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Full Length Article

Scrutinizing of Rhizobacterial Isolates for Improving Drought Resilience in Maize (*Zea mays*)

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Abstract

Food security due to scarcity of water to field crops is major concern now a days due to stress environments. A growth room trial was performed to screen potential bacterial inoculants for improving growth and physiology of maize (*Zea mays* L.) under water scarcity. Thirty fast growing rhizobacteria were isolated from rhizosphere of maize, cultivated in arid and semiarid areas of the province (Punjab). Isolates were evaluated for their plant growth promoting characters and drought tolerance, at various moisture levels developed in-vitro by using 0, 5, 10, 15, 20 and 25% polyethylene glycol (PEG-6000). Nine most efficient isolates (LK-2, LK-7, LK-9, LK-13, LK-16, LK-18, LK-21, LK-24, LK-29) were selected, having potential ability to survive in water stressed condition and were tested further in a jar experiment for their role in morpho-physiological improvements in maize seedlings, grown at different drought levels (100, 70 and 40% field capacity (FC). Results depicted that inoculation significantly ($P \leq 0.05$) enhanced root/shoot biomass & root/shoot ratio, chlorophyll a and b, starch content, soluble sugars, relative water content, photosynthetic rate, water use efficiency and stomatal conductance. Overall, isolate LK-13 and LK-16 were found more prominent in inducing drought tolerance in seedlings as compared to other isolates and uninoculated control. Identification of isolates through 16sRNA sequencing confirmed the both strains (LK-13 and LK-16) belong to *Bacillus* spp. Inoculation of maize seeds with rhizobacteria as plant growth promoting rhizobacteria enhanced growth and physiology of maize. So, inoculation of PGPR could be a potential approach for enhancing drought resilience in maize (*Zea mays* L.). © 2017 Friends Science Publishers

Keywords: Rhizobacteria; Physiology; Proline; Soluble sugars; Water use efficiency

Introduction

The major challenge of twenty first century is how to feed a burgeoning population sustainably? About 795 million people are undernourished worldwide (FAO, 2015). Current climate shift is expected to further increase challenges to food security (Umezawa *et al.*, 2006). Drought is a condition when water required to sustain growth, and development of the plant is un-available for a prolonged duration. It's a major constraint to the production of cereals (yield losses may up to 40.8%) in arid and semi-arid regions (Boyer, 1982) like Pakistan. Handful consumable water resources of the regions necessitated comprehensive and tedious efforts to strategize new avenues of crop management (Asghar *et al.*, 2015).

Several approaches have been reported to minimize the impact of drought stress on plant growth and physiology. Mulching, cover cropping, bed planting, deep tillage, and foliar spray of plant growth regulators have been studied to ameliorate adversities of drought stress (Hussain *et al.*, 2011). Breeders have made some excellent progress improving crop phenology, such as flowering time, height and other traits that can enhance water use efficiency through stress avoidance and tolerance (Passioura, 2007). Still, improvements are required to sustain crop productivity even under periodic and/or terminal drought stress, experienced in rain-fed agriculture. Breeding stress tolerant crops through conventional and biotechnical approaches can bring such improvements over a period of time (Hussain *et al.*, 1986; Ahmad *et al.*, 1987). Growing non-traditional crop with low delta of water can serve the purpose subject to adaptability to local cropping pattern and consumer acceptance. On the other hand, improving crop stress tolerance by exploiting beneficial plant-microbe interactions can be sustainable, economic and less time consuming to cope with water scarcity (Abolhasani *et al.*, 2010; Asghar *et al.*, 2015).

Plant growth promoting rhizobacteria have shown for improving drought tolerance in many plant hosts such as monocots, dicots and vegetables species (Kasim *et al.*, 2013; Asghar *et al.*, 2015). They have ability to confer more than one biotic and abiotic stress (Coleman-Derr and Tringe,

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2014). These rhizobacteria have excellent abilities to colonize plant roots extracellulary or intercellular and promote plant growth by producing phytohormones (Armada *et al.*, 2014), solubilizing different nutrients (Hussain *et al.*, 2009) and siderophore production (Arora *et al.*, 2001). Moreover, under drought stress these microbes release various kind of enzymes, metabolites accumulating in plant at compatible solutes which help host plants to escape stress (Berard *et al.*, 2015). These bacteria also synthesize sugars (Berjak, 2006), heat shock proteins (Feder and Hofmann, 1999), synthesize ACC-deaminase (1-aminocyclopropane 1-carboxylic acid) enzyme (Zahir *et al.*, 2009), and produce various amount of extracellular polymeric substances (EPS), which help them to survive in the water stressed environment (Rossi *et al.*, 2012) and improve crop growth, physiology and productivity. So, it is hypothesized that use of potential plant growth promoting rhizobacteria having survival ability under drought stress can improve growth and physiology of maize under water stressed conditions.

Material and Methodology

Isolation of Rhizobacteria

Various rhizobacterial strains were isolated from rhizosphere soil of maize, collected from different arid and semiarid areas of Punjab, Pakistan. Maize plants were up rooted and non-rhizosphere soil was removed using spatula, rhizosphere soil samples were taken to the laboratory in polythene bags. Isolation was done through dilution and plating technique by using sterilized general purpose medium (GPM). Thirty fast growing rhizobacterial strains were selected and streaked three to four times to obtain pure culture. These selected strains were coded as LK with numbers (1–30) and preserved at -40℃ in 60% glycerol for future use.

Drought Tolerance Assay

Selected isolates (LK-1, LK-2, LK-3 ……. LK-30) were tested for their survival ability against different drought levels using polyethylene glycol (PEG-6000) in GPM broth (Busse and Bottomley, 1989). Isolates were grown in sterilized test tubes containing GPM broth (10 mL each) with different drought levels (No PEG, 5, 10, 15, 20 and 25% PEG having osmotic potential -0.03, -0.24, -0.46, -0.77, -1.23 and -1.71 MPa, respectively, measured by Cryoscopic Osmometer (OSMOMAT- 030-D, Gonotec, Germany). For this purpose three set of test tubes were maintained for each isolate along with uninoculated control. Each test tube was inoculated with loop full culture of respective bacterial isolate and was incubated at 28 ± 1 for 96 hours, after that optical density (OD) at λ 540 nm was measured with the help of optical density meter (Dan-1 Densitometer, Mcfarland, UK). Isolates showing high OD (i.e. LK-2, LK-7, LK-9, LK-13, LK-16, LK-18, LK-21, LK-24 and LK-29) under each drought level were considered as drought tolerant isolate.

Characterization of Bacteria

The selected isolate of rhizobacteria were further characterized for various beneficial plant growth promoting traits and most efficient isolate were identified following standard protocols as described below.

