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SCREENING OF PGPR ISOLATES FROM SEMI-ARID REGION AND THEIR IMPLICATION TO ALLEVIATE DROUGHT STRESS

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Abstract

During the present study isolation of 24 plant growth promoting rhizobacteria isolates were made from rhizosphere soil of maize collected from semi-arid region of Kahuta, Pakistan having 12% soil moisture. These isolates varied considerably on the basis of colony morphology, Gram's test and catalase test. Most efficient bacterial isolates were screened on the basis of their positive activity for siderophore production, P-solubilization and bacteriocin production. The PGPR isolate 9K showed maximum P-solubilization index. Siderophore production was exhibited by 1K (1Kahuta) and KB (KahutaB) while (KahutaB) showed bacteriocin production also. The PGPR isolates1K, KB and 9K were selected for re-inoculation studies on maize under induced drought stress condition. The PGPR isolate 9K increased drought tolerance in maize plants by enhancing root proliferation and improving relative water content of leaves, significant (26%) increased in root to shoot dry weight ratio as compared to inoculated control. The PGPR isolate 9K can be selected in the formulation of bio-fertilizers for alleviating drought effects in arid and semi-arid region.

Introduction

Pakistan is situated in arid and semi-arid region of the world. It lies between 24° and 37° NL and between 61° and 78° E, covers 796099 Km². Different environmental stresses continuously effects plants in the field conditions (Sheng *et al.,* 2004). The decrease in rainfall, increase in atmospheric (CO2) and more extreme weather events accompanied by 1.5-4.5°C increase in temperature are expected in next 100 years (Anon., 2007). In arid and semi-arid areas all stresses predominantly drought limits the growth and productivity of crops particularly causing the most fatal economic losses in agriculture. This form of abiotic stress, affect the plant water relation at cellular and whole plant level reduce nitrogen and carbon metabolism lead to modulate plant physiology and photosynthetic activity (Benabdellah *et al*., 2011).The adaptation mechanism of plant drought tolerance may involve promotion of root extension, allowing an efficient water uptake (Potters *et al.,* 2007, Narusaka *et al*., 2003).

PGPR from stressed area can assist host plant to cope with stresses (Sandhya *et al.,* 2010)**.** Plant growth promoting bacteria can also make such changes in root morphology (Belimov *et al.,* 2005). Drought tolerance to the plants can be induced by PGPR inoculations which are adapted to water limited soil conditions (Marulanda *et al*., 2008). The term induced systemic tolerance (IST) has been proposed for PGPR-induced physical and chemical changes that result in enhanced tolerance of plants to abiotic stress (Sandhya *et al*., 2010). Phosphorus (P) is major essential macronutrients for biological growth and development and also promotes N2 fixation in legumes (Khan *et al.,* 2010). Microorganisms offer a biological rescue system capable of solubilising the insoluble inorganic P of soil and make it available to the plants. The ability of some microorganisms to convert insoluble phosphorus (P) to an accessible form, like orthophosphate, is an important trait in a PGPB for increasing plant yields (Saharan & Nehra, 2011). *Azospirillum* spp., isolated from arid areas can improve tolerance level in crop plants under water deficit condition (Ilyas & Bano, 2010).

PGPR significantly promote seedling emergence, vigor and yield by competing other rhizobacteria through production of antibiotics, lytic enzyme, hydrogen cyanide siderophore and bacteriocin (Herlache & Triplett, 2002; Antoun & Kloepper, 2001). Maize (*Zea mays* L.) can meet the rising demand of food for population as an alternative crop. Maize grain has high food value and its oil is used for cooking purposes while green fodder is quite rich in protein (Dowswell *et al*., 1996). In Pakistan, maize is the third most important cereal after wheat and rice. Water availability plays a crucial role in the life cycle of maize crop (Cakir, 2004). During the vegetative development drought stress decrease the plant height and leaf area (Muchow, 1989). Deficiency of water at flowering stage reduces the grain yield two to three times (Grant *et al*., 1989; Heisey & Edmeades, 1999). The aim of the study was to screen indigenous PGPR isolates from rhizosphere soils of maize growing under semi-arid fields of Kahuta on the basis of their growth promoting traits.

Materials and Methods

Soil sample collection: Soil samples were collected from rhizosphere soil of maize grown under semi-arid fields of Kahuta, Pakistan. Soil samples were taken from 6 inches depth from the rhizosphere of three months old maize plants at anthesis stage.

