, Islamabad, Pakistan (33°.28" N, 72°.48" E). Islamabad represents the humid agro-ecological region in Pakistan. The overall picture of daily maximum and minimum temperature of Islamabad during the cropping year is shown in Fig. 1. However, maize plants were grown in small sized pots (having size of 0.035 m^2) in the greenhouse under controlled temperature to minimize the adverse environmental effects on the growth and development of maize.

Soil nutrient and moisture content analysis: Samples of soil (20 g) up to uniform depth of 30 cm were taken from nearby field (soil of this field composed of clay and sand in the ratio of 3:1 and was used to fill the pots for growing maize). These soil samples were used to determine different physico-chemical properties of soil by using the method described by Mubeen *et al.*, (2013b).

Soil moisture content (SMC) was measured at 0.3 bar field capacity (FC) and 15 bar (permanent wilting point) by depths of 0–30, 30–60 cm soil before seeding (Thongsaga *et al*., 2010). Weight of fresh samples was noted down and dry weight was calculated after oven drying the soil for 72 h at 70°C till obtaining of constant weight.

$$Soil\ moisture\ \%age = \frac{weight\ of\ wet\ soil\ (g) - weight\ of\ dry\ soil\ (g)}{weight\ of\ dry\ soil\ (g)} X\ 100$$

Inoculation of PGPR strains: The Luria-Bertani (LB) media was inoculated with 24 h old culture of two PGPR sequenced strains viz., *Bacillus cereus* (strain. NBS- L49) and S2: *Pseudomonas putida* (Acc no. KX580766 and accession numbers: JN624926.1) and incubated in shaking incubator at 30°C for 72 h. The inoculum was centrifuged at 3000 revolutions per minute (rpm) for 10 min; the supernatant was discarded and pellet was put in distilled water to regulate the optical density (OD) 1 at 660 nm which was equivalent to 10^6 cells per ml. It is a general practice to maintain OD of the bacterial culture to 1 measured at 660nm. This has been calculated and found to have 108 CFU mL^{-1} (colony forming unit) (Asadullah & Bano, 2018). Then sterilized seeds were soaked in this bacterial inoculum for 2-3 h (Bano & Fatima, 2009; Hadi & Bano, 2010; Nasim & Bano, 2012).

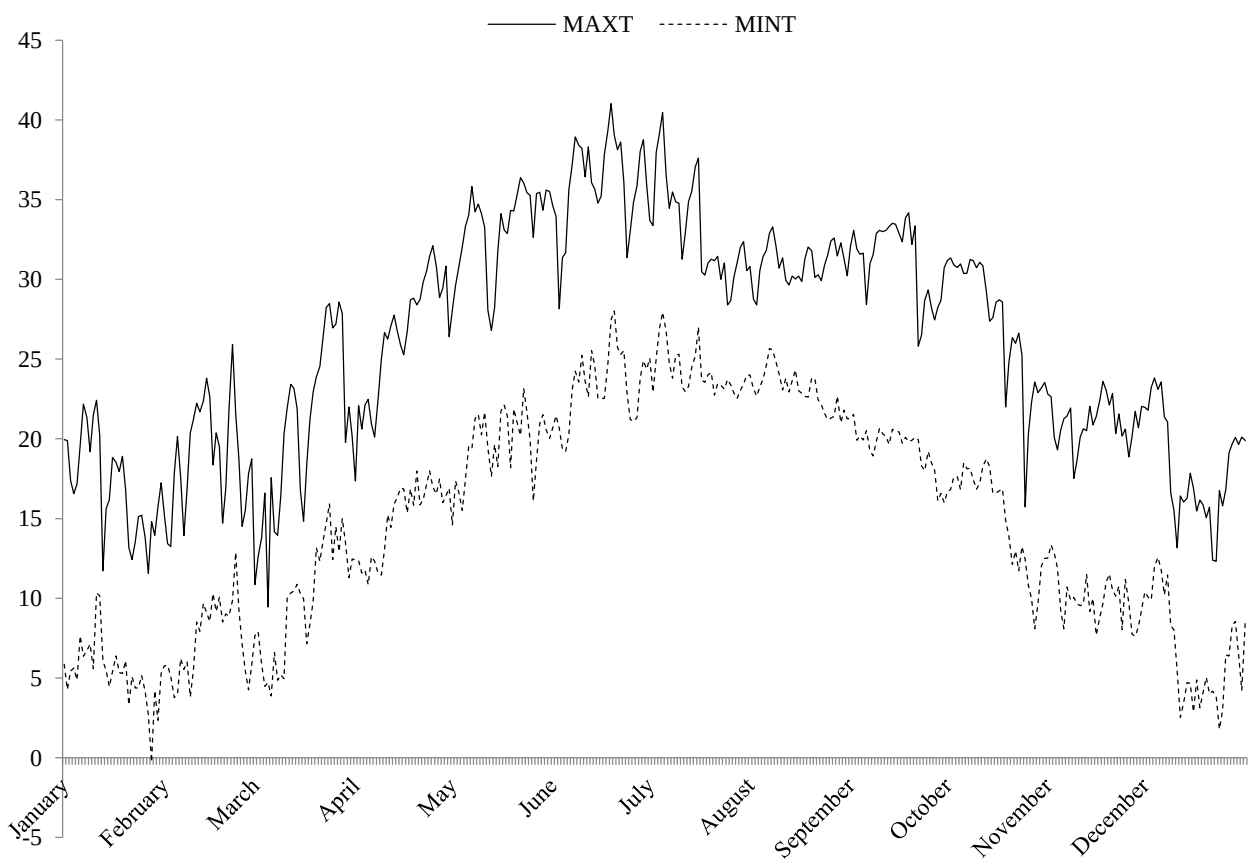


Fig. 1. Daily maximum and minimum temperature in Islamabad during the cropping year.