Characterization of Bacteria

Gram positive and negative isolates were identified using method Gram (1884). Solubilization of inorganic phosphate by selected strains of bacteria was evaluated on National Botanical Research Institute Phosphate Bromophenol Blue (NBRI-PBB) media (Mehta and Nautiyal, 2001). Siderophores production activity of the bacterial isolates was measured using siderophore production assay by Schwyn and Neilands (1987) and catalase activity was measured with method described by MacFaddin (1980). Exo-polysaccharides production assay was performed to measure the exopolysaccharides production by bacteria using RCV- glucose media (Ashraf *et al.*, 2004) and Indole-3-acetic acid (IAA) release was measured through method described by Sarwar *et al.* (1992), in the presence and absence of L-tryptophan (L.TRP).

Screening of Rhizobacteria for Growth Promotion under Drought Stress

Jar trial: Nine rhizobacterial isolates selected from drought tolerance assay were further screened for growth promotion activity in maize under drought stress. Jar were filled with double sterilized sandy clay loam soil (used for trial) and drought levels were adjusted to different field capacity (FC) levels (100, 70 and 40% FC). Where 100% FC represents the least stress level which subsequently increase at 70 and 40% FC. Amount of water to develop drought levels of 100, 70 and 40% field capacity was calculated following linear regression equation $P = lnP_e + b\ ln\frac{\theta}{\theta s}$ given by Imran *et al.* (2014).

Maize seeds were first surface sterilized with 5% sodium hypochlorite solution and then dipped in freshly prepared inoculum (broth culture) of each isolate e.g. LK-2, LK-7, LK9, LK13, LK16, LK18, LK-21, LK24 and LK29 (Gutierrez-Zamora and Martinez-Romero, 2001) in respective petri dish for half an hour. Seeds inoculated bacterial isolates were sown in soil while seed without dipping in broth culture (uninoculated) were sown for control treatment. Half strength Hoagland solution was applied for nutrient supply and drought levels 100, 70 and 40% were maintained through gravimetric method. Light and dark period was adjusted as 16 h day and 8 h night.

Temperature was maintained at 25–30ºC in controlled temperature room and crop was harvested after 25 days.

Growth Attributes

Shoot and root fresh biomass of maize seedling plants was measured using weighing balance and total fresh biomass was calculated by adding fresh shoot and root biomass (shoot fresh biomass + root fresh biomass). Root/shoot ratio was calculated through dividing root length with shoot length.

Physiological Attributes

Chlorophyll pigments were measured using 0.5 g of leaf sample from each treatment and homogenized in 80% acetone (v/v). Homogenate was filtered through filter paper and absorbance of filtrate was taken by spectrophotometer at 663 and 645 nm for chlorophyll a and b, respectively (Arnon, 1949). Chlorophyll a and b were calculated as under:

$$Chl_a \left({}^{mg}/_{g\ fresh\ leaf}\right) = \left[12.7(O.D\ 663) - 2.69(O.D\ 645) \times \frac{V}{1000} \times weight\ of\ sample\right]$$

$$Chl_b \left({}^{mg}/_{g\ fresh\ leaf}\right) = \left[22.9(O.D\ 645) - 4.68(O.D\ 663) \times \frac{V}{1000} \times weight\ of\ sample\right]$$

Relative water content (RWC) of plant leaves was determined following the formula described by Mayak *et al.* (2004).

$$Relative\ water\ content\ (RWC)\% = \frac{\text{FW} - \text{DW}}{\text{FTW} - \text{DW}}$$

Where: FW = fresh weight, DW = dry weight, FTW = fully turgid weight.

The fully turgid weight is defined as the weight of the leaf after it was held in 100% humidity conditions in the dark at 4ºC for 48 h. For measuring electrolyte leakage, uniform leaf discs were placed in test tubes containing 50 mL distilled water in each. Tubes were placed on shaker for 4 h at room temperature. Electrical conductivity (EC) of solution was measured through conductivity meter and recorded as Reading 1. Then samples were autoclaved at 121ºC for 20 minutes and after cooling second reading (Reading 2) was taken through conductivity meter. Electrolyte leakage was measured using formula:

$$\text{Electrolyte leakage (EL)}\% = \frac{\text{Reading 1}}{\text{Reading 2}} \times 100$$

The plant physiological parameters such as photosynthetic rate (A), transpiration rate (E), water use efficiency (WUE) and stomatal conductance (gs) of maize were recorded using portable photosynthesis system CIRAS-3 (PP-Systems International Inc. MA01913USA).

Biochemical Analysis

Proline contents from plant samples were determined according to the method described by Bates *et al.* (1973). One gram leaf sample was homogenized in 3% sulphosalyclic acid and after filtration samples were treated with acid ninhydrin and glacial acetic acid. Mixture was heated at 100°C for 1 h in water bath and at the end reaction was stopped by using ice bath. The mixture was extracted with toluene and the absorbance was taken at λ 520 nm. Starch content was determined using the method of phenol-sulfuric acid described by Dubois *et al.* (1956). For glycinebetaine 0.5 g plant sample was grounded and extract was prepared in distilled water. About 20 mL of extract was shaked for 48 at 25ºC, filtered and were diluted (1:1) with 2 N sulphuric acid. Aliquot (0.5 mL) was cooled in ice water for 1 h, after that cold potassium iodide reagent (0.2 mL) was added and the mixture was gently mixed with vortex mixture. The samples were stored at 4°C for 16 h and then centrifuge at 10,000 rpm for 15 min at 0°C. The supernatant is carefully aspirated with 1 mL micropipette. The periodide crystals were dissolved in 9 mL of 1, 2-dichloro ethane (reagent grade). Vigorous vortex mixing was done to effect complete solubility in developing solvent. After 2.0–2.5 hours the absorbance was measured at λ 365 nm with UV-visible spectrophotometer (Grieve and Grattan, 1983). Soluble sugars were determined (mg g^{-1} FW) based on the method of phenol sulfuric acid (Dubois *et al.*, 1956). Fresh weight of roots and shoots (0.5 g) was homogenized with deionized water, extract was filtered and treated with 5% phenol and 98% sulfuric acid, to leave for 1 h and absorbance was taken at λ485 nm by spectrophotometer (UV-VIS/1201, Shimadzu).

Statistical Analysis

Statistical analysis and data computations were made on Microsoft Excel 2013® (Microsoft Corporation, Redmond, WA, USA) and Statistix 8.1® (Analytical Software, Tallahassee, USA). Treatment means were compared by using Tukey's multiple comparison test ($p \leq 0.05$) (Steel *et al*., 1997). Cluster analysis was performed using Minitab software (Minitab™ version 16).

Identification of Selected Strains

Two most efficient PGPR isolate LK-13 and LK-16 showing better growth and physiology of maize seedlings in jar trial from growth room experiment were identified through 16S rRNA gene sequencing technology described by Yanagi and Yamasato (1993). The 16S rRNA gene of each isolate was amplified using universal PCR primers (27F and 1492R) and final product of ~1.5 kb was obtained.

This product was sequenced with DNA sequencer and then analyzed on http://www.ncbi.nlm.nih.gov. Fig. 2 represents the phylogenetic tree and accession numbers of LK-13 and LK-16 isolates while Table 9 showing different beneficial characters of rhizobacterial isolate.