Moisture content of soil: At the time of each sampling, moisture content of soil was determined. Soil (20 gm) sample was taken at a uniform depth of 6 inches from the surface of soil. Fresh weight of the samples was recorded. Dry weight was determined after drying the soil in oven for 72 hours at 70ºC till constant weight.

$$\text{Soil moisture (\%)} = \frac{\text{Weight of wet soil (g)-Weight of dry soil (g)}}{\text{Weight of dry soil (g)}} \times 100$$

Soil nutrients analysis: Rhizospheric soil was analyzed for macro (Na, Ca, Mg, N, P and K) and micronutrients following the ammonium bicarbonate-DTPA method developed by Soltanpour & Schwab (1977).

Isolation: Isolation of PGPR isolates were made from rhizosphere soil of maize grown in semi-arid region of Kahuta on Luria Bertani (LB) (g/L tryptone 10; yeast extract 5; NaCl 10, agar 18 and pH 7.5). Ten grams of rhizosphere soil were taken into 250mL conical flask, and 90 mL of sterile distilled water was added to it. The flask was shaken for 10 min on a rotary shaker at 120rpm. One milliliter of suspension was added to 10mL vial and shaken for 2 min. Serial dilution technique was performed up to 10^{-7} dilution. An aliquot (0.1 mL) of this suspension was spread on the plates of Luria Bertany (LB) agar medium.

Plates were incubated for 3 days at 28°C to observe the colonies of bacteria. Bacterial colonies were streaked to other LB agar plates and the plates were incubated at 28°C for 3 days. Morphologically different colonies were selected, marked and re-streaked until the pure cultures were obtained.

Morphology of isolates: Morphological characteristics of the colony of each isolate were examined on LB agar plates. To study the cell motility and shape, single colony from the agar plates were transferred on glass slide with a drop of sterile water and observed under light microscope (Nikon, Japan).

Catalase test: Fresh culture (24 h old) was used and bacterial colony was placed on a glass slide and one drop of H_2O_2 (30%) was dropped on the colony; appearance of gas bubbles is the indicator of catalase enzyme (McFadden, 1980).

Oxidase test: For observation under light microscope, slides of isolated bacterial cultures were prepared for Gram staining by the Vincent method (Vincent, 1970).

Oxidase test: Oxidase test was performed to determine the presence of oxidase enzyme in bacterial isolates (Steel, 1961).

PGP Characters

Phosphate solubilization: The culture of these isolates were streaked on Pikovskya's plates and incubated in an incubator at 28°C for 7 days. The plates were then examined and data were recorded (Pikovaskya, 1948).

$$SI = \frac{\text{Colony diameter + Halozone diameter}}{\text{Colony diameter}}$$

Siderophore production: The PGPR isolates were assayed for siderophore production on the chrome azurole S agar (CAS) described by Clark & Bavoil (1994). Chrome azurole S agar plates were prepared and spot inoculated with test organism and incubated at 30°C for 5 days. Development of yellow–orange halo around the colony was considered as positive for siderophore production.

Bacteriocin production: Bacteriocin activity was measured by using a saturated culture of indicator strain VF39 grown in TY medium, diluted (10^{-2}). One ml of the diluent was mixed with 25 ml of molten and soft TY agar (0.6% wt/vol) containing 5mM Ca^{+2}. The single colonies of strain to be verified for bacteriocin activity were inoculated into soft agar within two hours after agar solidification. Halozones are the indicators surrounding the stab inoculated cultures. The plates were incubated approximately for 48h and the result was recorded (Oresnik *et al*., 1999).

Inoculation of maize (*Zea mays*. L) by PGPR isolates: The Reinoculation studies of selected isolates 1K, 9K and KB from semi-arid region of Kahuta were performed for evaluating their drought tolerating capabilities on host plants maize (*Zea mays* L.). Maize seeds of variety "Islamabad White (Sawan 3)" were collected from National Agriculture Research Centre (NARC) Islamabad.

In preparation of inocula 24h old cultures were inoculated in 100ml broth of LB media and kept on shaker for 48h then centrifuged for 10 min at 10,000rpm. Supernatant was discarded and pellet was diluted with autoclaved distilled water up to 100ml. The optical density of the bacterial solution was adjusted to 1 at 600nm with spectrophotometer.

Seeds were surface sterilized with 95% ethanol for 3 min and then shaken in 10% solution of chlorox for 3 min and finally washed with sterilized water. Thereafter, sterilized seeds were soaked in broth bacterial culture (10^8cells/g) for 2-4 h and seeds were sown in plastic pots containing sterilized soil and sand in 3:1 ratio.