Experimental treatments: The research was conducted in completely randomized (CR) design having factorial arrangement with three replications. There were three factors; so the experimental treatments comprised three drought levels, two PGPR strains and four application methods. The drought levels comprised D_1: control (no drought), D_2: drought at 18 leaf stage (LS) or tasseling, D_3: drought at blister. The PGPR strains used were S_1: *Bacillus cereus* (strain. NBS- L49), S_2: *Pseudomonas putida* (Acc no. KX580766 and accession numbers: JN624926.1). The application methods were M_1: control i.e. no application of PGPR; M_2: seed treatment with PGPR; M_3: spray with PGPR at 3-4 leaf stage; M_4: PGPR application in the rhizosphere. For treatment M_2 (seed treatment with PGPR), seeds were soaked in broth culture of *B. cereus* and *P. putida* (having 10^6 cells/ml) for 2-3 h prior to planting. For treatment M_3, foliar spray of the two PGPR strains (having same number of cells as used for seed soaking) was done when the crop reached 3-4 LS. Similarly, for M_4, PGPR was diluted with 200% water and then applied in the rhizosphere at 40 days after sowing.

Crop husbandry: Grains of spring maize variety NARC-2704 (procured from Maize, Millet, Sorghum Research Program, National Agricultural Research Centre, NARC, Islamabad) were planted in small-sized pots (filled with soil having clay and sand in the ratio of 3:1) in the greenhouse during spring season. The sowing depth was kept 4 to 5 cm, as planting shallower than 4 cm in maize may increase the risk of poor or uneven germination during subsequent drainage of surface soils (Nielsen, 2010). Water was applied regularly to each pot till stand establishment. A known quantity of water was applied through each irrigation (measured by graduated beaker). A total of 400 mm water was applied in each treatment except in D_2 and D_3 in which the drought was imposed for the particular stage (i.e. tasseling and blister stages, respectively); in these treatments 350 mm water was applied. NPK (Nitrogen, Phosphate and Potassium) fertilizers were applied based on the recommendations of Department of Agriculture through urea, diammonium phosphate (DAP) and sulphate of potash (SOP). Half of N and complete P and K were added as side dressing before establishment. Half of remaining N was given as top dressing in two equal splits: initial at 20 days after sowing and another on tasseling. Greenhouse was covered with polyethene sheet of gauge-15 to maintain optimum temperature (15 to 35ºC). Plants were tagged soon after germination and growth stages (leaf and reproductive stages) were observed regularly. Pesticides were applied regularly (based on economic threshold level) to control the pests.

A thermometer was installed inside the greenhouse to note the temperature two times in a day (morning time at 7.0 am and in the afternoon at 3.0 pm) to take maximum and minimum temperatures in order to check for optimum growing conditions for maize.

Observations: Basically, two types of measurements are needed for growth analysis:

1. The plant weight- this is usually the oven dry weight (most often taken in kg) but it can be the organic matter or energy content.
2. The size of the assimilatory system- this is usually the leaf area (most often taken in m^{-2}) but it can be the leaf protein or chlorophyll content. If we specifically wish to consider the productivity of crops, it is convenient to express their performance per unit area.
3. This quantitative description of growth is based upon several terms. However, the terminology used here is based upon that of Hunt (1978).
   So, the following growth observations were taken:

Chlorophyll content: A SPAD Chlorophyll meter was used for measuring chlorophyll content on tagged plants. Chlorophyll contents were taken at one-month interval starting from one month after sowing till harvest maturity.

Time related leaf area index: Time related leaf area index (LAI) at one-month interval was observed. For this purpose, the length as well as width of every leaf was measured by hand and the area of every leaf was calculated as the product of length and maximum width after multiplying with 0.75. The LAI was then calculated through dividing the sum of leaf areas of every plant by the soil surface covered by every plant (Soler *et al*., 2007).

$$LAI = leaf\ area/land\ area$$

Time related total dry matter: Time related data of above ground total dry matter (TDM) at one-month interval was also observed.

Leaf area duration (days): Leaf area duration (LAD) is a measure of the persistence of the assimilatory surface. LAD was calculated following Hunt (1978).

$$LAD = (LAI_1 + LAI_2) \times (t_2 - t_1)/2$$

whereas LAI_1 and LAI_2 are the leaf area indices at sampling times t_1 (initial sampling) and t_2 (second sampling), respectively; this was LAD_1. Subsequent samplings provided LAD_2 (from the values of LAI_2 and LAI_3) and LAD_3 (from the values of LAI_3 and LAI_4), while adding the previous LAD(s). Cumulative LAD was the last one calculated by above method. As LAD is the product of a dimensionless unit and time, the units of LAD are time (usually expressed in days).

Crop growth rate (g m^{-2} d^{-1}): Crop growth rate (CGR) serves as a simple index of agricultural productivity and is expressed in terms of weight per unit area and time (kg m^{-2} s^{-1} or g m^{-2} d^{-1}). CGR was assessed following Hunt (1978).

$$CGR = (W_2 - W_1) / (t_2 - t_1)$$

W_1 and W_2 are the dry weights at sampling times t_1 and t_2, respectively.

Net assimilation rate (g m^{-2} d^{-1}): The mean net assimilation rate (NAR) was estimated by means of the formula given by Hunt (1978).

$$NAR = TDM/LAD$$

Here TDM and LAD are the last TDM and leaf area duration, respectively. In addition to growth, the following parameters were also taken:

Grain yield: Total plants in every pot were taken for resolving of various yield components. Total plants were threshed by hand for the approximation of pot yield and changed into t ha^{-1}.