Results

Drought Tolerant Assay

Isolates showed variable responses to when grown in broth medium supplemented with PEG 6000 (Fig. 1). Rhizobacterial strains LK2, LK7, LK9, LK13, LK16, LK18, LK21, LK24 and LK29 (Group 3) showed the maximum tolerance against drought stress having highest similarity index (red color) between optical density (OD) on all drought levels i.e. 0, 5, 10, 15, 20 and 25% PEG. Less drought tolerance was observed in strains LK6, LK10, LK14, LK28, LK11, LK20, LK26, LK17 and LK27 (Group 1) while strains LK1, LK5, LK8, LK22, LK23, LK15, LK3, LK4, L30, LK12 and LK19 (Group 2) showed moderate drought tolerance.

Growth Room Experiment

Growth Parameters: Inoculation with rhizobacteria significantly improved the shoot/root biomass as compared to control (Table 1). Maximum shoot fresh biomass (SFB) was observed with the inoculation of LK-16 which was 25% higher as compared to control at 100% field capacity (FC). However, when drought was increased to 70 and 40% FC it was observed that SFB tended to decrease still seedlings inoculated with bacterial strains sustained better biomass as compared to their respective control. LK-13 was found most efficient with maximum SFB at both stress levels which was 28% and 29% higher than un-inoculated controls at 70 and 40% FC levels. At 100% FC maximum increase in root fresh biomass (RFB) 12% more was observed with isolate LK-18 while at 70 and 40% FC maximum increase was calculated with treatment LK-13 which was 42 and 41% more, respectively, as compared to control. Moreover, highest total fresh biomass (4.73 g) was found with inoculation of rhizobacterial isolate LK-16 followed by LK-18 (4.65 g) and LK-13 (4.63 g), at 100% FC while at 70 and 40% FC with inoculation of LK-13 which was 33 and 35% more, respectively, as compared with uninoculated control (Table 2). Minimum root/shoot ratios 0.65, 0.57 and 0.60 were found with inoculation of rhizobacterial isolate LK-16 under all drought levels (100, 70 and 40% FC) as compared to control. However, these results were statistically at par among other inoculated isolates at 100% FC except uninoculated control.

Physiological Attributes

Results revealed (Table 3) that both chlorophyll a and b were decreased under drought stress and minimum value was in uninoculated control. Highest chlorophyll a (0.61 and 40 mg g^{-1} FW) and b (50 and 26 mg g^{-1} FW) content were measured with inoculation of bacterial isolate LK-13 at drought level 70 and 40% FC, as compared to control. Relative water content (RWC) were improved with inoculation with rhizobacteria non-significantly at 100% FC (Table 4). However, under drought stress there was significant increase in RWC with inoculation and maximum increase was observed with treatment LK-13 at 70 and 40% FC which was 21 and 51% more, respectively as compared to control. Percent electrolyte leakage (EL) was increased with increasing water stress from 100 to 40% FC and maximum increase was observed in control treatment without inoculation however, inoculation with rhizobacteria significantly reduced the EL under drought stress. Among all isolates LK-16 reduced EL (32 and 40% FC) as compared to control at both 70 and 40% FC than 100% FC (Table 4).

Physiological attributes i.e. photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs) and water use efficiency (WUE) were improved with inoculation of rhizobacterial isolates at 100% FC, as well as under drought stress at 70 and 40% FC (Table 7,8). Maximum improvement in photosynthetic rate (96, 95, 85 and 84%) was observed with inoculation of LK-16, LK-13, LK-29 and LK-7 respectively, as compared to uninoculated control at 70% FC. Moreover, at 40% field capacity LK-7, LK-16 and LK-13 improved E (226, 222 and 214%). At 100% FC transpiration rate (E) was maximum (4.40 µmol m^{-2} s^{-1}) in treatment inoculated with LK-13, while E was maximum (112 and 135%) at 70 and 40% FC with isolate LK-16 as compared to control, respectively. Similarly, stomatal conductance (gs) was also high with inoculation of LK-16 under drought stress and maximum improvement in gs (73 and 137% more) was measured at 70 and 40% FC. However, under normal field capacity (100%) there was non-significant difference between LK-16, LK-13, LK-7, LK-18, LK-21, LK24, LK-29 and results were statistically at par with each other (Table 8). Highest water use efficiency (WUE) was measured in the treatment inoculated with LK-13 both under normal and drought stress which was 134, 152and 244% high, respectively at 100, 70 and 40% FC, as compared to uninoculated control. However, these results are statistically at par with LK-16 at all drought levels.

Biochemical Attributes

There was non-significant difference in proline content at 100% FC between uninoculated control and inoculation of rhizobacterial isolates (Table 5). However, under drought stress at 70% FC isolate LK-13 showed the minimum proline content 1.73 mg g^{-1} FW followed by isolate LK-24 (1.93 mg g^{-1} FW), when compared with uninoculated control 3.13 mg g^{-1} FW.

Table 1: Effect of rhizobacteria inoculation on shoot fresh weight and root fresh weight of maize under drought stress (100, 70 and 40% FC)

Treatments	Shoot fresh weight (g)			Root fresh weight (g)		
	100% FC	70% FC	40% FC	100% FC	70% FC	40% FC
Control	2.06 ± 0.07c-g	1.57 ± 0.08i	0.80 ± 0.06j	1.96 ± 0.03b	0.99 ± 0.04ghi	0.83 ± 0.01i
LK2	2.28 ± 0.04a-d	1.87 ± 0.06f-i	0.94 ± 0.04j	1.98 ± 0.08b	1.26 ± 0.01cde	0.99 ± 0.04ghi
LK7	2.51 ± 0.07ab	1.88 ± 0.04e-i	0.99 ± 0.07j	2.09 ± 0.05ab	1.14 ± 0.04fgh	0.95 ± 0.01hi
LK9	2.28 ± 0.07a-d	1.77 ± 0.07ghi	0.88 ± 0.03j	2.09 ± 0.02ab	1.13 ± 0.03fgh	0.98 ± 0.02ghi
LK13	2.50 ± 0.08ab	2.01 ± 0.08d-h	1.03 ± 0.08j	2.06 ± 0.03ab	1.41 ± 0.02c	1.17 ± 0.01d-g
LK16	2.58 ± 0.03a	1.93 ± 0.03d-h	1.02 ± 0.09j	2.12 ± 0.06ab	1.37 ± 0.03cd	1.13 ± 0.02e-h
LK18	2.39 ± 0.06abc	1.82 ± 0.08ghi	0.84 ± 0.04j	2.19 ± 0.02a	1.22 ± 0.05c-f	1.10 ± 0.04e-h
LK21	2.18 ± 0.09b-f	1.85 ± 0.10f-i	0.88 ± 0.03j	1.98 ± 0.04b	1.14 ± 0.03e-h	0.99 ± 0.05ghi
LK24	2.28 ± 0.05a-d	1.66 ± 0.06hi	0.95 ± 0.03j	2.05 ± 0.04ab	1.21 ± 0.05c-f	1.04 ± 0.03fgh
LK29	2.22 ± 0.05b-e	1.74 ± 0.09ghi	0.81 ± 0.04j	2.08 ± 0.04ab	1.30 ± 0.03cde	1.03 ± 0.03f-i