Pots were placed in the green house of Quaid-i-Azam University and soil moisture (12.5% of dry weight of soil) was maintained during the experiment. After 7 days of germination, drought stress was induced in 24 replicated pots by withholding watering. Water-stressed seedlings and their corresponding non-stressed controls were harvested after 6 days of exposure to drought, and soil moisture in the pots was measured Length, fresh and dry weight of root and shoot leaf area and relative water content of leaves were recorded.

Relative water content of leaves: Relative water content of flag leaves was measured after 7d of induction of water stress, by the method of Weatherley (1950). Fresh weight of the leaves was recorded. The leaves were then immersed in distilled water in beakers and left for 24 hours. Thereafter, fully turgid leaves were weighed again. The leaves were dried in oven for 72h at 70°C, until constant weight of leaves was obtained. Relative water content was calculated by the following formula:

$$RWC\% = \frac{FW - DW}{FTW - DW} \times 100$$

where:
RWC = relative water content
DW = dry weight
FW = fresh weight
FTW = fully turgid weight.

Results

Isolation of PGPR isolates: Twenty four bacterial isolates designated as 1K, 2K, 3K, 4K, 5K, 6K, 7K, 8K, 9K, 10K, 11K, 12K, 13K, 14K, 15K, KA, KB, KC, KD, KE, KF, KG, KH and KI were isolated from rhizosphere soil of maize grown in semi-arid region of Kahuta (Table 2). Different types of colonies were obtained with varied morphological characters.

Physico chemical properties of rhizosphere soil of Maize grown in semi arid region of Kahuta are given in Table 1.

Table 1. Physico-chemical analysis of rhizosphere soil samples collected from maize grown under semi-arid region of Kahuta.

Soil	characteristics
pH	7.8
Soil moisture	12%
Ca	2.017(mg/g)
Mg	7.04 (mg/g)
K	2.73 (mg/g)
Na	5.29 (mg/g)
Ni	0.57 (mg/g)
Fe	0.13 (mg/g)
Cu	0.057 (mg/g)
Co	0.079 (mg/g)
Zn	0.039 (mg/g)
Cr	0.095 (mg/g)

Morphology of bacterial colonies obtained from rhizosphere soil of maize (*Zea mays* L.) grown in semi-arid region of Kahuta: Shape of the bacterial colonies isolated from rhizosphere soil of maize (*Zea mays* L.) grown in semi-arid region of Kahuta, varied from round to irregular, mostly bacterial isolates had colonies with wavy margins except 1K, 2K, 8K, 9K, KC, KD, KG, and KI which had entire margins. Size of all bacterial colonies ranged from 1- 6mm. These isolates varied greatly in their color ranging from skin, off white and green. All of them were odorless, had raised elevation. Most of the bacterial isolates had smooth and shiny surface but 3K, 7K, 8K, 10K and KA had rough and shiny surfaces. Most of them were Gram negative except 1K and 9K (Table 2).

Catalase test: 1K, 2K, 3K, 4K, 5K, 9K 11K, 12K, 15K, KB and KD were positive for catalase test while 6K, 7K, 8K, 10K, 13K, 14K, KA, KC, KE, KI, KG, KH and KI were negative for catalase test (Table 3).

Oxidase test: All the isolates from rhizosphere soil of maize collected from semi-arid region of Kahuta were positive for oxidase test (Table 3)

Siderophore production: All PGPR isolates from rhizosphere soil of Maize (*Zea mays* L.) grown in semi-arid zone of Kahuta were tested for sidreophore production on CAS agar. Only one bacterial isolate 1K was positive for siderophore production (Table 3).

Bacteriocin production: Out of 24 PGPR isolates from rhizosphere soil of maize (*Zea mays* L.) grown in semi-arid zone of Khauta, 5 isolates were positive for bacteriocin production. Among all tested bacterial isolates, maximum bacteriocin production was shown by 11K, 12K, and KB while minimum bacteriocin production was shown by bacterial isolates KC and KD (Table 3).

Solubilization index: Among 24 isolates from rhizosphere soil of maize grown in semi-arid zone of Kahuta, maximum phosphorous solubilization was shown by 9K. KB and KC showed insignificant difference among each other in P solubilization and exhibit significantly higher SI as compared to 5K. The ranking order of P solubilization in term of solubilization index by the isolates from arid zone of Kahuta is 9K > K5 > KB ≥ KC > K4 ≥ K2 ≥ K3 > K6 KF≥13 K ≥10 K ≥ KG ≥ KD ≥ KH≥K7≥K8≥14 K (Table 3).