Harvest index: Harvest index (HI) was measured through the following formula:

HI = (Grain yield / Total dry matter) x 100

Water use efficiency: Water use efficiency for grain yield and TDM was calculated using the following formulae.

WUE_{GY} = GY/Water applied

WUE_{TDM} = TDM/Water applied

where WUE_{GY} and WUE_{TDM} stand for water use efficiency for grain yield and total dry matter, respectively. This WUE is based on the criteria of absolute response (the amount of water applied).

Statistical analysis

Data collected on different variables were analyzed statistically with the statistical programme Statistix 8.1 (Muhae-Ud-Din *et al*., 2018). Analysis of variance method was applied to test the importance of the data, however Fisher's protected least significance difference (LSD) test on P = 0.05 was used to relate the differences between treatments means for various yield and yield components of the maize crop (Steel *et al*., 1997; Mubeen *et al*., 2013b).

Results and Discussion

Weather and soil characteristics: The mean greenhouse temperature during the maize growing period was ideal for the growth and development of maize. The soil at the experimental site (pH 7.34) contained organic matter 1.31%, nitrogen (N) 0.071%, phosphorus (P) 7.52 ppm and potassium (K) 115 ppm. Bulk density, field capacity (FC), and wilting point were 1.59 g cm^{-3}, 22.0%, and 13.0%, respectively.

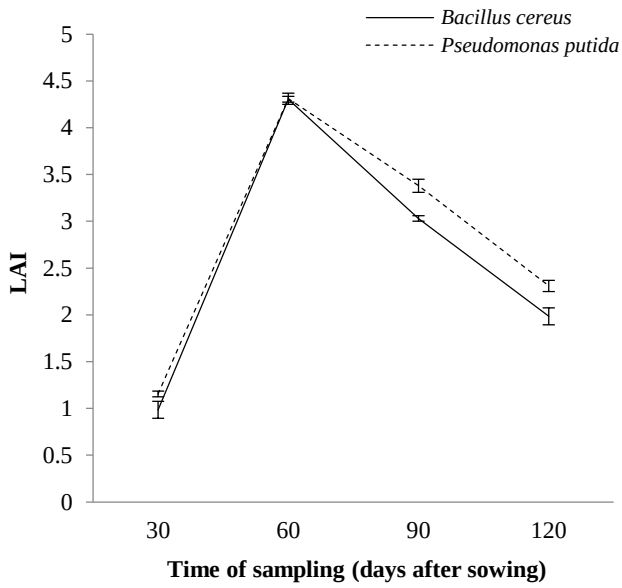
Growth parameters

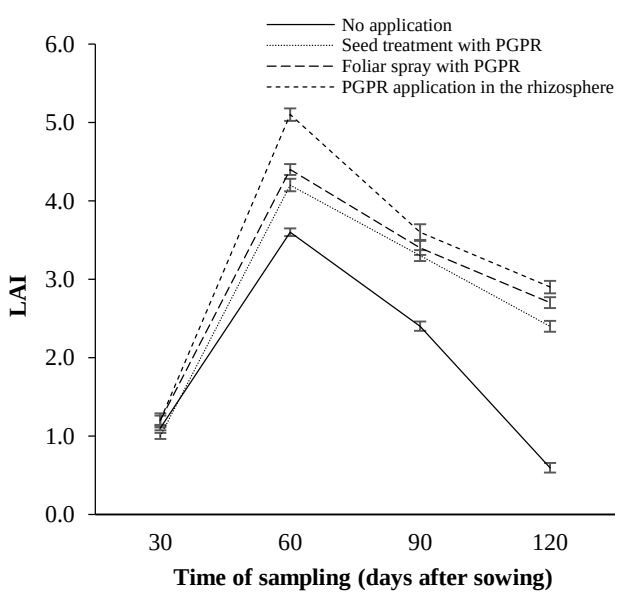
Time related Chlorophyll, LAI and TDM: Table 1 shows variation in time related chlorophyll content as an effect of PGPR strains and application methods and drought at different phenological stages of maize. It is clear from the table that maximum chlorophyll content was achieved at 60 days after sowing (DAS) with treatments of PGPR strains, application methods and drought at different growth stages of maize. After 60 days the chlorophyll content was declined. In case of PGPR strains, *Pseudomonas putida* showed statistically higher chlorophyll than *Bacillus cereus* at all the measurements except at first sampling when it was statistically at par with *B. cereus.* While discussing PGPR application methods, it was obvious from Table 1 that foliar spray with PGPR (M_3) was statistically at par with PGPR application in the rhizosphere (M_4) at all the sampling dates. These treatments were followed by Seed treatment with PGPR (M_2) and no application (M_1) (showing statistically non-significant results among each other). In case of drought levels, drought at blister was better in chlorophyll yield at 90 and 120 DAS.

Fig. 2 shows variations in leaf area index (LAI) with time due to application of PGPR strains. Maize achieved maximum LAI at 60 DAS in both the strains (Fig. 2). Fig. 2 also showed that maize behaviour was similar in LAI at the beginning in both PGPRs but different behaviour at the last developmental phase; *P. putida* produced slightly more LAI than *B. cereus* at these stages. Our results were somewhat in confirmation to those of Adjanohoun *et al.*, (2011) who reported that the overall effect of each group of PGPR on the emerging maize leaves was not statistically significant.