Means sharing similar letter's in the treatment of each parameter do not differ significantly at p≤0.05. Data is the average of three repeats ± standard error (SE). (HSD: 0.351; 0.208)

Table 2: Effect of rhizobacteria inoculation on total fresh biomass and root/shoot ratio of maize (*Zea mays* L.) under drought stress (100, 70 and 40% FC)

Treatment	Total fresh biomass (g)`			Root/shoot ratio		
	100% FC	70% FC	40% FC	100% FC	70% FC	40% FC
Control	4.02 ± 0.09d	2.57 ± 0.08hi	1.63 ± 0.06k	0.67±0.04cde	0.90±0.01ab	1.07±0.02a
LK-2	4.26 ± 0.09bcd	3.13 ± 0.05efg	1.92 ± 0.05jk	0.65±0.02cde	0.77±0.00b-e	0.85±0.04abc
LK-7	4.60 ± 0.11ab	3.03 ± 0.02efg	1.94 ± 0.06jk	0.67±0.03cde	0.69±0.05b-e	0.75±0.07b-e
LK-9	4.37 ± 0.08a-d	2.90 ± 0.05fgh	1.86 ± 0.04jk	0.68±0.02b-e	0.79±0.05b-e	0.76±0.06b-e
LK-13	4.57 ± 0.11abc	3.42 ± 0.07e	2.20 ± 0.09ij	0.66±0.03cde	0.60±0.04de	0.63±0.03cde
LK-16	4.70 ± 0.09a	3.30 ± 0.04ef	2.15 ± 0.10ij	0.65±0.03cde	0.57±0.03e	0.60±0.04de
LK-18	4.58 ± 0.04ab	3.04 ± 0.10efg	1.94 ± 0.06jk	0.68±0.01cde	0.71±0.02b-e	0.69±0.03b-e
LK-21	4.16 ± 0.09cd	3.00 ± 0.12fg	1.88 ± 0.05jk	0.69±0.02b-e	0.74±0.05 b-e	0.82±0.05bcd
LK-24	4.33 ± 0.01a-d	2.87 ± 0.09gh	1.99 ± 0.05jk	0.66±0.05cde	0.72±0.03 b-e	0.78±0.02b-e
LK-29	4.30 ±0.08a-d	3.04 ± 0.11efg	1.83 ± 0.01jk	0.66±0.04cde	0.74±0.06 b-e	0.71±0.03b-e

Means sharing similar letter's in the treatment of each parameter do not differ significantly at p≤0.05. Data is the average of three repeats ± standard error (SE). (HSD: 0.420; 0.207)

Table 3: Effect of rhizobacteria inoculation on chlorophyll a and chlorophyll b of maize (*Zea mays* L.) under drought stress (100, 70 and 40% FC)

Treatments	Chlorophyll a (mg g^{-1} FW)			Chlorophyll b (mg g^{-1} FW)		
	100% FC	70% FC	40% FC	100% FC	70% FC	40% FC
Control	0.53 ± 0.020de	0.41 ± 0.009gh	0.28 ± 0.012j	0.44 ± 0.013gh	0.30 ± 0.009ij	0.16 ± 0.012n
LK2	0.60 ± 0.021a-e	0.44 ± 0.015fg	0.33 ± 0.015hij	0.50 ± 0.012c-f	0.33 ± 0.015i	0.21 ± 0.015lmn
LK7	0.57 ± 0.015cde	0.51 ± 0.035ef	0.31 ± 0.009ij	0.47 ± 0.015e-h	0.34 ± 0.009i	0.20 ± 0.007mn
LK9	0.64 ± 0.006abc	0.43 ± 0.015fg	0.35 ± 0.015g-j	0.54 ± 0.006a-d	0.43 ± 0.015h	0.23 ± 0.009klm
LK13	0.68 ± 0.015ab	0.61 ± 0.012a-d	0.40 ± 0.012gh	0.59 ± 0.003a	0.50 ± 0.012c-f	0.26 ± 0.006jk
LK16	0.65 ± 0.023abc	0.58 ± 0.012b-e	0.38 ± 0.007ghi	0.52 ± 0.015b-e	0.46 ± 0.007fgh	0.24 ± 0.007j-m
LK18	0.66 ± 0.025abc	0.51 ± 0.035ef	0.35 ± 0.015g-j	0.55 ± 0.003abc	0.48 ± 0.004efg	0.24 ± 0.007j-m
LK21	0.68 ± 0.006a	0.52 ± 0.019def	0.35 ± 0.012g-j	0.55 ± 0.007abc	0.48 ± 0.004d-g	0.26 ± 0.005jkl
LK24	0.66 ± 0.022abc	0.54 ± 0.015de	0.36 ± 0.009g-j	0.57 ± 0.008ab	0.50 ± 0.004ef	0.24 ± 0.007j-m
LK29	0.58 ± 0.012b-e	0.44 ± 0.012fg	0.31 ± 0.015hij	0.49 ± 0.012efg	0.33 ± 0.012i	0.19 ± 0.015klm

Means sharing similar letter's in the treatment of each parameter do not differ significantly at p≤0.05. Data is the average of three repeats ± standard error (SE). (HSD: 0.094; 0.055)

Moreover, at 40% FC, isolate LK-13 and LK-16 showed minimum proline content (4.70 and 4.90 mg g^{-1} FW) respectively, as compared to control (7.50 mg g^{-1} FW) while these results were statistically at par with treatment LK-18 and LK-24. Similarly, as compared to 100% FC concentration of soluble sugars was less at drought level 70 and 40% FC in the treatments inoculated with rhizobacterial isolate LK-16, which was 16 and 23% less respectively, as compared to control. However, there was non-significant difference among all the treatments except control at both drought levels i.e. 70 and 40% FC (Table 5). Glycine betaine was increased under drought stress while starch content were decreased in uninoculated control (Table 6). Minimum glycine betaine (58 and 82 µmol g^{-1} DW) was measured with LK-16 and maximum increase in starch content with LK-16 was 19 and 64% more as compared to control at 70 and 40% FC, respectively. While under normal field capacity (100%) there was non-significant difference among all the treatment i.e. inoculation and uninoculation (Table 6).