Effect of PGPR isolates from rhizosphere soil of Maize grown in semi- arid region of Kahuta on growth of maize under drought stress: This experiment was conducted to screen the most efficient growth promoting and drought tolerant rhizobacteria isolated from rhizosphere soil of maize (*Zea mays* L.) grown in semi-arid region of Kahuta. Reinoculation studies of three bacterial isolates, 1K, KB and 9K from rhizosphere soil of maize grown in semi- arid region of Kahuta was conducted on maize (*Zea mays* L.) exposed to induced drought stress.

Exposure of maize (*Zea mays* L.) seedling to drought stress affect the health of plant in terms of low stature, yellowing and wilting of leaves, inhibit leaf formation and decreased seedling vigor. After 7 days of exposure to drought stress soil moisture decline to 1.32%, un-inoculated plants showed drastic effects of drought stress as compared to inoculated seedlings. Among 3 bacterial inoculants isolated from semi-arid region of Kahuta 9K delayed symptoms of wilting which indicate more drought tolerance as compared to 1K and KB.

Effect of PGPR isolates on shoot and root length of *Zea mays* L. under drought stress: All the inoculated treatments showed significant enhancement in shoot length. The inoculation treatment with PGPR isolates 1K, 9K and KB showed increase in shoot length by 31.5 - 73.6% and 38-58.2% as compared to un-inoculated control under non-stressed and drought stressed conditions respectively (Table 4) The PGPR isolate 9K performed better and enhanced shoot length with (73.6%) under non-stressed and with (58.2%) under drought stressed condition.

All the inoculated treatments showed significantly enhanced root length. The inoculation treatment with PGPR isolates 1K,9K and KB showed increase in root length by 25-87.5 and 16.4-43.3% as compared to un-inoculated control under non-stressed and drought stressed conditions respectively(Table 4). PGPR isolate 9K performed better and enhanced root length (87.5%) under non-stressed and (43.3%) under drought stressed condition as compared to un-inoculated control.

Table 2. Morphological characteristics of 3 day old colonies of PGPR isolates.

Bacterial isolates	Shape	Size (mm)	odor	Color	Elevation	Surface	Margins	Cell shape	Arrangement	Grams Test
1K	irregular	6	odorless	Dirty green	Raised	Smooth shiny	Entire	Round	Paired	Gram positive
2K	Round	5	odorless	Yellowish green	Raised	Smooth shiny	Entire	Round	Scattered	Gram Negative
3K	irregular	3	odorless	Off white	Raised	Rough shiny	wavy	Round	Scattered	Gram Negative
4K	Round	5	odorless	white	Raised	Smooth shiny	wavy	Round	Paired	Gram Negative
5K	Round	1	odorless	Light yellow	Raised	Smooth shiny	wavy	Irregular	Scattered	Gram Negative
6K	Round	3	odorless	Light yellow	Raised	Smooth shiny	wavy	Irregular	Rods	Gram Negative
7K	Round	4	odorless	White	Raised	Rough shiny	wavy	Irregular	Scattered	Gram Negative
8K	Round	3	odorless	Sea green	Raised	Rough shiny	Entire	Irregular	Rods	Gram Negative
9K	Round	4	odorless	Light yellow	Raised	Smooth shiny	Entire	Round	Scattered	Gram positive
10K	Round	2	odorless	white	Raised	Rough shiny	wavy	Round	Rods	Gram Negative
KA	Round	6	odorless	Yellowish green	Raised	Rough shiny	wavy	Irregular	Scattered	Gram Negative
KB	irregular	6	odorless	Dirty green	Raised	Smooth shiny	wavy	Round	Scattered	Gram Negative
KC	Round	5	odorless	Pale yellow	Raised	Smooth shiny	Entire	Round	Paired	Gram Negative
KD	Round	3	odorless	yellow	Raised	Smooth shiny	Entire	Irregular	Scattered	Gram Negative
KE	irregular	4	odorless	white	Raised	Smooth shiny	wavy	Irregular	Scattered	Gram Negative
KF	Round	6	odorless	Off white	Raised	Smooth shiny	wavy	Round	Paired	Gram Negative
KG	Round	5	odorless	Off white	Raised	Smooth shiny	smooth	Irregular	Scattered	Gram Negative
KH	Round	5	odorless	Off white	Raised	Smooth shiny	wavy	Irregular	Rods	Gram Negative
KI	Round	2	odorless	Sea green	Raised	Smooth shiny	Entire	Round	Scattered	Gram Negative

where,
1K,2K,3K,4K,5K,6K,7K,8K,9K,10K,KA,KB,KC,KD,KE,KF,KG,KH,KI: Bacterial isolates from rhizosphere soil of maize (*Zea mays* L.) fields in semi-arid region of Kahuta. All such means which share a common English letter are similar, otherwise they differ significantly $p>0.05$, Where, - ve = stands for negative in test, +ve = positive in test

