Egypt. J. Agron. **Vol. 45**, No. 1, pp. 99-110 (2023)

Egyptian Journal of Agronomy
http://agro.journals.ekb.eg/

Role of Resilient Soil Microbial Consortia on Growth and Yield Attributes of Chickpea under Water Stress

Azhar Mahmood Aulakh[(1)#], Ghulam Qadir[(1)], Fayyaz Ul Hassan[(1)], Rifat Hayat[(2)], Tariq Sultan[(3)], Riffat Bibi[(4)], Sarfraz Ahmed[(4)], Muhammad Yunas[(4)], Muhammad Itikhar[(3)], Safina Noreen Malik[(4)]

[(1)]Department of Agronomy, PMAS Arid Agriculture University, Rawalpindi 46000, Pakistan; [(2)]Soil Science Research Institute, PMAS Arid Agriculture University, Rawalpindi 46000, Pakistan; [(3)]Land Resources Research Institute(LRRI), National Agricultural Research Centre, Islamabad 44000, Pakistan; [(4)]Soil and Water Conservation Research Institute (SAWCRI), Chakwal, Pakistan.

DRYLAND crop production is restraining due to severe effects of drought spells and natively derived resilient isolates can support crop yield enhancement under abiotic stress. This study elaborated for isolation, morpho-physiological, biochemical characterization and molecular identification of indigenous soil microbes from Thal desert of Pakistan and their application to elucidate the efficacy of desert isolates on yield of chickpeas growing on three farmer fields (Kakri, Adhi Kot and Jara) of native area. Isolates were identified through 16sRNA sequencing and used to formulate eight consortia with different combination. Four isolates (*Bacillus subtilis* RP-01, *Enterobacter cloacae* RP-08, *Bacillus mojavensis* RS-14 and *Providencia vermicola* RS-15) have ability to produce indole-3-acetic acid (IAA), Protease, catalase, ACC-deaminase and EPS, among, the two strains RP-01 and RP-08 were found phosphate solubilizer and others two isolates *Mesorhizobium ciceri* RZ-11 and *Mesorhizobium ciceri* RZ-22 were appeared as ammonia producing. The consortia in T7 showed highest grain yield (1201kg ha^{-1}) followed by T2 (1126kg ha^{-1}) at same location (Kakri) comparatively to other consortia and control (557kg ha^{-1}). Similarly, T2 found most promising consortia regarding grain yield (1072.1kg ha^{-1} and 961.8kg ha^{-1}) at locations Adhi Kot and Jara, respectively during the 1st year. However, T4 also performed with par to T2 and T7 consortium and same trend was seen in the next year field experiments. The present study confirmed that T2 (*Mesorhizobium ciceri RZ-11* + *Bacillus subtilis* RP-01 + *Bacillus mojavensis RS-14*), T4 (*Mesorhizobium ciceri RZ-11* + *Enterobacter cloacae* RP-08 + *Providencia vermicola RS-15*) and T7 (*Mesorhizobium ciceri RZ-22* + *Enterobacter cloacae* RP-08 + *Providencia vermicola RS-15*) are promising consortia for chickpeas yield in Thal desert of Punjab, Pakistan.

Keywords: ACC-deaminse, Bacterial consortia, Chickpea, Thal desert, PSB, Yield.

Introduction

In the new epoch, water stress decreased crop production (Kavadia et al., 2020). It is expected that the world will gain 9 billion human populations by 2050, and will face the un-sufficiency of food. Thus, the global food security is highlighted as a burning issue, so, it is need of the day to focus on mitigating the inverse effects of desiccation through adopting alternate technology under limited available recourses (Yang et al., 2009; Mancosu et al., 2015; Aalipour et al., 2020; Khan et al., 2020). Therefore, the soil microbial activities are placed as top priority of alternate technology for enhancing crop yield under stressful conditions.

#Corresponding author e-mail: azuaulakh@gmail.com
Received 01/09/2022; Accepted 25/06/2023
DOI: 10.21608/AGRO.2023.159807.1336

Many experiments have been designed in all over the world to highlight plant-bacteria symbiotic relationship and to derive efficient strains for sustainable crop production in desiccated area (FAOSTAT, 2020). Chickpea (*Cicer arietinum* L.) is an important pulse crop and rich in grain protein which is a leading part of human diet (Aslam et al., 2000). The worldwide very low chickpea production (17.19 million tons) is reported from total cultivated area of 17.81 million hectares (Kantar et al., 2007). The low production of chickpeas may be attained due to prolonged spell of drought (Khan & Bano, 2019a). To date, the way forward is to use of resilient microbes to enhance dry land chickpea production under desiccation environment. All leguminous crops including chickpea conserve free environmental nitrogen in the root nodules having *Rhizobium* sp. However, chickpea fix about 60 % nitrogen during crop season through the process of biological nitrogen fixation (Khan & Bano, 2019b). The combination of *Rhizobium* and phosphate solubilizing isolates in a consortium enhance growth rate of inoculated chickpea plants instead of inoculating with single strain (Billah et al., 2019). Among macronutrients the phosphorus is most important nutrient after nitrogen for plant growth but mostly fixed in the soil profile due to phosphatase enzyme inactivity especially under stressful condition of drought and unavailable to plants, therefore, it is necessary to make sure its availability through application of phosphate solubilizing bacteria (PSB) (Heydari et al., 2019). Root growth and development occurred through cell division and elongation with the help of soil phosphorus. Phosphorus increases plant growth and yield attributes by improving roots architecture, thus it is indirectly involved in absorbing water from untapped soil profile (Nannipieri et al., 2011). Phosphatase enzyme released by PSB, converts fixed phosphorus to soluble form from fixed soil P-pools for plant growth requirements (Sharma et al., 2013; Kour et al., 2019). Thus, application of PSB play a key role for availability of phosphorous to plants under abiotic stress in desiccated dry areas (Khan & Bano, 2019c). Keeping in view the vital role of phosphorus in root architecture improvement which helps an increase in water absorption from soil profiles (Kour et al., 2019) and fixation of free environmental nitrogen in roots nodules to produce higher chickpea yield under stressful conditions of Thal desert, Punjab, Pakistan.