Table 4: Effect of rhizobacteria inoculation on relative water content (RWC) and electrolyte leakage (EL) of maize (*Zea mays* L.) under drought stress (100, 70 and 40% FC)

Treatments	Relative water content (%)			Electrolyte leakage (%)		
	100% FC	70% FC	40% FC	100% FC	70% FC	40% FC
Control	62 ± 1.53a-e	52 ± 3.21d-i	39 ± 2.60i	4.67 ± 0.60hi	7.33 ± 0.55c-g	10.60 ± 0.38a
LK2	64 ± 2.08a-d	56 ± 1.15a-h	46 ± 1.02hi	4.67 ± 0.60hi	6.33 ± 0.33e-j	8.60 ± 0.46a-e
LK7	66 ± 1.53ab	58 ± 3.28a-h	53 ± 3.02c-h	4.77 ± 0.43hi	6.33 ± 0.88e-j	8.37 ± 0.34a-e
LK9	64 ± 2.65a-d	59 ± 2.08a-g	47 ± 2.03ghi	4.47 ± 0.29i	6.73 ± 0.56d-j	9.00 ± 0.35a-d
LK13	68 ± 1.53a	63 ± 2.89a-e	59 ± 1.45a-g	4.83 ± 0.60g-j	5.50 ± 0.29f-j	7.00 ± 0.29d-i
LK16	67 ± 1.76a	61 ± 1.53a-f	56 ± 2.08a-h	4.70 ± 0.44hij	4.97 ± 0.20g-j	6.33 ± 0.55e-j
LK18	66 ± 1.15ab	54 ± 3.46b-h	54 ± 2.85b-h	4.90 ± 0.32g-j	6.67 ± 0.88d-j	9.53 ± 0.32abc
LK21	65 ± 1.00abc	58 ± 1.84a-h	51 ± 2.65e-i	4.83 ± 0.44g-j	5.67 ± 0.33f-j	8.67 ± 0.18a-e
LK24	61 ± 2.65a-f	56 ± 4.48a-h	53 ± 2.03c-h	4.50 ± 0.29ij	7.10 ± 0.49c-h	10.00 ± 0.32ab
LK29	64 ± 1.53a-d	59 ± 2.08a-g	49 ± 1.73f-i	4.83 ± 0.22j	5.60 ± 0.45f-j	8.00 ± 0.29b-f

Means sharing similar letter's in the treatment of each parameter do not differ significantly at p≤0.05. Data is the average of three repeats ± standard error (SE). (HSD: 12.845; 2.531)

Table 5. Effect of rhizobacteria inoculation on proline content and soluble sugars of maize (*Zea mays* L.) under drought stress (100, 70 and 40% FC).

Treatments	Proline content (mg g^{-1} FW)			Soluble Sugars (mg g^{-1} FW)		
	100% FC	70% FC	40% FC	100% FC	70% FC	40% FC
Control	1.53 ± 0.29kl	3.13 ± 0.24g	7.50 ± 0.15a	46 ± 2.08gh	55 ± 2.31b-g	66 ± 2.52a
LK2	1.50 ± 0.21kl	2.87 ± 0.22gh	6.90 ± 0.17abc	47 ± 1.53fgh	52 ± 1.73c-h	63 ± 2.31ab
LK7	1.53 ± 0.15kl	2.57 ± 0.12g-k	6.30 ± 0.15bcd	45 ± 1.15gh	50 ± 1.53fgh	59 ± 1.73a-e
LK9	1.47 ± 0.18l	2.70 ± 0.21ghi	6.93 ± 0.13ab	46 ± 2.52gh	53 ± 1.15b-h	63 ± 1.53ab
LK13	1.53 ± 0.25kl	1.73 ± 0.23i-l	4.70 ± 0.15f	45 ± 1.53gh	47 ± 1.53fgh	54 ± 2.08b-h
LK16	1.57 ± 0.15jkl	2.03 ± 0.15h-l	4.90 ± 0.12ef	45 ± 2.00gh	46 ± 1.00gh	51 ± 1.15d-h
LK18	1.57 ± 0.27jkl	2.13 ± 0.30g-l	5.57 ± 0.09def	47 ± 1.00fgh	52 ± 2.08c-h	61 ± 1.53a-d
LK21	1.47 ± 0.26l	2.63 ± 0.23g-j	6.70 ± 0.12abc	45 ± 1.53gh	51 ± 2.31d-h	59 ± 2.08a-e
LK24	1.50 ± 0.18kl	1.93 ± 0.15h-l	5.83 ± 0.17cde	44 ± 2.00h	49 ± 2.52e-h	57 ± 1.73a-f
LK29	1.50 ± 0.23kl	2.67 ± 0.23ghi	6.43 ± 0.15a-d	45 ± 1.53gh	52 ± 1.53c-h	62 ± 2.00abc

Means sharing similar letter's in the treatment of each parameter do not differ significantly at p≤0.05. Data is the average of three repeats ± standard error (SE). (HSD: 1.091, 10.161)

Table 6: Effect of rhizobacteria inoculation on glycine betaine and starch content of maize (*Zea mays* L.) under drought stress (100, 70 and 40% FC)

Treatments	Glycinebetaine (µmol g^{-1} DW)			Starch (mg g^{-1} FW)		
	100% FC	70% FC	40% FC	100% FC	70% FC	40% FC
Control	50 ± 3.61hi	75 ± 4.36d-g	115 ± 5.77a	67 ± 2.60a	54 ± 2.91b-e	33 ± 2.08g
LK2	49 ± 2.52hi	68 ± 3.46f-i	105 ± 7.51ab	67 ± 1.53a	54 ± 2.65b-e	39 ± 1.73fg
LK7	48 ±2.08i	66 ± 3.21f-i	102 ± 6.00abc	66 ± 2.03ab	58 ± 1.45a-d	42 ± 1.45efg
LK9	49 ±3.00hi	70 ± 2.52e-h	110 ± 4.51ab	66 ± 1.86ab	54 ± 1.53b-e	40 ± 2.19fg
LK13	48 ±2.31i	62 ± 3.61f-i	90 ± 5.03b-e	66 ± 1.45ab	62 ± 2.08abc	50 ± 1.73e-f
LK16	47 ±2.52i	58 ± 2.89ghi	82 ± 5.13ef	68 ± 2.85a	64 ± 2.65ab	54 ± 2.00b-e
LK18	51 ±2.89hi	67 ± 2.08f-i	108 ± 7.02ab	69 ± 4.48a	59 ± 1.53abc	43 ± 2.65efg
LK21	51 ±2.00hi	67 ± 2.65f-i	104 ± 6.03ab	69 ± 2.33a	56 ± 1.45a-d	40 ± 1.86fg
LK24	49 ±1.73hi	62 ± 2.00f-i	96 ± 3.51a-d	68 ± 1.73a	59 ± 2.33abc	46 ± 2.52def
LK29	50 ±2.08hi	64 ± 2.08e-h	106 ± 4.00ab	68 ± 3.84a	57 ± 1.86a-d	41 ± 1.86efg

Means sharing similar letter's in the treatment of each parameter do not differ significantly at p≤0.05. Data is the average of three repeats ± standard error (SE). (HSD: 21.860, 12.680)

Characterization and Identification of Isolates

Various characters of bacterial isolates are shown in Table 9. Isolate LK-2, LK-9, LK18 and LK-21 were grams positive while LK-7, LK-13, LK16, LK24 and LK-29 were gram negative bacteria. All bacterial strains produced siderophore except bacterial strain LK-24 which did not has siderophore production activity. Catalase activity was observed in LK-7, LK-13, LK-16, LK-24 and LK-29 but strains LK-2, LK-9, LK-18 and LK-21 showed no catalase activity. Release in exopolysaccharides was positive with LK-7, LK-13, LK16, LK-18 and LK-24, while negative with LK-2, LK-9, LK-21 and LK-29. Bacterial isolates LK-7, LK-16 and LK-21 showed organic acid release but no release was observed with LK-2, L-9, LK-13, LK-18, LK-24 and LK-29. Moreover, all the strains showed P-solubalization and Indole acetic acid (IAA) production however, maximum P-solmization and IAA production was

observed with LK-13 and LK-16. Bacterial strains LK-13 and LK-16 both were identified as were identified as *Bacillus* spp. (Fig. 2).