Effect of PGPR isolates on shoot fresh and dry weight of *Zea mays* L. under drought stress: All the inoculated treatments showed significantly enhanced shoot fresh weight. The inoculation treatment with PGPR isolates 1K, 9K and KB showed increase in shoot fresh weight by 54.8-99.3 and 55-117.5% as compared to un-inoculated control under non-stressed and stressed conditions respectively (Table 4). The PGPR isolate 9K had maximum increase in shoot fresh weight by 99.3% under non-stressed and by 117.5% under drought stressed condition as compared to un-inoculated control.

The inoculation treatments with PGPR isolates 1K, 9K and KB showed increase in shoot dry weight by 6.6-24.4 and 78-146.5% as compared to un-inoculated control under non-stressed and stressed conditions respectively (Table 5). The PGPR isolate KB enhanced shoot dry weight by 24.4% under non-stressed and by 146.5% under drought stressed condition as compared to un-inoculated control.

Effect of PGPR isolates on root fresh and dry weight of *Zea mays* L. under drought stress: The inoculation treatment with PGPR isolates 1K, 9K and KB showed increase in root fresh weight by 2.7-56.4% as compared to un-inoculated control under non-stressed conditions (Table 4). The PGPR isolate 9K enhanced root fresh weight by 56.4% under non-stressed and by 35.4% under drought stressed condition as compared to un-inoculated control. All the inoculated treatments showed significant enhancement in root dry weight. The inoculation treatment with PGPR isolates 1K, 9K and KB showed increase in root dry weight by 59-128.7 and 218-381% as compared to un-inoculated control under non-stressed and drought stressed conditions respectively. The PGPR isolate 9K exhibited maximum increase in root dry weight by 128.7% under non-stressed and by 381% under drought stressed condition as compared to un-inoculated control.

Table 3. Solubilization index, Catalase test, Siderophore production and bacteriocin production of PGPR isolates from the rhizosphere soil of maize grown in semiarid region of Kahuta against VF39 strain of *Rhizobium leguminosarum*

Bacterial isolates	Solubilization index	Catalase test	Oxidase test	Siderophore production	Bacteriocin inhibition zone(mm)
1K	2.43HI	+	+	+++	Not detected
2 K	3.83e	+	+	Not detected	Not detected
3K	3.63e	+	+	Not detected	Not detected
4K	4.33d	+	+	Not detected	Not detected
5K	4.83C	+	+	Not detected	Not detected
6K	3.32f	-	+	Not detected	Not detected
7K	2.31I	-	+	Not detected	Not detected
8K	2.27I	-	+	Not detected	Not detected
9K	5.83a	+	+	Not detected	Not detected
10K	2.63gh	-	+	Not detected	Not detected
11K	Not detected	+	+	Not detected	11.34A
12K	Not detected	+	+	Not detected	11.620A
13K	Not detected	-	+	Not detected	Not detected
14K	2.76I	-	+	Not detected	Not detected
15K	2.23i	+	+	Not detected	6.620B
KA	Not detected	-	+	Not detected	Not detected
KB	5.46B	+	+	Not detected	11.333A
KC	5.40B	-	+	Not detected	5.38C
KD	2.4633HI	+	+	Not detected	5.37C
KE	Not detected	-	+	Not detected	Not detected
KF	2.83G	-	+	Not detected	Not detected
KG	2.46HI	-	+	Not detected	Not detected
KH	2.38I	-	+	Not detected	Not detected
KI	Not detected	-	+	Not detected	Not detected

Table 4. Effect of inoculation with the PGPR isolates from semi-arid region of Khauta on Length and fresh weight of root and shoot of maize (*Zea mays* L.) under non-stressed and drought stress conditions.