Studies revealed that promising PGPR strains having 1-aminocyclopropane-1-caboxylate (ACC) deaminase, exopolysaccharides (EPS) and phytohormones releasing characters, induce drought tolerance in dry land chickpea plants (Vurukonda et al., 2016). Thal desert is located (31_30000.0" N 71_40000.0" E) in Tehsil Nurpur Thal, Mankaira, Bhakkar and Chobara along with some part of districts Jhang and Muzafargarh, Punjab, Pakistan. This area is major chickpea producing rain fed tract and chickpea yield depends on annual rainfall, however, few acres irrigated by tube wells also. The average chickpea production from Thal desert is much lower than crop yield potential. Leguminous crops consist a specific symbiotic systems which are capable to exist in extreme conditions of alkalinity, temperature and moisture stress (Khan & Bano, 2019a), which shows its adaptability to different soil and environmental conditions. Atmospheric nitrogen fixation through symbiotic association in the root nodules of leguminous crops is the major substitute of inorganic nitrogenous fertilizers in the vast tract of Thal desert, Pakistan. However, considering the scarcity of water and issues of reduced crop yield from the deserts, the present study was designed to examine the performance of resilient soil microbes to increase the chickpea production in Thal Desert under prolonged drought spells. The hypothesis of our study is that isolated bacteria may be helpful to increase the drought tolerance in plants by secreting different compounds like ABA, exopolysaccharides, producing ACC-deaminase and enzymatic activities. Moreover, rhizobium species may increase nodulation for better productivity in chickpeas through the process of biological nitrogen fixation. Building consortia between PGPR, including those having P-solubilizing activity and rhizobium species might increase significantly chickpea crop production.

Material and Methods

Isolation and microbial strains collection

Fresh nodules and rhizosphere along with rhizoplane soils were collected from different located sand dunes during prolonged drought spells (soil moisture < 7%) of chickpea producing area of Thal desert, Punjab, Pakistan in winter crop season 2017–2018. *Rhizobium* was isolated from fresh colored nodules on Yeast Mannitol Agar (YMA). PSB and other PGPRs were isolated from rhizoplane (RP) and rhizospheric

soil (RS) of collected roots on Pikovskaya agar and Luria Bertani medium (Mainatis et al., 1982), respectively. The isolates RP-01, RP-08, RS-14, RS-15, RZ-11 and RZ-22 were biochemically characterized by using standard methods (Cappucino & Sherman, 1992).

Morpho-physiological characterization and molecular identification of isolates

The *rhizobium* isolates (RZ-11, RZ-22) and PGPRs (RP-01, RP-08, RS-14, RS-15) were characterized for their shape, margin and color (Smibert, 1994) and molecular identification was done by amplification and 16sRNA gene sequencing. Pure isolates were mixed with 20μL Tris-EDTA buffers in Polymerase Chain Reaction (PCR) strips and kept for 10min at 95^0C in a PCR apparatus (Thermal Cycler PCR PEQSTAR, Munich, Germany) for centrifuged template DNA extraction. Same apparatus was used for DNA amplification with the help of universal primers as 2μL forward 9F (50-GAGTTGATCCTGGCTCAG-30)andreverse 1510R (50-GGCTACCTTGTTACGA-30), 25μL TAKARA Pre-mix Ex-Taq, 20μL PCR water and 1μL of DNA template. The sequencing was done from Macrogen, Seoul Korea and each isolate were allotted accession numbers from gene bank after submission of sequences.

In vitro biochemical characterization of isolates

IAA (indole-3-acetic acid), Phosphate solubilization assay and its solubility index were carried according to respective methodology (Qureshi et al., 2011; Hussain et al., 2019). Enzymatic activities (catalase and amylase) were evaluated through the methods given by Macfaddin (2000) and Ajayi & Fagade (2006), respectively. Isolates were tested for NH_3 production (Dinesh et al., 2015) and Siderophore assay by using procedure given by Schwyn & Neilands (1987). Also, ACC deaminase enzyme activity and production of Exopolysaccharide (EPS) were examined by methodology of Honma & Shimomura (1978) and Subair (2015), respectively.

Isolates compatibility in consortium

Three isolates in each consortium were cultured side by side (1cm apart) in a petri plate having agar medium (3.0g yeast extract, 20.0g agar L^{-1} and 5.0g peptone) to observe the compatibility among each other. These petri plates (triplicate) were kept in incubator at 28-30^0C for 72h. The isolates were examined visually and culture images were taken to record the inhibiting effect of isolates on each other along with close ups of the overlapping areas of expanding colonies (Hashmi et al., 2019).

Seed inoculums preparation

The chickpea (*Cicer arietinum* L.) var. Bhakkar-2011 was arranged from Arid Zone Research Institute (AZRI), Bhakkar, Punjab, Pakistan. The varietal seeds were mixed with sticky materials (gum acacia), then, for each isolate the broth cultures (10mL) were prepared in their respective medium. Separate broth cultures were mixed in 1:1 ratio for consortium treatments and kept it for 5h. From this mixture, 1g sample (seed) was used for serial dilution and spread on yeast extract mannitol agar and nutrient agar. The plates were kept for incubation at 28 ± 2°C for 3-4 days to make bacteria population as 10^7 CFU/ seed.

Experimental soil analysis

Three composite samples of experimental sites were used for analysis; soil texture (Koehler et al., 1984), organic matter (Walkley & Black, 1934) soil phosphorus (Olsen et al., 1982), soil nitrogen (Jackson, 1962), available phosphorus and potassium (Soltanpour & Schwab, 1977) and soil pH (1:5 soil-water) were determined following the methodology described by Mcclean (1982) and given in Table 1.

Field experiment

The farmer field experiments were laid out with 8 consortium treatments (T1= RZ22 + RP01+ RS15, T2= RZ11 + RP01 + RS14, T3= RZ11+ RP08 + RS14, T4= RZ11 + RP08 + RS15, T5= RZ11 + RP01 + RS15, T6= RZ22 + RP08 + RS14, T7= RZ22 + RP08 + RS15, T8= RZ22 + RP01 + RS14, T9= control, where RZ11= *Mesorhizobium ciceri*, RP08= *Enterobacter cloacae*, RS14= *Bacillus mojavensis*, RS15= *Providencia vermicola*, RP01= *Bacillus subtilis*, RZ22= *Mesorhizobium ciceri*) along with un-inoculated control and replicated thrice according to Randomized Complete Block Design (RCBD) by using the following set (Table 3) of treatments. The trial was laid out during winter crop season of 2018-19 and 2019-20 at 3 farmer field (Kakri, Jara and Adhi kot) of Thal desert. Crop sowing was done with single row gram drill with 30cm distance among rows. The plants were harvested at maturity and data regarding growth and yield attributes such as nodule number $plant^{-1}$, nodule

dry weight plant^{-1} (mg), Root dry weight plant^{-1} (cm), Shoot dry weight plant^{-1} (mg), plant height (cm) and number of pods plant^{-1} were recorded.

Statistically data analysis

All recorded data was analyzed using analysis of variance (ANOVA). The mean comparisons were carried out by the Tukey's honestly significant difference test (Steel et al., 1997) at P= 0.05.