Correlations

Table 10 showed the correlations between different attributes. Shoot fresh weight (SFW), root fresh weight (RFW) and total fresh biomass (TFB) revealed a significant positive correlation with relative water content (RWC), chlorophyll a and b (CHLa and b), photosynthetic rate (PR), stomatal conductance (SC), transpiration rate (TR), water use efficiency (WUE) and starch (STA) whereas, significant negative correlation with electrolyte leakage (EL), root/shoot ratio (RTS), proline (PRO) sugars (SUG) and glycine betaine (GLY). Root/shoot ratio showed negative but significant correlation with RWC, CHLa and b, PR, SC, TR, WUE and STA however, there was positive but significant correlation with EL, PRO, SUG and GLY. RWC presented significant positive correlation with CHLa and b, PR, SC, TR, WUE and STA while, significant but negative correlation with EL, PRO, SUG and GLY. EL revealed significant negative correlation with CHLa and b, PR, SC, TR, WUE and STA whereas, positive correlation with SUG, GLY and PRO. Both CHLa and b showed significant positive correlation with PR, SC, TR, WUE and STA however, significant but negative correlation with GLY, PRO and SUG. Furthermore PR, SC and TR showed significant positive correlation with each other as well with STA but significant but negative correlation with PRO, SUG and GLY. Similarly, WAUE revealed the significant negative correlation with PRO, SUG, GLY and STA. Both PRO and SUG presented positive significant correlation with GLY as well as with each other however, showed significant but negative correlation with starch (STA).

Discussion

In present study thirty rhizobacteria were isolated from maize rhizosphere grown in arid and semiarid areas of the Punjab, Pakistan. Out of thirty, nine isolates (LK2, LK7, LK9, LK13, LK16, LK18, LK21, LK24 and LK29) showed efficiency to grow under drought stress (Fig. 1). The reason of these microbial isolates to grow under drought stress might be due their ability of producing osmoprotactent, antioxidants e.g. catalase, siderophore and exopolysaccharides production which helps them to grow efficiently and tolerate drought stress. Hussain *et al.* (2014) reported that bacteria produce oxidase and catalases that help them to grow under water stress condition through by protecting cell and its organelles. They also observed that bacteria can grow in the broth medium containing 25% PEG-6000, as documented by Asghar *et al.* (2015). (These bacteria also produce exopolysaccharides (Nautiyal *et al.*, 2013; Ali *et al.*, 2014), which increase water availability and form biofilm to protect them from drought

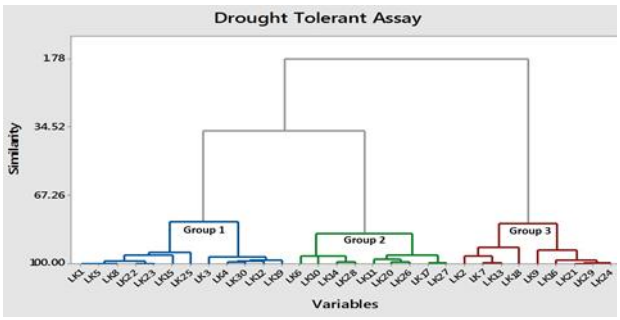

Fig. 1: Cluster analysis of rhizobacterial isolates on the basis of similarity index between optical densities (OD) from low (left- group 1) to high (wright-group 3) at different levels of drought (PEG 0, 5, 10, 15, 20 and 25%). Data is the average of three repeats

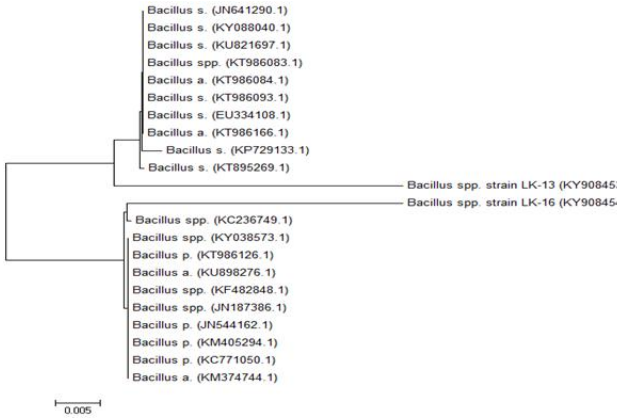

Fig. 2: Phylogenetic tree of bacterial strain LK-13 and LK-16

(Vanderlinde *et al.*, 2010). In addition, siderophore production is beneficial characteristics of bacteria for their survival under drought stress (Arzanesh *et al.*, 2011; Nautiyal *et al.*, 2013). Other studies also showed the survival of beneficial bacteria under water stressed environment (Marulanda *et al.*, 2007; Benabdellah *et al.*, 2011).

Inoculation of crop plants with rhizobacteria maintained normal plant growth, resulting in improved productivity under drought stress. Selected efficient isolates were further tested for improving growth and physiology of maize seedlings under drought stress. We observed inoculation with rhizobacteria significantly improved shoot, root and total biomass compared to un-inoculated control under drought stress, which might be due to reason that bacteria are capable for supplying nutrients under water deficit conditions through different mechanisms like phosphorous solubalization, biofilm formation which act as a channels for supplying nutrients, phytohormone production and siderophore production as reported by Vardharajula *et al.* (2011). Lim and Kim (2013) observed

Table 7: Effect of rhizobacteria inoculation on photosynthetic rate (A) and transpiration rate (E) of maize (*Zea mays* L.) under drought stress (100, 70 and 40% FC)