Treatment	SL	SLD	RL	RLD	SFW	SFWD	RFW	RFWD
1K	16.3B ±0.17	13.6 D ±0.2.617	20.0E ±1.7	33.33BC ±1	0.7C ±0.17	0.62D ±0.07	0.87DE ±0.1	1.24 BC ±0.08
9K	19.8A ±0.17	15.6BC ±1	30.0CD ±0.17	41A ±5.19	0.95A ±0.17	0.87B ±0.045	1.33B ±0.1	1.70A ±0.11
KB	15C± 0.17	14.7C ±0.34	28D ±0.17	36.3B ±7.2	0.88 B ±0.17	0.63D ±0.027	0.94D ±0.1	1.22C ±0.15
Control	11.4E ±0.1	9.96F ±0.2.6	16F ±0.17	28.6D ±2.6	0.48E ±0.17	0.403F ±0.01	0.85E ±0.043	1.2BC ±0.1

1K, 9K, and KB are the **b**acterial isolates from rhizosphere soil of maize (*Zea mays* L.) fields in semi-arid region of Kahuta.
SL=shoot length, SLD=Shoot length under drought stress, RL=Root length, RLD=Root length under drought stress, SFW=Shoot fresh weight, SFWD=Shoot fresh weight under drought stress, RFW=Root fresh weight, RFWD=Root fresh weight under drought stress, SDW=Shoot dry weight, SDWD=Shoot dry weight under drought stress, RDW=Root dry weight, RFWD=Root dry weight under drought stress, R:S=Root to shoot dry weight ratio, R:S=Root to shoot dry weight ratio under drought stress, LA=Leaf area, LAD=Leaf area drought
All such means which share a common English letter are similar, otherwise they differ significantly ($p>0.05$)

Effect of PGPR isolates on root to shoot dry weight ratio of maize (*Zea mays* L.) under drought stress: All the inoculated treatments showed significantly enhanced root to shoot dry weight ratio. The inoculation treatment with PGPR isolates 1K, 9K and KB showed increase in root to shoot dry weight ratio by 36.5-100 and 26-127% as compared to un-inoculated control under non-stressed and drought stressed conditions respectively (Table 5). The PGPR isolates 9K performed better and significantly increase root to shoot dry weight ratio by 100% under non-stressed and by 127% under drought stress condition as compared to un-inoculated control.

Effect of PGPR isolates on leaf area of *Zea mays* L. under drought stress: All the inoculated treatments showed significantly enhanced leaf area. The inoculation treatment with PGPR isolates 1K, 9K and KB showed increase in leaf area by 47-71 and 66.5-116.4% as compared to un-inoculated control under non-stressed and drought stressed conditions respectively (Table 5). The PGPR isolates 9K significantly enhanced leaf area by 71% under non-stressed condition while PGPR isolates 9K and KB showed maximum increase under drought stress with 116.4 and 113.7% respectively as compared to un-inoculated control.

Table 5. Effect of inoculation with the PGPR isolates from semi-arid region of Khauta on dry weight of shoot and root, root to shoot dry weight ratio, leaf area and relative water content of leaves of maize (*Zea mays* L.) under non-stressed and drought stress

Treatment	SDW	SDWD	RDW	RDWD	R/S	R/S D	LA	LAD	RWC	RWCD
1K	0.16B ±0.017	0.13C ±0.017	0.35C ±0.034	0.45B ±0.072	2.19C ± 0.02	3.48A ± 0.06	7.78BC ±0.5	7.33C ±1.1	64.26B ±8.9	64.21B ±4.9
9K	0.18A ±0.017	0.15B ±0.017	0.5AB ±0.026	0.53A ±0.017	2.79B ± 0.05	3.54A ± 0.01	9.066A ±0.6	9.40A ±1.3	70.79A ±4.47	60.86B ±0.73
KB	0.18A ±0.01	0.18A ±0.017	0.3C ±0.026	0.35C ±0.10	1.91C ± 0.01	1.93C ± 0.03	8.22B ±0.18	9.52A ±0.5	63.73B ±7.6	43.3D ±0.3
Control	0.15B ±0.017	0.073D±0.01	0.22D ±0.17	0.11E ±0.09	1.46D ± 0.01	1.54D ±0.01	5.366D ±0.1	4.4E ±0.6	60.73B ±7.5	50.0C ±3.2

1K, 9K, and KB are the bacterial isolates from rhizosphere soil of maize (*Zea mays* L.) fields in semi-arid region of Kahuta.