Results and Discussion

Isolation and morpho-physiological characterization

Two *rhizobia* (RZ-11 and RZ-22) from root nodules, Two PGPR (RS-14 and RS-15) from rhizospheric and two PSB (RP-01 and RP-08) from rhizoplane soil of chickpea were isolated. MIRA3, Tescan Libušina tˇrída, Brno, Czech Republic SEM (scanning electron microscope) was used to characterize colony shape, form, elevation, color and margin (Table 1). Root nodule isolates (RZ-11 and RZ-22) showed negative gram reaction, however, positive in ammonia production. Results coincided with findings of (Naseem et al., 2018), who revealed that ammonia producing microbes play a vital role to enhance crop growth attributes. Two *PSB* strains (RP-01 and RP-08) appeared as positive gram reaction, siderophore, oxidase citrate test, Catalase, Protease, and Carbohydrates (glucose, lactose and sucrose). Two, gram negative isolates (RP-08 and RS-14) showed similar characteristics as positive for Catalase, oxidase citrate test and Protease. All strains showed positive test for Carbohydrates (glucose, lactose and sucrose) activities (Table 1).

Identification of the microbial strains

Most efficient *rhizobium* (RZ-11 and RZ-22) and PGPRs (RP-01 and RP-08, RS-14 and RS-15) isolates were recognized through 16sRNA gene sequencing analysis. The phylogenetic tree (Fig. 1) was made by identifying nucleotides through MEGA 7 (Kumar et al., 2016b). *Rhizobium* strain RZ-11 resembles to RZ-22 (*Mesorhizobium ciceri*), SS1 (5) and CM-25. Same trend was seen among RS-14, *Bacillus mojavensis,* RS-1, PMCC-9 and LMB3G43 in the phylogenetic tree and others strains congregated together with 3 Providencia strains Mum1, Ag1 and OF6. PGPR isolates having P-solubilizing ability coded RP-01 and RP-08 resembles to *Bacillus subtilis* strain XGL205 and *Enterobacter cloacae* strain MSK, respectively.

In vitro biochemical characterization of isolates

Most resilient isolates (RP-01, RP-08, RS-14 and RS-15) released ACC-deaminase, Exopolysaccharides (EPS) and Indole acetic acid (IAA) in sufficient quantity (Tables 1, 2). Enzymatic activity in two isolates RS-14 (*Bacillus mojavensis*) and RS-15 (*Providencia vermicola*) were positive for IAA. Similar findings were also evaluated by other researchers (Majeed et al., 2015; Khan & Bano, 2016). However, the highest value of ACCD (0.84μM/mg protein/h), EPS (0.80mg/mL) and IAA (86μg/mL) were recorded by RP-08. Isolate RP-01 showed the highest value (3.0) of P-solubilization index, followed by RP-08 (2.90), compared to other strains. Similar findings were resulted by Thakur & Putatunda (2017), who found *Enterobacter* sp., have maximum phosphate solubilizing ability. In this study, we found very resilient microbes (RZ-11, RZ-22, RP-01, RP-08, RS-14, RS-15), those induced drought resistance in chickpea through exopolysaccharides (EPS), phytohormones, 1-aminocyclopropane-1-1 carboxilate (ACC) deaminase. Our results are in agreement with Kumar et al. (2016a) and Khan et al. (2018), who evaluated the mechanism to induce drought tolerance in wheat and chickpea grown on dry lands under abiotic stressful conditions with the help of these bio-chemicals.

Isolates formulated consortium impact on growth, grain yield and grain protein of chickpea growing under field conditions

The isolates in the consortium were found compatible to each other, therefore, each consortium showed positive impact on nodulation, growth and yield attributes of chickpea at different locations of Thal desert, during 1st year of field experiments. The highest number of nodules plant^{-1} (35.09) were showed by T7 at location Kakri, followed by T2 with nodules plant^{-1} 32.91 and 31.34 at Kakri and Adhi kot, respectively, compared to un-inoculated and all others treatment (Table 3). Similar results were reported by Dey et al. (2004), that chickpea seed inoculation with *Mesorhizobium ciceri gave more* nodulation plant^{-1} comparatively to un-inoculated. Similar trend was seen regarding this attribute (nodules plant^{-1}) during 2nd year of experiments. Similarly, in the 1st year, the highest nodular dry weight plant^{-1} (124.60mg) were resulted by T7 at location Kakri, followed by T2 with nodular dry weight plant^{-1} 114.13mg and 107.13mg at Kakri and Adhi kot, respectively. However, during 2nd year experimental results, maximum nodular dry weight plant^{-1} (129.91mg) was measured by T4 at

Kakri, followed by T7 (118.40mg) at same location. Our experimental results are in conformity with the results found by Romdhane et al. (2007), Khan & Bano (2019b), who evaluated that *Mesorhizobium ciceri* inoculation increased significantly the nodulation of chickpea plants through symbiotic relationship. During the 1st year, the maximum root dry weight plant^{-1} (27.67mg) were showed by T7 at location Kakri, followed by T2 with root dry weight plant^{-1} 25.34mg and 25.20mg at Adhi kot and Kakri, respectively, compared to un-inoculated and all others treatment. However, during 2nd year experimental results, maximum root dry weight plant^{-1} (24.43mg and 23.70mg) was measured by T2 at Jara and Adhi kot, respectively.

TABLE 1. Biochemical properties of microbial isolates

Biochemical characters	***Bacillus subtilis***	***Enteroabacter cloacae***	***Bacillus mojavensis***	***Providencia vermicola***	***Mesorhizobium ciceri***	***Mesorhizobium ciceri***
Colony morphology	Irregular, dry/ rough undulate margin, yellowish	Round, smooth, entire margin, yellowish	Round, moist, convex, undulate margin, yellowish	Round,moist, entire margin, light yellow	Round, sticky raised, white	Round, sticky/ gummy, raised, white
Gram reaction	+v	–	–	–	–	–
NH_3	–	–	–	–	+v	+v
Catalase, oxidase citrate test	+v	+v	+v	+v	–	–
Siderophore	+v	+v	+v	+v	–	–
Amylase	–	+v	+v	–	–	
Protease	+v	+v	+v	–	–	
Catalase						
hydrated carbon						
Glucose	+v	+v	+v	+v	+v	+v
Lactose	+v	+v	+v	+v	+v	+v
Sucrose	+v	+v	+v	+v	+v	+v

"+v" pointed positive test; "–"pointed negative test.