Treatments	Photosynthetic rate (µmol m^{-2} s^{-1})			Transpiration rate (µmol m^{-2} s^{-1})		
	100% FC	70% FC	40% FC	100% FC	70% FC	40% FC
Control	12.10 ± 0.55ef	7.90 ± 0.05g-i	2.62 ± 0.34l	2.37 ± 0.12g-j	1.40 ± 0.10mn	1.00 ± 0.06n
LK2	13.97 ± 0.50de	9.67 ± 0.54f-h	3.83 ± 0.21kl	2.71 ± 0.10efg	1.79 ± 0.17klm	1.67 ± 0.09lm
LK7	18.37 ± 0.84ab	14.52 ± 0.56ab	8.52 ± 0.74ghi	3.70 ± 0.09bc	2.32 ± 0.10g-k	2.08 ± 0.11h-l
LK9	14.47 ± 0.41cde	10.16 ± 0.56fg	4.24 ± 0.21jkl	3.24 ± 0.13cde	2.08 ± 0.10h-l	1.96 ± 0.09i-m
LK13	20.20 ± 0.35a	15.43 ± 0.32cd	8.20 ± 0.28ghi	4.40 ± 0.15a	2.67 ± 0.09fg	2.20 ± 0.06g-l
LK16	20.60 ± 0.51a	15.47 ± 0.75cd	8.41 ± 0.40ghi	4.20 ± 0.13ab	2.96 ± 0.05def	2.35 ± 0.10g-k
LK18	16.43 ± 0.37bcd	11.98 ± 0.88ef	5.87 ± 0.57ijk	3.72 ± 0.09bc	2.54 ± 0.06fgh	1.96 ± 0.05e-h
LK21	16.87 ± 0.49bc	12.37 ± 0.63ef	5.91 ± 0.15ijk	3.80 ± 0.15bc	2.49 ± 0.07klm	2.09 ± 0.11h-l
LK24	18.43 ± 0.62ab	13.81 ± 0.43de	6.96 ± 0.30hij	3.37 ± 0.08cd	2.23 ± 0.12g-l	2.07 ± 0.07h-l
LK29	18.77 ± 0.39ab	14.61 ± 0.38cde	7.58 ± 0.16ghi	3.80 ± 0.06bc	2.24 ± 0.13g-k	1.90 ± 0.10j-m

Means sharing similar letter's in the treatment of each parameter do not differ significantly at p≤0.05. Data is the average of three repeats ± standard error (SE). (HSD: 2.731, 0.569)

Table 8: Effect of rhizobacteria inoculation on stomatal conductance (gs) and water use efficiency (WUE) of maize (*Zea mays* L.) under drought stress (100, 70 and 40% FC)

Treatment	Stomatal conductance (mmol m^{-2} s^{-1})			Water use efficiency (nmol CO_2 mol^{-1} H_2O)		
	100% FC	70% FC	40% FC	100% FC	70% FC	40% FC
Control	203 ± 10g-j	135 ± 5mn	78 ± 3o	2.17 ± 0.07p	1.81 ± 0.09p	1.22 ± 0.09q
LK-2	238 ± 9d-g	166 ± 6j-m	111 ± 4no	2.85 ± 0.08lmn	2.25 ± 0.09op	1.88 ± 0.07pq
LK-7	276 ± 8a-d	198 ± 9g-j	147 ± 4lmn	4.14 ± 0.12b-f	3.66 ± 0.09e-j	3.18 ± 0.12i-m
LK-9	248 ± 13c-f	175 ± 8j-m	120 ± 6n	3.35 ± 0.19i-l	2.76 ± 0.07mo	2.35 ± 0.08nop
LK-13	306 ± 10a	222 ± 9fi	172 ± 5j-m	5.07 ± 0.18a	4.57 ± 0.09abc	4.19 ± 0.13b-e
LK-16	295 ± 7ab	234 ± 6e-h	185 ± 3i-l	4.69 ± 0.14ab	4.10 ± 0.10c-f	3.63 ± 0.09e-j
LK-18	263 ± 12b-e	188 ± 6ijk	135 ± 2mn	3.97 ± 0.11d-g	3.37 ± 0.12h-l	3.10 ± 0.05j-m
LK-21	270 ± 9abc	195 ± 7hij	142 ± 6mn	4.00 ± 0.16c-g	3.45 ± 0.09g-k	2.89 ± 0.07k-n
LK-24	280 ± 9abc	203 ± 7g-j	151 ± 3k-n	3.73 ± 0.09e-i	3.47 ± 0.03g-j	3.15 ± 0.09j-m
LK-29	274 ± 9abc	198 ± 7g-j	145 ± 5lmn	4.34 ± 0.09bcd	3.93 ± 0.09d-h	3.62 ± 0.05f-j

Means sharing similar letter's in the treatment of each parameter do not differ significantly at p≤0.05. Data is the average of three repeats ± standard error (SE). (HSD: 40.678; 0.576)

that inoculation of pepper plant with PGPR enhanced biomass up to 50%, over control. Moreover, up to 75% increase in wheat plant biomass under stress was reported by Timmusk *et al.* (2014).

Plant root also play important role to endure drought, root system architecture is most important (Huang *et al.*, 2014) including biomass of roots (Vacheron *et al.*, 2013). Previously, scientist documented that roots are associated with sustaining crop productivity under drought stress (Comas *et al*., 2013). Similar effect was observed in present study with the inoculation of rhizobacterial isolates under drought stress as compared to un-inoculated control which could be due to more available nutrient as microbes have ability to solubilize different nutrient (e.g. P), auxin production, sidrophore production, release exo-polysaccharides flavonoid (through root exudation) and action of ACC-deaminase enzyme (Cesco *et al*., 2012). Inoculation of crop plants with bacterial strains showed increase in root surface area and resultantly enhanced nutrient and water uptake from rhizosphere with positive effect on plant growth as a whole (Timmusk *et al.*, 2014). Root growth and alteration in root architecture has also been noticed by Ngumbi (2011), in plants treated with PGPR. Similarly, maize plant inoculated with *Burkholderia phytofirmans* strain PsJN showed significant increase in root biomass by 70 and 58% in Mazurka and Kaleo cultivars of maize (Naveed *et al*., 2014). The relation between deeper root system drought tolerance has also been observed by other researchers in maize crop (Hund *et al.*, 2011; Naseem and Bano, 2014).

Physiological parameters of maize (*Zea mays* L.) were adversely affected under drought stress, which could be due to water stress as water deficiency reduces the plant capacity to utilize energy, ultimately reduced CO_2 influx, photosynthetic and transpiration rate (Wang *et al*., 2003). However, inoculation with drought tolerant rhizobacteria (especially LK-13 and LK16) significantly improved the physiological attributes under drought stress. The results are similar to the findings of Naveed *et al.* (2014). They documented that inoculation of maize cultivar (Mazurka) with FD17 showed 53% improvement in photosynthesis, while inoculation of PsJN strain increased stomatal conductance up to 87% and transpiration rate up to 84% upon exposure to drought stress as compared to un-inoculated control. Similarly, *Pseudomonas fluorescens* inoculation improved photosynthetic activity in *Pinus halepensis* (Rincon *et al*., 2008) and *Azospirillum* inoculation increased photosynthetic activity in rice (Ruiz-Sanchez *et al*., 2011). Moreover, inoculation with *Phyllobacterium brassicacearum* strain STM196 increased drought tolerance in *Arabidopsis thaliana* through improved transpiration rate (Bresson *et al*., 2013). Wang *et al.* (2012)