SL=Shoot length, SLD=Shoot length under drought stress, RL=Root length, RLD= Root length under drought stress, SFW=Shoot fresh weight, SFWD= Shoot fresh weight under drought stress, RFW=Root fresh weight, RFWD=Root fresh weight under drought stress, SDW=Shoot dry weight, SDWD= shoot dry weight under drought stress, RDW=Root dry weight, RFWD=Root dry weight under drought stress, R: S=Root to shoot dry weight ratio, R: S= Root to shoot dry weight ratio under drought stress, LA=Leaf area, LAD=Leaf area drought

All such means which share a common English letter are similar,otherwise they differ significantly ($p>0.05$)

Effect of PGPR isolates on relative water content of *Zea mays* L. under drought stress: All the inoculated treatments showed significantly enhanced relative water content. The inoculation treatment with PGPR isolates 1K, 9K and KB showed increase in relative water content by 5.8-16.6% under non-stressed and by 21.7-28.4% under drought stressed conditions. The PGPR isolates 9K performed better and significantly increase relative water content by 16.6 % under non-stressed and by 28.4% under drought stress condition as compared to un-inoculated control.

Discussion

Drought tolerance is a multi gene activity and has a complex signal transduction pathways (Nakashima *et al*., 2000, Kidokoro *et al*., 2009). The PGPR isolates from semi-arid area of low soil moisture when used as bio inoculants increase plant growth and resistance to soil water deficit. These PGPR inocula provided tolerance against drought stress. The solubilization of P in the rhizosphere is the most common mode of action implicated in PGPR that increase nutrient availability to host plants (Richardson, 2001). Iron is an essential nutrient of plants, but it is relatively insoluble in soil solutions. According to Salisbury & Ross (1992) plant roots absorb iron preferably in reduced ferrous (Fe^{2+}) ion, but the ferric (Fe^{3+}) ion is more common in well aerated soil although it is easily precipitated in iron-oxide forms. Soluble organic compounds siderophores are generally excreted by a plant which binds Fe^{3+} and mediates its delivery to the root surface where it is reduced to Fe2+ resulting in quick absorption. There is evidence that Siderophores can also produce by PGPR and a number of plant species can absorb bacterial Fe^{3+}-siderophore complexes (Wang *et al.,* 1993). Hafeez *et al*., (2004) reported that inoculant strains of *Rhizobiaceae* on the basis of bacteriocin production showed antagonism in mixture. The isolate KB combine the properties of P-solubilization, siderophore production and also can compete with the native microflora by producing bacteriocin which is a narrow range antibiotic. Plants are constantly exposed to abiotic stress, such as drought, which is one of the most serious problems associated with plant growth and development affecting agricultural demands (Hamayun *et al*., 2010). Inoculation with PGPR has been found effective under drought stress environment to increase productivity (Sandhya *et al.,* 2010).

Inoculation with PGPR isolates 1K,9K, KB for plant growth study under drought stress condition resulted in increase in root and shoot length, fresh and dry weight of root and shoot, R/S dry weight ratio, leaf area and leaf RWC of maize plants. Marulanda *et al.,* (2008) reported that rhizobacteria which are native resident of water scarce area are more adapted to the stress as compared to the isolates of irrigated area. Sandhya *et al.,* (2010) determined that drought-exposed maize plants tolerated stress for 9 days when inoculated with PGPR as compared to un-inoculated control maize seedlings which can tolerate drought just for 5 days.

According to Ilyas *et al*., (2012) PGPR isolated from water-stressed conditions produced low concentration of Indole acetic acid (IAA), Gibberellic acid (GA), trans-Zeatin riboside (tzr) but higher concentration of Abscisic acid (ABA), this work strongly supports the present results.

Our results showed that our isolates 1K, 9K and KB produced significant positive increase in dry weight of root under drought stressed condition by 218-381% than under non-stressed conditions by 59-128.7% over un-inoculated control. The PGPR isolate 9K showed increase in root length by 36.6%, root dry weight by 5.3%, root to shoot dry weight ratio by 26.5% as compared to inoculated control when exposed to drought stress condition. Lazarovits & Norwak (1997) reported that under ideal climatic conditions seed inoculation with PGPR has been reported to slightly increase yields. However it can be helpful when plants are under stressful conditions for prolonged periods (Egamberdiyeva & Hoflich, 2004).

The microbial inoculation increased the relative water content of leaves under drought stress. Increase in shoot water content of maize seedlings under drought stress when inoculated with the PGPR isolates may be

due to decrease in stomatal conductance. This result in increase in water use efficiency and ultimately growth of plant. Benabdellah *et al*., (2011) reported that stomatal conductance was highly decreased in inoculated plants particularly in those treated with AMF or B2 inoculants. The PGPR isolate 9K had better RWC under drought stressed condition which indicate the better water use efficiency and turgidity maintenance by 9K.