Similar to PGPR strains, LAI values progressively increased in all the PGPR application methods and reached at maximum value at 60 DAS (Fig. 2). After 60 DAS, LAI reduced in all application treatments and reached its smallest values in range of 0.6 to 2.9. Such LAI decrease was clearer in treatments getting no PGPR; this was due to further senescence of leaves in such treatments. Similarly, in situation of irrigation levels, the maximum LAI value was obtained at the irrigation treatment where no drought was applied. However, the minimum LAI was obtained when irrigation was withheld at blister stage. These results validate the studies by Mansouri-Far *et al.*, (2010) who also described that in maize, LAI value was significantly decreased when the water deficit was given on V8 (8-leaf) stage, but, then, this decrease was compensated in the treatments in which water was given on the R3 (dough) stage.

Total dry matter (TDM) production improved increasingly to physiological maturity in the two PGPR treatments (Fig. 3). It was evident from the figure that initially TDM values of the two PGPR were close but with the passage of time, *P. putida* showed greater TDM production (than *B. cereus*) reaching a value of 1583 g m^{-2} at physiological maturity. These results confirmed the findings of Adjanohoun *et al.*, (2011) who reported that *P. putida* (among a no. of PGPR strains) showed more promotory effect on maize plant height and could be considered a promising PGPR to improve maize crop development. More TDM was obtained in the PGPR methods of application in which PGPR foliar spray was done (M_3) and PGPR was applied in the rhizosphere (M_4), (as contrast to seed treatment with PGPR).





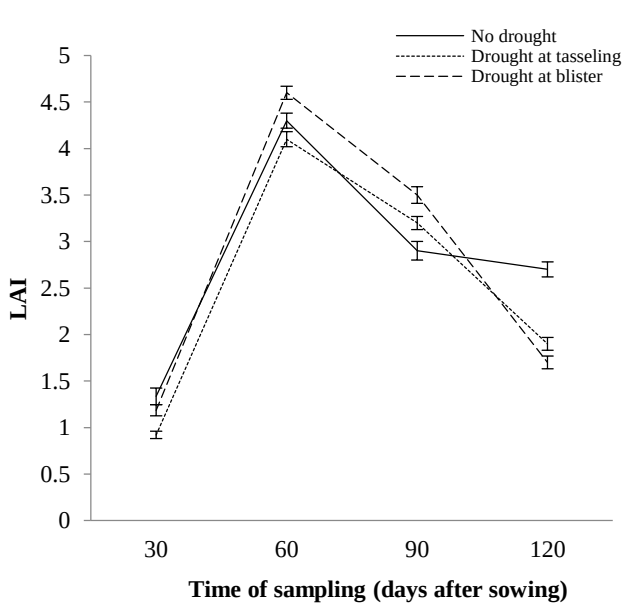
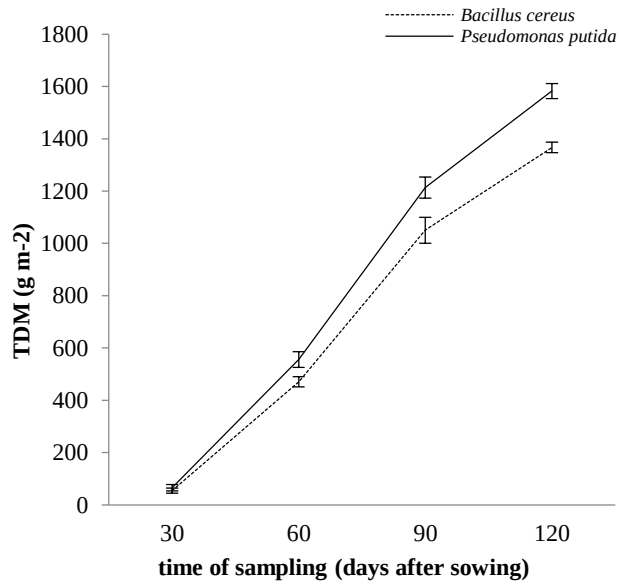
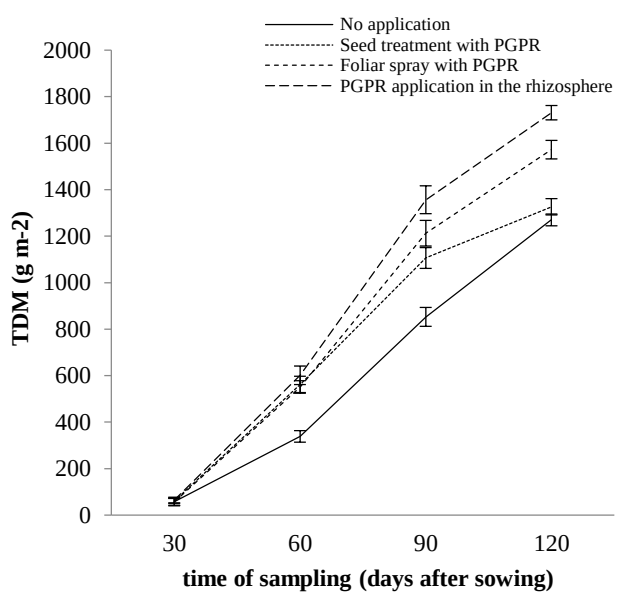


Fig. 2. Changes in LAI as affected by PGPR strains and application methods and drought at different maize stages.