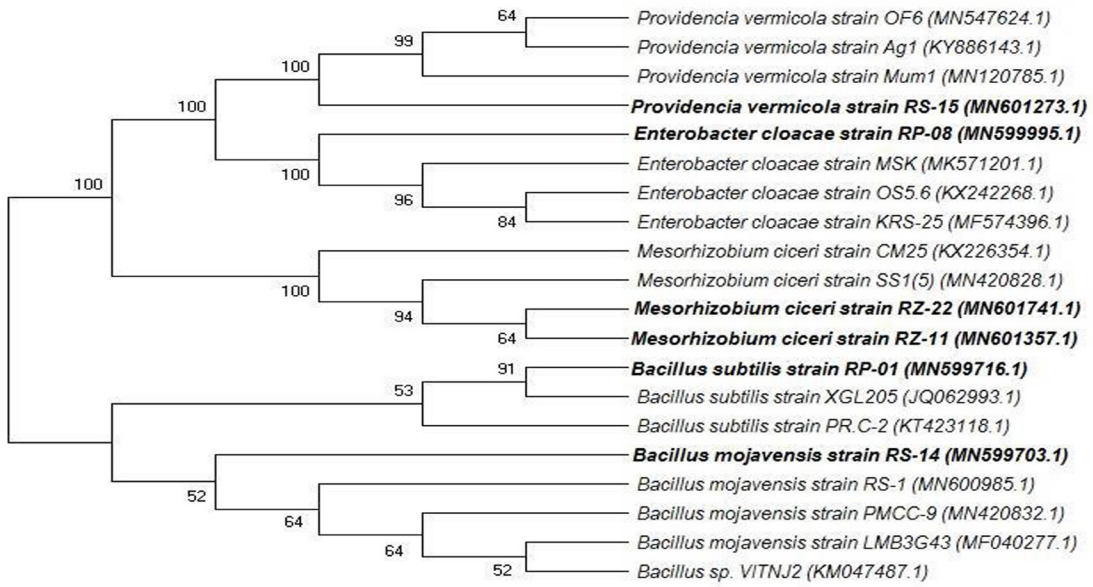

Fig. 1. Phylogenetic tree having arrangement of the screened isolates [Bootstrap values (n= 100) are displayed at the nodes]

TABLE 2. Plant growth promoting properties of Isolates

Bacterial strain	IAA (µg/mL) at different tryptophan (µg/mL)	Solubilization index	Exopolysaccharide (mg/mL)	ACC-deaminase (μM/mg protein/h)
Bacillus subtilis RP-01	79 ± 1.60	3.00	0.74 ± 0.032	0.77 ± 0.024
Enteroabacter cloacae RP-08	86 ± 1.99	2.90	0.80 ± 0.030	0.84 ± 0.016
Bacillus mojavensis RS-14	63 ± 1.40	2.58	0.61 ± 0.062	0.66 ± 0.022
Providencia vermicola RS-15	71 ± 1.80	2.72	0.68 ± 0.038	0.69 ± 0.012
Mesorhizobium ciceri RZ-11	NF	NF	NF	NF
Mesorhizobium ciceri RZ-22	NF	NF	NF	NF

NF, Mean Not Found

In both the year, the increasing trend regarding plant height was observed as T7>T2>T4 (Table 4). Our results are matched with the findings of Gull et al. (2004), who found that shoots and roots architecture increased by phytohormone releasing isolates. Similar findings were reported by Marasco et al. (2012), who evaluated that plant-root system increases up to 40% higher in PGPRs treated plants as compared to untreated controls. Similarly, in the 1st year, the number of pods plant^{-1} (34.73) were showed by T7 at location Kakri, followed by T2 with number of pods plant^{-1} 32.00 and 31.93 at Adhi kot and Kakri, respectively. However, during 2nd year experimental results, maximum number of pods plant^{-1} (36.20) was measured by T2 at Adhi kot, followed by T7 (35.60) at Kakri. Previously researchers also reported higher number of pods per plant of chickpea under *vivo* and *vitro* conditions due to co-inoculation of *rhizobium* and PGPRs (. Similarly, in the 1st year, the maximum biological yield (2428.8kg ha^{-1}) were resulted by T7 at location Kakri, followed by T2 with biological yield 2315.1kg ha^{-1} and 2263.8kg ha^{-1} at Adhi kot and Kakri, respectively, and lowest biological yield kg ha^{-1} (795.3) was obtained by un-inoculated (control) from Jara. However, during 2nd year experimental results, maximum biological yield (2574.5kg ha^{-1}) was measured by T7 at Kakri, followed by T2 (2479.1kg ha^{-1}) at Jara, while minimum biological yield kg ha^{-1} (485.9) was seen by control from Jara experimental site.

In the 1st year, the highest grain yield (1032kg ha^{-1}) was recorded for consortium in T2 treatment followed by treatment T7 which responded to yield of 1012.7kg ha^{-1}, while the lowest grain yield (455kg ha^{-1}) was recorded for T9. Thus, the T2 showed 1.90% and 126.8% higher biological yield (kg ha^{-1}) over the plants treated with T7 and untreated (T9) respectively. The data pertaining to locations, the maximum grain yield (894.1kg ha^{-1}) was recorded at Kakri with percent increase of 23% and 47.2% over Adhi Kot and Jara respectively. Interaction among treatments, year and locations showed highest grain yield by T7 (1200.4kg ha^{-1}) at Adhi Kot followed by T2 (1126.3kg ha^{-1}) at Kakri. Moreover, T2 also performed better by giving (1072kg ha^{-1} & 960.8kg ha^{-1}) at Adhi Kot and Jara, while minimum grain yield (198.3kg ha^{-1}) at Jara.

In the 2nd year, T7, T2 and T4 resulted as most promising consortia for grain yield (1152kg ha^{-1}, 1147.1kg ha^{-1} & 1110.8kg ha^{-1}) respectively followed by T3 (897.6kg ha^{-1}), while minimum grain yield (437.4kg ha^{-1}) was recorded by T9 (uninoculated). In case of percent, increase the T7, T2 and T4 showed 163%, 162% and 154% higher grain yield respectively than untreated (control). The data regarding locations, the maximum grain yield (961.3kg ha^{-1}) was recorded at Kakri with percent increase of 10.4% and 10.9% over Adhi Kot and Jara respectively. Interactively (treatments x year x locations) effect on tabulated attribute showed that maximum grain yield was recorded by T7 and T2 (1275.3kg ha^{-1} & 1221.4kg ha^{-1}) at Kakri and Adhi Kot respectively followed by T4 (1179.2kg ha^{-1}) at Kakri. Moreover, T2

gave 1080.4kg acre^{-1} grain yield at Jara during 2nd year field trial while lowest grain yield was recorded by (348.3kg ha^{-1}) at Jara.