This drought tolerant ability of PGPR may be due to their ability to produce exopolysachrides under stress conditions. Exopolysacchrides forms a mucilaginous sheath around the cells, which leads to an increase in micro aggregates as an indirect additional effect, which improves the plant growth under drought stress by increasing aggregate stability and help in the survival of plant under drought stress (Alami *et al*., 2000).

The correlation between root lengths under drought stress conditions was positive and highly significant with root length, shoot fresh weight and leaf area under non-stressed condition. Shoot fresh weight was positively correlated with the root length under drought stress conditions (r= 0.98). The correlation between root dry weight and root to shoot dry weight ratio under non-stressed condition was positive and highly significant (r=0.97). Length and dry weight of root under drought stressed condition was positively and significantly correlated (r=0.98) with relative water content under non-stressed conditions. The correlation between relative water content was positive and highly significant (r=.95, 0.98) with shoot fresh weight and root to shoot dry weight ratio under non-stressed conditions respectively (Table 6).

Table 6. Correlation coefficient of growth parameters of inoculated maize plant with PGPR isolates from rhizosphere soil of maize grown under semi-arid region of Kahuta.

	SL	SLD	RL	RLD	FWS	FWSD	FWR	FWRD	R:S	R:SD	DWS	DWSD	DWR	DWRD	LA	LAD	RWC	RWCD
SL	1.0																	
SLD	0.90	1.0																
RL	0.78	0.92	1.00															
RLD	0.92	**0.95***	**0.96***	1.0														
FWS	0.88	**0.99***	**0.96***	**0.97***	1.00													
FWSD	**0.98***	0.93	0.87	**0.98***	0.92	1.00												
FWR	0.84	0.68	0.77	0.87	0.72	0.89	1.00											
FWRD	0.77	0.52	0.60	0.75	0.55	0.80	0.97	1.00										
R:S	**0.99***	0.85	0.73	0.90	0.83	**0.97***	0.86	0.82	1.00									
R:SD	0.88	0.67	0.40	0.63	0.61	0.78	0.58	0.59	0.90	1.00								
DWS	0.61	0.87	**0.96***	0.85	0.91	0.72	0.57	0.35	0.54	0.22	1.00							
DWSD	0.62	0.90	0.87	0.78	0.91	0.69	0.40	0.17	0.54	0.33	**0.95***	1.00						
DWR	**0.98***	0.92	0.87	**0.98***	0.92	**1.00***	0.89	0.80	**0.97***	0.78	0.72	0.68	1.00					
DWRD	**0.96***	0.92	0.72	0.86	0.88	0.92	0.67	0.58	0.94	0.91	0.60	0.69	0.92	1.00				
LA	0.92	**1.00***	0.90	**0.95***	**0.99***	0.94	0.70	0.55	0.88	0.72	0.83	0.87	0.94	0.94	1.00			
LAD	0.79	**0.97***	**0.95***	0.92	**0.99***	0.85	0.61	0.42	0.72	0.49	**0.95***	**0.97***	0.84	0.81	**0.96***	1.00		
RWC	**0.96***	0.82	0.79	0.93	0.82	**0.98***	**0.95***	0.91	**0.98***	0.78	0.59	0.52	**0.98***	0.86	0.85	0.72	1.00	
RWCD	0.59	0.24	-0.05	0.23	0.16	0.44	0.34	0.45	0.64	0.88	-0.27	-0.14	0.44	0.61	0.31	0.02	0.51	1.00

All such means with * showed significantly positive correlation at p>0.05

SL=Shoot length, SLD=Shoot length under drought stress, RL=Root length, RLD= Root length under drought stress, SFW=Shoot fresh weight, SFWD= Shoot fresh weight under drought stress, RFW=Root fresh weight, RFWD=Root fresh weight under drought stress, SDW=Shoot dry weight, SDWD= Shoot dry weight under drought stress, RDW=Root dry weight, RFWD=Root dry weight under drought stress, R:S=Root to shoot dry weight ratio, R:S=Root to shoot dry weight ratio under drought stress, LA=Leaf area, LAD=Leaf area drought, RWC=Relative water content, RWCD=Relative water content under drought stress

Conclusion

It can be concluded that there is diverse soil micro flora well adapted to water scarce environment. The PGPR isolate 9K is very efficient Phosphate solubilizer and could be very effective to be used as bio-inoculant to induce plant growth under drought condition as well as in phosphorous deficient soil. The PGPR 1K and KB which were siderophore and bacteriocin producers could also be used as biocontrol agent against plant pathogens.

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