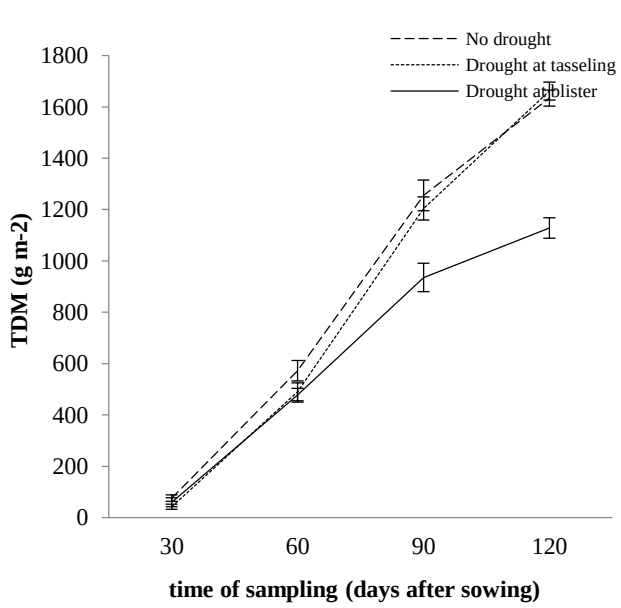


Fig. 3. Changes in TDM as affected by PGPR strains and application methods and drought at different maize stages.

Table 1. Time related Chlorophyll contents in maize as influenced by PGPR strains and application methods and drought at different maize stages.

Treatments	30 DAS†	60 DAS	90 DAS	120 DAS
PGPR strains (S)				
Bacillus cereus	27.0	36.5 b	31.4 b	27.6 b
Pseudomonas putida	28.5	39.2 a	34.0 a	29.9 a
LSD (p≤0.05)	1.6	1.5	1.8	1.9
PGPR method of application (M)				
No application	26.7 b	35.6 b	29.7 b	26.7 b
Seed treatment with PGPR	25.9 b	35.7 b	30.7 b	26.8 b
Foliar spray with PGPR	29.8 a	40.5 a	35.1 a	31.1 a
PGPR application in the rhizosphere	28.6 a	39.7 a	35.3 a	30.5 a
LSD (p≤0.05)	2.1	2.1	2.5	2.8
Drought levels (D)				
No drought	27.1	37.6	36.3 a	31.7 a
Drought at tasseling	28.1	37.7	30.0 b	26.3 c
Drought at blister	28.0	38.3	31.7 b	28.3 b
LSD (p≤0.05)	1.9	1.8	2.0	1.9

†DAS = Days after sowing

Table 2. Leaf area duration (LAD), Crop growth rate (CGR) and Net assimilation rate (NAR) in maize as influenced by PGPR strains and application methods and drought at different maize stages.

Treatments	LAD (Days)	CGR (g m^{-2} d^{-1})	NAR (g m^{-2} d^{-1})
PGPR strains (S)			
Bacillus cereus	265	14.6 b	5.16
Pseudomonas putida	283	16.9 a	5.60
LSD (p≤0.05)	45.2	2.2	0.95
PGPR method of application (M)			
No application	205 c	13.5 b	6.18 a
Seed treatment with PGPR	276 b	14.1 b	4.80 b
Foliar spray with PGPR	293 a	16.8 a	5.38 a
PGPR application in the rhizosphere	323 a	18.5 a	5.37 a
LSD (p≤0.05)	45.3	2.5	0.85
Drought levels (D)			
No drought	277	17.3 a	5.91 a
Drought at tasseling	261	18.0 a	6.36 a
Drought at blister	286	11.9 b	3.94 b
LSD (p≤0.05)	38	3.3	1.02

Table 3. Grain yield (GY), harvest index (HI) and water use efficiency (WUE_{GY} and WUE_{TDM}) as influenced by PGPR strains and application methods and drought at different maize stages.

Treatments	GY (t ha^{-1})	HI	WUE_{GY}	WUE_{TDM}
PGPR strains (S)				
Bacillus cereus	5300 b	38.77	1.33	3.42 b
Pseudomonas putida	5880 a	37.16	1.47	3.96 a
LSD (p≤0.05)	492	2.91	0.31	0.51
PGPR method of application (M)				
No application	3785 c	29.80 c	0.95 b	3.18 b
Seed treatment with PGPR	5165 b	38.96 b	1.29 ab	3.31 b
Foliar spray with PGPR	6695 a	42.58 a	1.67 a	3.93 ab
PGPR application in the rhizosphere	6715 a	38.80 b	1.68 a	4.33 a
LSD (p≤0.05)	615	3.15	0.59	0.91
Drought levels (D)				
No drought	6080 a	37.21 b	1.52 ab	4.09 a
Drought at tasseling	6165 a	37.09 b	1.76 a	4.75 a
Drought at blister	4515 b	40.02 a	1.29 b	3.22 b
LSD (p≤0.05)	689	2.72	0.48	0.75

When drought was applied at different phenological stages of maize, drought at blister showed least TDM at physiological maturity. The TDM production at final stages was almost equal to the treatments D_1 (no drought) and D_2 (drought at tasseling). Results of this study confirmed the studies of Khan *et al.*, (2003) who described increased TDM through increasing amount of irrigations (2004 g m^{-2} in 6 irrigations and 1333 g m^{-2} in 4 irrigations). This indicated that irrigation during reproductive period was a key component of biomass production in maize. These outcomes verified the results of other studies (in studies of Mubeen *et al.*, 2013b and Mubeen *et al*., 2016), related effect of water deficit in maize have also been discussed.

LAD, CGR, NAR: Differences in total dry matter as well as grain yield, for instance, influenced through various agronomic treatments may or may not be described through differences in their greatest leaf area indices (LAIs). Therefore, leaf area duration (LAD) values were also calculated to study the significance of photosynthetic area for the period of growth (planting to maturity) (Table 2). PGPR strains variations in LAD were non-significant in our experiment. This is likely due to similar LAI production by the two strains initially and slightly different LAI at later stages. PGPR application methods showed a significant effect on LAD. Treatment M_4 (PGPR application in the rhizosphere) showed the maximum LAD; on the other hand, it was statistically similar to treatment M_3 (Foliar spray with PGPR). These two were followed by M_2 (PGPR application to seed). This can be ascribed to enhanced LAI in such treatments. The lowest LAD was obtained in treatment M_1 (No PGPR application) (Table 2). There was no significant difference in LAD of various drought levels.