All consortia gave positive response to grain yield at each location during both year (2018-19 & 2019-20) field trials. Grain yield (kg ha^{-1}) was increased (21.2%) in 2nd year conducted field experiment than the Grain yield (kg ha^{-1}) obtained during 1st year at same locations (Kakri, Adhi Kot & Jara). Similarly, experiments were laid out to study the impact of efficient bacterial strains producing resistance capabilities in crop plants to maintain productivity under drought stress environment. Results revealed that drought severity is considered main obstacle to crop productivity on wide range of arable land in the world. Many strategies are adopted to mitigate drought stress effects. PGPR has significant positive response to alleviate drought stress in dry land crops by secreting exopolysaccharides (EPS), phytohormones, 1-aminocyclopropane-1-1carboxilate (ACC) deaminase along with adjusting root architecture and growth dynamics to enhance drought tolerance. The microorganisms (PGPR) caused several physiological and chemical adaptations in dry land crop plants to Induce Systemic Tolerance (IST) against worse effects of abiotic stress especially drought (Vurukonda et al., 2016). A similar field study was designed at two different locations on chickpea crop to evaluate the effect of seed inoculation with Rhizobium strains along with nitrogen application @ 30kg ha^{-1} by Khattak et al. (2006). Results revealed as both treatments gave significantly positive response to grain yield of chickpea at both experimental sites. Yadav et al. (2011) took samples of Rhizospheric soil and root nodules from a selected chickpea field. They screened eight rhizobium strains from fifty pre-isolated strains for field experiments. Results revealed that grain yield and yield regarding attributes were increased by inoculation with screened out *Rhizobium* strains significantly in comparison to un-inoculated treatment. Quantity of nodules, their dry weight, economic yield and biological yield were increased by 73.53%, 78%, 31.76% and 24.37% respectively. In our study, most promising strains (RZ-11, RZ-22, Rp-01, RP-08, RS-14, RS-15) produced exopolysaccharides (EPS), phytohormones, 1-aminocyclopropane-1-1carboxilate (ACC) deaminase and P-solubilizing activities and helped the chickpea in acquisition of drought tolerance at 55 % field capacity. Similar studies were conducted by Timmusk et al. (2014) and Kumar et al. (2016a) on the mechanism to induce drought tolerance in wheat and chickpea grown on dry lands. They highlighted the role of microorganisms to manage abiotic and biotic stress by producing indole acidic acid (IAA) and ACC-deaminase to reduce levels of ethylene in roots.

The physico-chemical and biological properties of composite soils of initial and final

The physico-chemical properties of the pre-sowing soil and postharvest soil were recorded as sandy loam in texture, ratio of sand, silt, clay, soil pH and electronic conductivity given in Table 5. The content of organic matter, pre-sowing, postharvest soil available P, N and K were determined (Table 5).

Conclusion

Extreme drought events are expected to be one of the main challenges for agriculture and, a threat for global food security. Exploration and utilization of desert microbes to cope with the issue of drought through experimentation on desert soil is quite a novel idea being adopted in the present study. Series of experiments revealed growth promotion as well as substantial nodulation characteristics in chickpea to enhance their grain yield under drought stress. This approach indicates the vital role of isolated strains to be utilized as bio-fertilizers under drought spell in Thal desert; main chickpea producing area in Pakistan, which is 4th largest chickpea producing country. So, it is concluded that the consortia T_2 (*Mesorhizobium ciceri* RZ-11 + *Bacillus subtilis* RP-01+ *Bacillus mojavensis* RS-14) and T_7 ((*Mesorhizobium ciceri* RZ-22 + *Enteroabacter cloacae RP-08* + *Providencia vermicola RS-15*) can perform best in drought conditions of Thal desert, Punjab, Pakistan. Hence, microbial combination in T2 and T7 could be used to make effective biofertilizers for chickpea growing areas under rainfed conditions to cope with drought spells.

Acknowledgements: We thank to Land Recourses Research Institute, National Agricultural Research Centre, Islamabad, for providing the lab facilities to carry out this research work and to farmers giving experimental sites.

TABLE 3. Effect of microbial consortia on the chickpea growth attributes at 3 locations of farmer fields during years

Treatment	Locations	Nodule number/ plant		Nodule dry weight/ plant (g)		Root dry weight/ plant (cm)		Shoot dry weight/ plant (g)	
		Year-1	Year-2	Year-1	Year-2	Year-1	Year-2	Year-1	Year-2
	1	26.63 g-n	30.92 d-i	90.20 g-o	94.61 e-l	19.93 a-j	18.37 b-k	49.83 e-m	52.77 d-m
T_1	2	21.42 m-s	26.64 g-n	72.53 n-w	81.67 j-s	17.61 b-1	18.50 b-k	40.67 m-r	54.77 c-k
	3	17.88 q-v	20.57 n-s	60.53 t-a	66.60 s-z	15.78 d-1	18.57 b-k	33.67 qrs	50.73 e-m
	1	32.91 b-f	36.30 a-d	114.13 a-d	110.13 b-f	25.20 abc	21.60 a-h	65.87 abc	61.93 a-e
T_2	2	31.34 c-h	37.68 ab	107.13 b-g	110.24 b-e	25.34 ab	23.70 a-e	60.60 a-g	71.13 a
	3	28.10 f-l	31.57 b-g	95.13 e-k	108.07 b-g	23.45 a-e	24.43 a-d	53.60 c-l	64.13 a-d
	1	22.99 k-q	27.99 f-l	77.85 k-t	82.81 j-s	18.53 b-k	18.27 b-k	51.88 d-m	53.88 c-l
T_3	2	17.78 q-v	27.84 f-l	60.20 t-b	77.73 k-t	16.21 d-1	17.50 b-l	42.77 k-q	53.17 d-m
	3	14.23 t-x	27.83 f-l	48.20 zab	80.47 j-s	14.35 f-1	19.83 a-j	35.77 o-s	52.87 d-m
	1	31.09 d-i	27.83 f-l	106.13 c-h	129.91 a	22.30 a-g	22.67 a-f	60.77 a-g	71.30 a
T_4	2	26.19 g-o	32.99 b-f	88.67 h-p	110.53 b-e	19.98 a-j	21.93 a-h	51.73 d-m	64.17 a-d
	3	22.64 k-r	31.89 b-g	76.67 l-u	103.3 c-i	17.75 b-1	21.97 a-h	44.73 i-q	60.47 a-g
	1	25.36 h-p	29.81 e-	85.87 i-r	90.63 g-n	21.67 a-h	18.27 b-k	53.90 c-l	56.93 c-i
T_5	2	20.17 o-t	22.22 l-r	68.33 r-y	68.40 r-y	19.33 a-j	20.17 a-j	44.17 j-q	58.07 b-h
	3	16.64 r-v	26.98 f-m	56.33 w-b	78.54 j-d	17.45 b-1	20.20 a-j	37.17 n-s	53.27 d-l
	1	25.90 g-o	30.25 d-j	87.71 h-q	91.79 f-m	15.90 d-1	17.37 b-l	50.53 e-m	52.87 d-m
T_6	2	20.59 n-s	24.69 j-p	69.72 q-y	74.83 m-v	13.57 g-1	19.13 a-j	41.53 l-r	55.47 c-j
	3	17.05 q-v	27.85 f-l	57.72 v-b	83.70 j-s	11.62 jkl	19.93 a-j	34.53 o-s	55.27 c-k
	1	35.09 a-e	40.26 a	124.60 ab	118.40 abc	27.67 a	22.27 a-g	69.60 ab	71.53 a
T_7	2	28.63 f-k	34.79 a-e	96.93 d-j	111.43 b-e	22.88 a-f	22.37 a-g	56.17 c-j	65.87 abc
	3	25.08 i-p	30.94 d-i	84.93 j-s	104.40 c-h	20.71 a-i	22.97 a-f	49.17 f-n	61.50 a-f
	1	21.33 m-s	26.46 g-n	72.20 o-x	77.67 k-u	16.80 b-1	17.43 b-l	46.23 h-p	48.70 g-n
T_8	2	15.97 s-w	26.13 g-o	54.07 x-b	70.57 p-x	14.47 f-1	19.30 a-j	37.23 n-s	52.60 d-m
	3	12.42 vwx	21.24 m-s	42.07 bc	59.30 u-v	13.07 h-1	18.87 a-j	30.23 rs	49.50 e-n
	1	13.76 u-x	19.27 p-u	46.60 abc	55.12 w-b	12.20 i-1	16.80 b-l	42.93 k-q	46.30 h-o
$T_{9©}$	2	8.60 xy	13.91 u-x	29.13cd	52.13 y-b	9.88 kl	15.10 e-l	33.77 p-s	46.77 h-o
	3	5.79 y	10.18 wxy	17.13 d	41.80 bc	9.34 -1	16.33 c-l	26.77 s	46.23 h-p