Table 9: Plant growth promoting characteristics of rhizobacterial isolates

Characteristics	LK-2	LK-7	LK-9	*LK-13	**LK-16	LK-18	LK-21	LK-24	LK-29
Colony color	White	White	Yellow	Yellow	Creamy white	Yellow	Creamy white	Light yellow	Light yellow
Gram's staining	—	+	—	+	+	—	—	+	+
Siderophore production	+	+	+	+	+	+	+	—	+
Catalase activity	—	+	—	+	+	—	—	+	+
Exopolysaccharides	—	+	—	+	+	+	—	+	—
Organic acid	—	+	—	—	+	—	+	—	—
P solubalization Hollow dia. (mm)	8.67±1.20	15.10±1.99	13.04±1.70	20.00±1.15	18.33±2.73	16.33±0.88	13.67±2.67	16.67±2.33	15.63±1.21
IAA production (mgL^{-1}) without (L-TRP)	1.6±0.32	2.6±0.23	1.8±0.21	3.1±0.13	3.4±0.31	2.4±.30	1.9±0.23	2.1±0.44	2.5±0.26
with L-TRP	20±2.08	25.4±2.84	17.7±1.76	32.7±2.85	35.3±3.18	24.3±1.45	27.0±3.06	24.3±2.60	19.7±1.20

Most efficient isolates *LK-13 and **LK-16 were identified as *Bacillus* spp. LK-13 and *Bacillus* spp. LK-16. Positive sign (+) represents the presence and negative sign (—) represents the absence of character

Table 10: Correlation matrix among different attributes of maize (*Zea mays* L.) hybrid

Attributes	SFW	RFW	TFB	RTS	RWC	EL	CHLa	CHLb	PR	SC	TR	WUE	PRO	SUG
RFW	0.853***													
TFB	0.972***	0.951***												
RTS	-0.411***	-0.462**	-0.450***											
RWC	0.785***	0.774***	-0.810***	-0.622***										
EL	-0.834***	-0.780***	-0.841***	0.555***	-0.763***									
CHLa	0.915***	0.859***	0.924***	-0.510***	0.777***	-0.806***								
CHLb	0.905***	0.807***	0.895***	-0.468***	0.747***	-0.795***	0.952***							
PR	0.908***	0.825***	0.905***	-0.563***	0.802***	-0.821***	0.898***	0.871***						
SC	0.876***	0.884***	0.913***	-0.606***	0.840***	-0.827***	0.903***	0.873***	0.940***					
TR	0.779***	0.863***	0.847***	-0.575***	0.761***	-0.725***	0.831***	0.798***	0.871***	0.923***				
WUE	0.501***	0.513***	0.526***	-0.674***	0.657***	-0.543***	0.604***	0.558***	0.753***	0.752***	0.763***			
PRO	-0.928***	0.763***	-0.889***	0.506***	-0.787***	0.847***	-0.893***	-0.906***	-0.876***	-0.835***	-0.685***	-0.512***		
SUG	-0.845***	0.758***	-0.838***	0.577***	-0.751***	0.826***	-0.834***	-0.827***	-0.855***	-0.851***	-0.748***	-0.583***	0.875***	
GLY	-0.934***	0.804***	-0.911***	0.518***	-0.778***	0.856***	-0.891***	-0.886***	-0.889***	-0.857***	-0.731***	-0.515***	0.945***	0.883***
STA	0.895***	0.832***	0.900***	-0.529***	0.806***	-0.848***	0.893***	0.877***	0.868***	0.875***	0.754***	-0.515***	-0.924***	-0.86***

***shows the significant at $p \leq 0.05$; SFW: Shoot fresh weight, RFW: Root fresh weigh, TFB: Total fresh biomass, RTS: Root to shoot ratio, RWC: Relative water content, EL: Electrolyte leakage, CHLa: Chlorophyll a, CHLb: Chlorophyll b, PR: Photosynthetic rate, SC: Stomatal conductance, TR: Transpiration rate, WUE: Water use efficiency, PRO: Proline, SUG: Sugars, GLY: Glycine betaine, STA: Starch

investigated that cucumber inoculation with plant growth promoting rhizobacterium consortium (*Bacillus cereus* AR156, *Bacillus subtilis* SM21 and *Serratia* sp. XY21) showed increased photosynthetic activity under drought stress. Inoculation of plants with rhizobacteria also decreased electrolyte leakage in present study (Table 4). Our results are according to the findings of Naveed *et al*. (2014) who used *Burkholderia phytofirmans* strain PsJN and noticed that this strain reduced the electrolyte leakage under reduced water condition. Similarly, Sandhya *et al.* (2010) documented that treatment with GAP-P45 inoculation resulted in minimum electrolyte leakage 68% followed by WAPP53 (70%). Other researchers also showed increased electrolyte leakage under drought however, bacterial inoculation significantly decreased the electrolyte leakage under drought stress (Armada *et al*., 2015; Ortiz *et al.*, 2015). In present study, we observed that bacterial inoculants also increased the chlorophyll a and b under drought stress (Table 3) and this increase in chlorophyll with inoculation of PGPR are responsible for improved photosynthetic efficiency under drought stress (Gururani *et al*., 2013). Increase in chlorophyll content due to bacterial inoculation was also reported by Naveed *et al*. (2014.

Osmotic adjustment is key adaptation at cellular level that improves drought tolerance in plants (Farooq *et al.*, 2009), protect enzymes, proteins, cellular organelles and membranes from oxidative damage (Huang *et al*., 2014). Osmotic adjustment is the accumulation of certain organic and inorganic solutes (compatible solutes) that includes glycine betaine, sugars proline etc. (Farooq *et al*., 2008). These compatible solutes helped the plants to maintain their cellular turgor and lower water potential without decreasing original water content (Serraj and Sinclair, 2002). Serraj and Sinclair (2002) also observed that proline content was increased under drought stress while inoculation showed increased proline content but as compared to un-inoculated control increased was lower. The results are similar to the finding of previous researchers (Vardharajula *et al.*, 2011; Naseem and Bano, 2014) who reported that inoculation of maize crop with PGPR increased the proline content. Armada *et al.* (2015) documented that the lower level of proline in maize inoculated with Arbuscular mycorrhiza (AM) and *Bacillus* sp. compared to control. In addition significant decrease in starch content was noticed under water limited conditions, while inoculation of rhizobacterial strain *Pseudomonas* sp. showed significantly higher starch

content under stressed condition as compared to un-inoculated control (Sandhya *et al.*, 2010). Bacteria also produce several organic solutes such as amino acids which help in regulation of plant physical and biochemical processes under water stressed condition (Vardharajula *et al.*, 2011).

Conclusion

Plant growth promoting rhizobacteria showed ability to sustain their growth under drought stress even at swear stress. Taking all the parameters into consideration, drought tolerant PGPR, especially isolates having exo-polysaccharides (EPS) producing ability along with siderophore production and P-solubalization ability improved more growth of maize seedlings under drought stress than control. Furthermore, *Bacillus* spp. LK-13 and *Bacillus* spp. LK-16 were most drought tolerant isolates as most of parameters were improved with inoculation of both isolates under drought stress, as compared to control as well as among other PGPR isolates. So, inoculation of exo-polysaccharides producing bacterial inoculants could be a novel approach for better growth and production of maize (*Zea maize* L.) under water stressed environment. However, field experiments of such bacterial inoculants should be done for further evaluation.

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