PGPR strains significantly differed in mean CGR (Table 2). *P. putida* showed higher CGR (16.9 g m^{-2} d^{-1}) than *B. cereus*. This may be due to greater differences in TDM among the two strains throughout the growing season. Foliar spray with PGPR was statistically at par with PGPR application in the rhizosphere at all the sampling dates (Table 2). These treatments were followed by Seed treatment with PGPR and no application (showing statistically non-significant results among each other).

Irrigation levels significantly affected mean CGR (Table 2). The higher mean CGR was recorded in pots irrigated throughout and in those in which drought was applied only at tasseling; these two treatments were statistically similar in mean CGR. A greater CGR for the duration of flowering (anthesis) may be a pre-requisite to get a maximum grain yield. The deficit irrigation at blister showed a smaller mean CGR. These results revealed that optimum water application till the final growth of crop had good effect on crop growth rate. Similar results were obtained by Mubeen *et al*., (2013b) who demonstrated that greater decrease in dry matter yield was obtained in the maximum water deficit given in maize.

The average net assimilation rate (NAR) in a crop characterizes the average photosynthetic production for each unit leaf area duration (Hunt, 1978). It was clear from Table 2 that PGPR differences regarding NAR were found to be non-significant in our experiment. However, there were significant differences among various PGPR application methods. The higher NAR was observed in treatments M_1, M_3 and M_4 which were statistically similar to each other. The lowest NAR was observed in M_2 (seed treatment with PGPR). In overall, average NAR values presented positive response to applications of irrigation. Irrigation level (no drought) and drought at tasseling showed more NAR due to higher production of TDM in such treatments (see previous section). The range of NAR given by these treatments was 5.91 to 6.36 g m^{-2} d^{-1}. Limiting levels of irrigation reduced NAR and smallest values were found for treatment D_3 (drought at blister). To explain the reasons determinative of grain yield in various treatments, the growth of plants was observed during the growing period. The importance of LAI and leaf greenness to clarify the differences is dependent on development phases at which maize suffers water deficit. In fact, LAI and leaf greenness decide the capture and use of solar radiation intercepted by maize plant, hence they influence the conversion rate of available radiation to dry matter accumulation (Mansouri-Far *et al*., 2010).

Yield and harvest index: Table (3) showed that PGPR differences in grain yield were significant in this experiment. Grain yield value for *P. putida* (5880 kg ha^{-1}) was statistically higher than *B. cereus*, (5300 kg ha^{-1}). The difference in grain yield of these two PGPR strains may be due to chlorophyll content, TDM and CGR. While discussing PGPR application methods, one could see from Table 3 that the grain yield of foliar spray with PGPR 6695 kg ha^{-1}) was statistically similar to PGPR application in the rhizosphere (6715 kg ha^{-1}). These treatments were followed by Seed treatment with PGPR (5165 kg ha^{-1}) and the least grain yield (3785 kg ha^{-1}) was observed in no application. It means treatment with PGPR in one way or the other increases the grain yield. The behaviour of PGPR in soil in terms of water stress has been discussed by Ansary *et al*., (2012) who described that *Pseudomonas* survived under stress conditions due to the production of exopolysaccharides (EPS), which protected microorganisms from water stress and fluctuations in water potential by enhancing water retention and regulating the diffusion of carbon sources in microbial environment.

Drought at tasseling and no drought treatments were statistically similar in terms of grain yield (6165 and 6080 kg ha^{-1}, respectively). These treatments were followed by water stress at blister giving 4515 kg ha^{-1}. Less grain yield in this treatment was due to lesser CGR and NAR that was a cause of decreased growth rate, number of grains for each cob, mean grain weight and radiation use efficiency, and therefore less grain yield. In contrast, it is evident from the Table 3 that treatment D_2 (drought at tasseling) was equally efficient and statistically similar to the treatment having greater number of irrigations (D_1). These results validated the results of Khan *et al.*, (2003) who obtained greater grain yield at 6 irrigations as compared to 7 irrigations. Therefore, it is determined that giving increased number of irrigation is not a good methodology for obtaining higher yields. Instead, research should focus on irrigation scheduling under varied environmental situations.

Harvest index (HI) shows the physiological productivity of plants to variation in the fraction of photo-assimilates to grain yield. The two PGPR strains presented non-significant differences regarding HI (Table 3). However, there was statistical difference among various PGPR application methods and drought levels. In case of application methods, Foliar spray with PGPR (M_3) produced highest HI and it was followed by the treatments of M_2 and M_4 giving HI values of 38.96 and 38.80, respectively. The lowest HI was found in treatment where no PGPR was applied.

Levels of irrigation also showed varied behavior for this parameter of yield (Table 3). Higher HI was obtained in treatment having drought at blister (40.02). This was followed by the other two treatments (drought at tasseling and no drought giving HI values of 37.09 and 37.21, respectively). Results of the study recommended that an optimal irrigation supply was important for enhancing dry matter partitioning between grain and other parts in maize.

Water use efficiency: The calculation of water use efficiency was based on the criteria of absolute response (the quantity of irrigation water applied).