Mean values under each treatment with the same leter (s) do not differ statistically by LSD (P≤0.05), where T1 = RZ22 + RP01 + RS15, T2 = RZ11 + RP01 + RS14, T3 = RZ11 + RP08 + RS14, T4 = RZ11 + RP08 + RS15, T5 = RZ11 + RP01 + RS15,T6 = RZ22 + RP08 + RS14, T7 = RZ22 + RP08 + RS15, T8 = RZ22 + RP01 + RS14, T9 = control, where RZ11= *Mesorhizobium ciceri,* RP08= *Enterobacter cloacae,* RS14 = *Bacillus mojavensis,* RS15= *Providencia vermicola,* RP01= *Bacillus subtilis,* RZ22= *Mesorhizobium ciceri,* Location 1= Kakri, Location 2= Adhi Kot, Location 3= Jara.

TABLE 4. Effect of microbial consortia on the chickpea yield attributes at 3 locations of farmer fields during years

Treatment	Locations	Plant height (cm)		No. of pods/plant		Biological yield (kg/ha)		Grain yield (kg/ha)	
		Year-1	Year-2	Year-1	Year-2	Year-1	Year-2	Year-1	Year-2
	1	37.27 l-t	40.51 g-p	24.00 d-m	26.67 a-k	1959.9 a-e	2127.2 a-i	911.65 c-f	955.79 d-i
T_1	2	32.43 o-u	42.17 e-n	18.27 h-o	26.27 a-k	1723.8 b-h	1888.3 d-l	733.29 f-j	843.51 i-l
	3	28.93 tuv	39.11 h-r	14.27 l-p	21.47 f-n	1568.5 c-i	1587.5 h-p	611.99 h-l	704.22 k-p
	1	51.60 a-d	51.86 a-h	31.93 a-f	33.53 a-e	2263.8 ab	2474.2 a-d	1126.60 ab	1140.03 a-d
T_2	2	47.63 a-i	51.02 a-h	32.00 a-f	36.20 ab	2315.1 ab	2479.1 a-d	1072.1 abc	1221.34 ab
	3	44.13 c-l	49.71 a-f	28.00 a-i	33.53 a-e	2127.9 abc	2474.9 a-d	961.77 b-e	1080.35 a-f
	1	38.31 j-s	41.48 f-o	23.87 d-m	27.20 a-j	1861.6 a-f	2043.7 b-j	787.07 e-h	952.48 d-i
T_3	2	33.37 n-u	41.51 f-o	17.80 i-o	24.47 c-m	1595.6 c-i	1980.9 c-k	608.61-l	884.56 f-l
	3	29.87 s-v	40.78 f-p	13.80 m-p	22.80 e-m	1235.0 g-j	1917.2 c-l	487.3 klm	855.51 h-l
	1	44.45 c-l	56.72 a	30.80 a-f	37.27 a	2173.8 abc	2710.33 a	1064.1 a-d	1179.25 abc
T_4	2	39.33 h-q	49.55 a-g	25.47 b-l	31.60 a-f	2047.9 a-d	2483.1 abc	896.41 c-g	1091.8 a-f
	3	35.83 l-u	46.94 b-j	21.47 f-n	28.00 a-i	1774.7 b-g	2302.0 a-g	775.09 e-i	1061.11 b-h
	1	38.80 h-s	42.79 d-m	27.00 a-j	29.73 a-g	2056.8 a-d	2085.4 b-i	868.11c-g	917.68 e-j
T_5	2	33.80 m-u	44.95 c-l	21.27 f-n	28.40 a-i	1738.9 b-h	1932.8 c-k	690.24 g-k	923.38 e-j
	3	30.30 q-v	41.14 f-o	17.27 i-p	24.20 c-m	1408.6 e-i	1775.2 f-m	569.51 i-l	692.32 l-q
	1	37.48 k-t	40.20 h-p	22.87 e-m	25.53 b-i	1867.6 a-f	2091.1 b-i	886.71 c-g	932.67 d-j
T_6	2	32.47 o-u	42.83 d-m	17.93 i-o	27.20 a-j	1471.1 d-i	2010.8 b-j	704.86 f-j	953.32 d-i
	3	28.97 tuv	42.83 d-m	13.93 m-p	24.73 c-m	1467.2 d-i	1906.8 c-l	583.54 h-l	776.59 i-o
	1	53.06 abc	55.13 ab	34.73 a-d	35.60 abc	2428.8 a	2574.48 ab	1201.00 a	1275.34 a
T_7	2	46.60 b-k	50.92 a-e	28.13 a-i	29.47 a-h	2158.3 abc	2450.2 a-e	979.97 b-e	1122.9 a-e
	3	43.10 d-l	47.67 a-i	24.13 d-m	30.27 a-f	2023.4 a-e	2391.7 a-e	858.65 d-g	1058.86 b-h
	1	38.60 i-s	37.56 k-t	18.33 g-o	21.07 f-n	1622.5 c-i	1893.5 c-l	729.93 f-j	803.12 i-m
T_8	2	33.60 n-u	40.41 g-p	13.33 m-p	22.27 e-m	1332.1 f-i	1763.8 f-m	546.59 jkl	826.07 i-l
	3	30.10 r-v	38.37 j-s	9.33 op	20.73 f-o	1060.4 ijk	1609.2 h-p	425.28 lm	726.82 j-p
	1	31.86 p-v	35.93 l-t	15.87 j-p	17.67 i-o	1146.8 hij	1159.8 n-r	471.13 lm	556.79 p-s
$T_{9©}$	2	26.70 uv	36.17 l-t	10.07 nop	16.53 j-p	692.3 jk	1061.4 p-s	294.52 mn	407.55 r-u
	3	23.20 v	36.12 l-t	6.07 p	15.33 k-p	485.9 k	795.34 qrs	188.24 n	348.27 stu

Mean values under each treatment with the same leter (s) do not differ statistically by LSD (P≤0.05), where T1= RZ22 + RP01 + RS15, T2= RZ11 + RP01 + RS14, T3= RZ11 + RP08 + RS14, T4= RZ11 + RP08 + RS15, T5= RZ11 + RP01 + RS15,T6= RZ22 + RP08 + RS14, T7= RZ22 + RP08 + RS15, T8= RZ22 + RP01 + RS14, T9= control, where RZ11 = *Mesorhizobium ciceri,* RP08 = *Enterobacter cloacae,* RS14 = *Bacillus mojavensis,* RS15 = *Providencia vermicola*, RP01 = *Bacillus subtilis,* RZ22 = *Mesorhizobium ciceri,* Location 1 = Kakri, Location 2 = Adhi Kot, Location 3 = Jara.

TABLE 5. The physico-chemical properties of soil at field experimental sites

Characteristics	Value					
	Kakri	Adhi Kot	Jara	Kakri	Adhi Kot	Jara
Sand (%)	65.3	66.7	67.9	NT	NT	NT
Silt (%)	20	17.5	18.1	NT	NT	NT
Clay (%)	14.7	15.8	14	NT	NT	NT
Texture	Sandy loam	Sandy loam	Sandy loam	NT	NT	NT
Ph	8.3	8.1	8.5	NT	NT	NT
EC (dSm^{-1})	0.45	0.44	0.47	0.46	0.46	0.48
Available P ($kgha^{-1}$)	24	22	21	31	29	27
Available K ($mgkg^{-1}$)	68	64	66	NT	NT	NT
Organic matter (%)	0.25	0.26	0.21	0.28	0.28	0.25
Nitrogen (%)	0.013	0.014	0.012	3.89	3.74	3.62

NT: Not Tested, Data are presented as mean (n=3), Mean values in each column with the same superscript (s) do not differ significantly by honestly significant difference (HSD) test (P≤0.05).

References

Aalipour, H., Nikbakht, A., Etemadi, N., Rejali, F., Soleimani, M. (2020) Biochemical response and interactions between arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria during establishment and stimulating growth of Arizona cypress (*Cupressus arizonica* G.) under drought stress. *Scientia Horticulturae*, **261**, 108923.

Ajayi, A.O., Fagade, O.E. (2006) Growth pattern and structural nature of amylases produced by some Bacillus species in starchy substrates. *African Journal of Biotechnology*, **5**(5), 440-444.

Aslam, M., Mahmood, I.A., Sultan, T., Ahmad, S. (2000) Inoculation approach to legume crops and their production assessment in Pakistan-A Review. *Pakistan Journal of Biological Sciences*, **3**(2), 193-195.

Billah, M., Khan, M., Bano, A., Hassan, T.U., Munir, A., Gurmani, A.R. (2019) Phosphorus and phosphate solubilizing bacteria: Keys for sustainable agriculture. *Geomicrobiology Journal*, **36**(10), 904-916.

Cappucino, J.C., Sherman, N. (1992) "*Microbiology: A Laboratory Manual*," 3rd ed., Benjamin/Cumming Pub. Co., New York.

Dey, R.K.K.P., Pal, K.K., Bhatt, D.M., Chauhan, S.M. (2004) Growth promotion and yield enhancement of peanut (Arachis hypogaea L.) by application of plant growth-promoting rhizobacteria. *Microbiological Research*, **159**(4), 371-394.

Dinesh, R., Anandaraj, M., Kumar, A., Bini, Y.K., Subila, K.P., Aravind, R. (2015) Isolation, characterization, and evaluation of multi-trait plant growth promoting rhizobacteria for their growth promoting and disease suppressing effects on ginger. *Microbiological Research*, **173**, 34-43.

FAOSTAT, (2020) Production Statistics of Agriculture Crops. 2020. Available online: http://faostat.fao.org/site.

Gull, M., Hafeez, F.Y., Saleem, M., Malik, K.A. (2004) Phosphorus uptake and growth promotion of chickpea by co-inoculation of mineral phosphate solubilising bacteria and a mixed rhizobial culture. *Australian Journal of Experimental Agriculture*, **44**(6), 623-628.

Hashmi, I., Paul, C., Al-Dourobi, A., Sandoz, F., Deschamps, P., Junier, T., et al. (2019) Comparison of the plant growth-promotion performance of a consortium of Bacilli inoculated as endospores or as vegetative cells. *FEMS Microbiology Ecology*, **95**(11), fiz147.

Heydari, M.M., Brook, R.M., Jones, D.L. (2019) The role of phosphorus sources on root diameter, root length and root dry matter of barley (Hordeum vulgare L.). *Journal of Plant Nutrition*, **42**(1), 1-15.

Honma, M., Shimomura, T. (1978) Metabolism of 1-aminocyclopropane-1-carboxylic acid. *Agricultural and Biological Chemistry*, **42**(10), 1825-1831.

Hussain, A., Kamran, M. A., Javed, M. T., Hayat, K., Farooq, M. A., Ali, N., et al. (2019) Individual and combinatorial application of *Kocuria rhizophila* and citric acid on phytoextraction of multi-metal contaminated soils by *Glycine max* L. *Environmental and Experimental Botany*, **159**, 23-33.

Jackson, M.L, (1962) Nitrogen determination from soils and plant tissues. In: "*Soil Chemical Analysis*"; Constable and Company Ltd.: London, UK; pp. 183–204.

Kantar, F., Hafeez, F.Y., Shivakumar, B.G., Sundaram, S.P., Tejera, N.A., Aslam, A., et al. (2007) Chickpea: Rhizobium management and nitrogen fixation. In: "*Chickpea Breeding and Management*", pp. 179-192. Wallingford UK: CABI.

Kavadia, A., Omirou, M., Fasoula, D., Trajanoski, S., Andreou, E., Ioannides, I.M. (2020) Genotype and soil water availability shape the composition of AMF communities at chickpea early growth stages. *Applied Soil Ecology*, **150**, 103443.

Khan, N., Bano, A. (2016) Role of plant growth promoting rhizobacteria and Ag-nano particle in the bioremediation of heavy metals and maize growth under municipal wastewater irrigation. *International Journal of Phytoremediation*, **18**(3), 211-221.

Khan, N., Bano, A. (2019a) Exopolysaccharide producing rhizobacteria and their impact on growth and drought tolerance of wheat grown under rainfed conditions. *PLoS One*, **14**(9), e0222302.