The water use efficiency for grain yield (WUE_{GY}) was non-significant for the two PGPR strains (Table 3). The values of WUE_{GY} for *B. cereus* and *P. putida* were 1.33 and 1.47 g m^{-2} mm^{-1}, respectively. As far as PGPR methods of application are concerned, PGPR application in the rhizosphere and the foliar spray with PGPR were the more efficient treatments for utilizing water in terms of grain yield. These two treatments were, however, at par with seed treatment with PGPR. The least WUE_{GY} was obtained where no PGPR was applied. Similar to grain yield, higher values of WUE_{GY} were obtained in treatments of no drought and drought at tasseling.

The result of treatments on WUE for TDM production (WUE_{TDM}) are represented in Table 3. There was statistical difference in WUE_{TDM} values between the two PGPR strains. The WUE_{TDM} produced by *B. cereus* and *P. putida* were 3.42 and 3.96 g m^{-2} mm^{-1}, respectively. Similar pattern as was found in WUE_{GY} was also observed by PGPR application methods in WUE_{TDM}. No PGPR application was the least efficient user of water; however, seed treatment with PGPR was also statistically similar to this treatment. As regards irrigation levels, WUE_{TDM} followed the similar trend as was shown by WUE_{GY}. Higher values of WUE_{GY} were obtained in treatments of no drought and drought at tasseling.

The results showed that there was statistically no difference in treatments of D_1 i.e. no drought (in which we have to go for further amount of irrigations) and the treatment D_2 (drought at tasseling stage). So deficit irrigation can be used as a useful principle for obtaining appreciable yield if a smaller amount of irrigation is applied by some optimum level for a given type of soil and weather conditions.

Kumar *et al.*, (1996) determined that grain yield and TDM production at various irrigation regimes were connected with conforming evapotranspiration values. Zelikovick *et al.*, (1997) reported that WUE was greater in maize when 290 mm water was applied all through the growing period than the maize grown under no irrigation. Pandey *et al.*, (2000) showed that WUE did not increase when irrigation was withheld for the duration of vegetative and reproductive phases compared to completely irrigated.

Conclusions

Pseudomonas putida was found more efficient than *Bacillus cereus*. The previous research in the Lab of Prof. Asghari Bano demonstrated that the PGPR survived and proliferated but this test was not done in the present investigation. However, the significant differences in physiological and biochemical effects observed in plants due to PGPR (as compared to uninoculated control) clearly demonstrate the association of PGPR with plants and their proliferation when used as Bioinoculants. The foliar spray of PGPR (at 3-4 leaf stage) > rhizosphere application of PGPR was more promotory for TDM, HI and yield than that of seed treatment. The effect of PGPR was maximized at 60 DAS. Water stress at blister was found to be more detrimental for maize crop.

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References

Adjanohoun, A., M. Allagbe, P. Noumavo, H. Gotoechan-Hodonou, R. Sikirou and K. Dossa. 2011. Effects of plant growth promoting rhizobacteria on field grown maize. *J. Anim & Plant Sci.*, 11(3): 1457-1465.

Ahmad, M., O.C. Turgay, M. Farooq and R. Hayat. 2016. Seed biopriming with plant growth promoting rhizobacteria: a review. *FEMS Microbiol. Ecol.*, 92: 1-14. doi: 10.1093/femsec/fiw112.

Ansary, M.H., H.A. Rahmani, M.R. Ardakani, F. Paknejad, D. Habibi and S. Mafakheri. 2012. Effect of Pseudomonas fluorescent on Proline and Phytohormonal Status of Maize (*Zea mays* L.) under Water Deficit Stress. *Ann. Biol. Res.,* 3(2): 1054-1062.

Asadullah and A. Bano. 2018. Role of PGPR in the reclamation and revegetation of saline land. *Pak. J. Bot*., 51(1) DOI: 10.30848/PJB2019-1(43)

Bano, A. and M. Fatima. 2009. Salt tolerance in *Zea mays* (L). following inoculation with Rhizobium and Pseudomonas. *Biol. Fertil. Soils*, 45(4): 405-413. DOI: 10.1007/s00374-008-0344-9.

Figueiredo, M.d.V.B., L. Seldin, F.F. de Araujo and R.d. L.R. Mariano. 2010. Plant growth promoting rhizobacteria: fundamentals and applications, Plant growth and health promoting bacteria. Springer. p. 21-43. DOI 10.1007/978-3-642-13612-2_2,

Gerpacio, R.V. and P.L. Pingali. 2007. Tropical and Subtropical maize in Asia: production systems, constraints, and research priorities. *Mexico, D.F.: CIMMYT*.

Hadi, F. and A. Bano. 2010. Effect of diazotrophs (Rhizobium and Azatebactor) on growth of maize (*Zea mays* L.) and accumulation of lead (Pb) in different plant parts. *Pak. J. Bot*., 42: 4363-4370.

Herman, M.A.B., B.A. Nault and C.D. Smart. 2008. Effects of plant growth promoting rhizobacteria on bell pepper production and green peach aphid infestations in New York. *Crop Prot*., 27: 996-1002. doi:10.1016/j.cropro.2007.12.004

Hunt, R. 1978. Plant growth analysis. Edward Arnold, U.K, p. 26-38.

Khan, M.B., M. Asif and M. Aman. 2003. Response of some maize (*Zea mays* L.) genotypes to different irrigation levels. *Int. J. Agric. Biol.,* 5: 17-18.

Kloepper, J.W., C.M. Ryu and S. Zhang. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology*, 94: 259-1266. doi: 10.1094/PHYTO. 2004.94.11.1259.

Kumar, J., N.K. Tyagi, D.R. Dahiya, A. Yadau and O.P.S. Khola. 1996. Summer maize production function in relation to irrigation regimes. *Ind. J. Soil Conser*., 26: 207-210.

Mahmood, A., O.C. Turgay, M. Farooq and R. Hayat. 2016. Seed biopriming with plant growth promoting rhizobacteria: A review. *FEMS Microbiol. Ecol*., 92, 2016, fiw112. doi: 10.1093/femsec/fiw112.

Mansouri-Far, C., S.A.M.M. Sanavy and S.F. Saberali. 2010. Maize yield response to deficit irrigation during low-sensitive growth stages and nitrogen rate under semi-arid climatic conditions. *Agri. Water Manag.,* 97: 12-22.

Marulanda, A., R. Azco´n, J.M. Ruı´z-Lozano and R. Aroca. 2008. Differential effects of a *Bacillus megaterium* strain on *Lactuca sativa* plant growth depending on the origin of the arbuscular mycorrhizal fungus coinoculated: physiologic and biochemical traits. *J. Plant Growth Regul*., 27: 10-18. DOI 10.1007/s00344-007-9024-5.

Muhae-Ud-Din, G., M.A. Ali, M. Naveed, K. Naveed, A. Abbas, J. Anwar and M.H. Tanveer. 2018. Consortium application of endophytic bacteria and fungi improves grain yield and physiological attributes in advanced lines of bread wheat. *Turk. J. Agri. Food Sci. & Tech.,* 6(2): 136-144. DOI: https://doi.org/10.24925/turjaf.v6i2.136-144.1416.

Mubeen, M., A. Ahmad, T. Khaliq, S.R. Sultana, S. Hussain, A. Ali, H. Ali and W. Nasim. 2013a. Effect of growth stage-based irrigation schedules on biomass accumulation and resource use efficiency of wheat cultivars. *Amer. J. Plant Sci.,* 4: 1435-1442.

Mubeen, M., A. Ahmad, A. Wajid and A. Bakhsh. 2013b. Evaluating different irrigation scheduling criteria for autumn-sown maize under semi-arid environment. *Pak. J. Bot.,* 45: 1293-1298.

Mubeen, M., A. Ahmad, A. Wajid, T. Khaliq and A. Bakhsh. 2013c. Evaluating CSM-CERES-Maize model for irrigation scheduling in semi-arid conditions of Punjab, Pakistan. *Int. J. Agri. Biol*., 15: 1-10.

Mubeen, M., A. Ahmad, A. Wajid, T. Khaliq, H.M. Hammad, S.R. Sultana, S. Ahmad, W. Nasim and S. Fahad. 2016. Application of CSM-CERES-Maize Model in Optimizing Irrigated conditions. *Outlook Agri.,* 45: 173-184. DOI: 10.1177/0030727016664464.

Nasim, W. and A. Bano. 2012. Impact of nitrogen and plant growth promoting rhizobacteria on yield and yield components of sunflower in a glasshouse environment. *J. Crop Sci. Biotechnol.,* 15: 319-324. DOI No. 10.1007/ s12892-012-0043-9

Nielsen, R. 2010. Requirements for uniform germination and emergence of corn. Corny News.

Pandey, R.K., J.W. Maranville and A. Admou. 2000. Deficit irrigation and nitrogen effects on maize in a Sahelian environment I. Grain yield and yield components. *Agri. Water Manag.,* 46: 1-13.

Panitnok, K., S. Tubngeon, S. Techapinyawat, T. Somwang, S. Lim-Aroon and N. Udomprasert. 2005. Effect of water deficit on yield of three maize cultivars. In: Proceedings of international conference on maize adaptation marginal environments 25th anniversary of the cooperation between Kasetsart University and Swiss Federal Institute of Technology, Bangkok: *Asksorn Siam Printing*. pp. 140-144.

Sandhya, V., S.K.Z. Ali, M. Grover, G. Reddy and B. Venkateswarlu. 2010. Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. *Plant Growth Regul*., 62: 21-30. DOI: 10.1007/s10725-010-9479-4.

Shaharoona, B., M. Arshad, Z.A. Zahir and A. Khalid. 2006. Performance of *Pseudomonas* spp. containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. *Soil Biol. Biochem.* 38: 2971-2975. DOI: 10.1016/j.soilbio.2006.03.024.

Soler, C.M.T., P.C. Sentelhas and G. Hoogenboom. 2007. Application of the CSM-CERES-Maize model for planting date evaluation and yield forecasting for maize grown off-season in a subtropical environment. *Eur. J. Agron.,* 27: 165-177. doi:10.1016/j.eja.2007.03.002.

Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1997. Principles and procedures of statistics: A biometrical approach. 3rd ed., McGraw Hill Book. Int., Co., New York, 400-428.

Thongsaga, K., S.L. Ranamukhaarachchi, S. Jampatong, L. Samarakoon, A. Noomhorm, R. Clemente and D. Hannaway. 2010. Comparison of crop simulation and field performance of maize under 20-day dry period imposed during selected critical growth periods in Nakhon Ratchasima province, Thailand. Recent Research in Science and Technology 2.

Wu, S., Z. Cao, Z. Li, K. Cheung and M. Wong. 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma* 125:155-166. DOI: 10.1016/j.geoderma. 2004.07.003.

Yasmin, H., A. Bano and A. Samiullah. 2013. Screening of PGPR isolates from semi-arid region and their implication to alleviate drought stress. *Pak. J. Bot.,* 45: 51-58.

Zelikovick, L.E., V.J. Zelikovick and O.P. Perez. 1997. Irrigation scheduling for maize in the Northern region of province of Buenos Aires. In: Proc. "1st Latin American Agrometeorology conference" Argentina, 51-63.

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