Khan, N., Bano, A. (2019b) Growth and yield of field

crops grown under drought stress condition is influenced by the application of PGPR. In: "*Field Crops: Sustainable Management by PGPR*", pp. 337-349. Springer, Cham.

Khan, N., Bano, A. (2019c) Rhizobacteria and abiotic stress management. In: "*Plant Growth Promoting Rhizobacteria for Sustainable Stress Management*", pp. 65-80. Springer, Singapore.

Khan, N., Bano, A., Zandi, P. (2018) Effects of exogenously applied plant growth regulators in combination with PGPR on the physiology and root growth of chickpea (*Cicer arietinum*) and their role in drought tolerance. *Journal of Plant Interactions*, **13**(1), 239-247.

Khan, N., Bano, A., Curá, J.A. (2020) Role of beneficial microorganisms and salicylic acid in improving rainfed agriculture and future food safety. *Microorganisms*, **8**(7), 1018.

Khattak, S.G., Khan, D.F., Shah, S.H., Madani, M.S., Khan, T. (2006) Role of rhizobial inoculation in the production of chickpea crop. *Soil & Environment*, **25**(2), 143-145.

Koehler, F.E., Moodie, C.D., McNeal, B.L. (1984) "*Laboratory Manual for Soil Fertility*", Washington State Uni. Pullman Washington.

Kour, D., Rana, K. L., Yadav, A. N., Yadav, N., Kumar, V., Kumar, A., et al. (2019) Drought-tolerant phosphorus-solubilizing microbes: biodiversity and biotechnological applications for alleviation of drought stress in plants. In: "*Plant Growth Promoting Rhizobacteria for Sustainable Stress Management*", pp. 255-308. Springer, Singapore.

Kumar, M., Mishra, S., Dixit, V., Kumar, M., Agarwal, L., Chauhan, P.S., Nautiyal, C.S. (2016a) Synergistic effect of *Pseudomonas putida* and *Bacillus amyloliquefaciens* ameliorates drought stress in chickpea (Cicer arietinum L.). *Plant Signaling & Behavior*, **11**(1), e1071004.

Kumar, S., Glen, S., Koichiro, T. (2016b) MEGA-7, Molecular evolutionary genetics version 7.0 for bigger data sets. *Molecular Biology and Evolution*, **33**, 1870–1874.

MacFaddin, J.F. (2000) "*Biochemical Tests for Identification of Medical Bacteria*", Williams and Wilkins. Philadelphia, PA, 113p.

Majeed, A., Abbasi, M.K., Hameed, S., Imran, A., Rahim, N. (2015) Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. *Frontiers in Microbiology*, **6**, 198.

Mancosu, N., Snyder, R.L., Kyriakakis, G., Spano, D. (2015) Water scarcity and future challenges for food production. *Water*, **7**(3), 975-992.

Maniatis, T., Fritsch, E.F., Sambrook, J. (1982) Cold "*Molecular Cloning: A Laboratory Manual*", Spring Harbor; NY.

Marasco, R., Rolli, E., Ettoumi, B., Vigani, G., Mapelli, F., Borin, S., et al. (2012) A drought resistance-promoting microbiome is selected by root system under desert farming. *PloS One*, **7**(10), e48479.

Mcclean, E.O. (1982) Soil pH and lime requirement. In: "*Methods of Soil Analysis*" Part 2, 2nd ed.; Page, A.L., Miller, R.D., Keeney, D.R. (Eds.), pp. 199–208. Agronomy: Madison, WI, USA.

Nannipieri, P., Giagnoni, L., Landi, L., Renella, G. (2011) Role of phosphatase enzymes in soil. In: "*Phosphorus in Action*", pp. 215-243. Springer, Berlin, Heidelberg.

Naseem, H., Ahsan, M., Shahid, M.A., Khan, N. (2018) Exopolysaccharides producing rhizobacteria and their role in plant growth and drought tolerance. *Journal of Basic Microbiology*, **58**(12), 1009-1022.

Olsen, S.R., Sommers, L.E., Page, A.L. (1982) "*Methods of Soil Analysis*" Part 2; Soil Science Society of America, Inc.: Madison, WI, USA; American Society of Agronomy, Inc.: Madison, WI, USA; pp. 403–430.

Qureshi, M.A., Ahmad, Z.A., Akhtar, N., Iqbal, A., Mujeeb, F., Shakir, M.A. (2011) Role of phosphate solubilizing bacteria (PSB) in enhancing P availability and promoting cotton growth. *Journal of Animal and Plant Sciences,* **22**(1), 204-210.

Romdhane, S., Tajini, F., Trabelsi, M., Aouani, M.E., Mhamdi, R. (2007) Competition for nodule formation between introduced strains of Mesorhizobium ciceri and the native populations of rhizobia nodulating chickpea (*Cicer arietinum*) in Tunisia. *World Journal of Microbiology and Biotechnology*, **23**(9), 1195-1201.

Schwyn, B., Neilands, J.B. (1987) Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, **160**(1), 47-56.

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., Gobi, T.A. (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus*, **2**(1), 1-14.

Smibert, R.M. (1994) Phenotypic characteization. In: "*Methods for General and Molecular Bbacteriology*", pp. 607-654.

Soltanpour, P.N., Schwab, A.P. (1977) A new soil test for simultaneous extraction of macro-and micro-nutrients in alkaline soils. *Communications in Soil Science and Plant Analysis*, **8**(3), 195-207.

Steel, R.G.D., Torrie, J.H., Dicky, D. (1997) "*Principles and Procedures of Statistics. A Biometrical Approach*", 3rd ed.; McGraw-Hill Book Company, Inc.: New York, NY, USA.

Subair, H. (2015) Isolation and screening bacterial exopolysaccharide (EPS) from potato rhizosphere in highland and the potential as a producer Indole Acetic Acid (IAA). *Procedia Food Science*, **3**, 74-81.

Thakur, I.B., Putatunda, C. (2017) In vitro phosphate solubilization by Enterobacter spp. isolated from wheat rhizosphere. *Journal of Pure and Applied Microbiology*, **11**(4), 2007-2015.

Timmusk, S., Islam, A., Abd el, D., Lucian, C., Tanilas, T., Kannaste, A., et al. (2014) Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: Enhanced biomass production and reduced emissions of stress volatiles. *PLoS One*, **9**, 1–13.

Vurukonda, S.S. K.P., Vardharajula, S., Shrivastava, M., Skz, A. (2016) Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiological Research*, **184**, 13-24.

Walkley, A., Black, C.A. (1934) A critical examination of a rapid method for determination of organic carbon in soils. Effect of variations in digestion conditions and organic soil constituents. *Soil Science,* **63**, 251–263.

Yadav, J., Verma, J.P., Rajak, V.K., Tiwari, K.N. (2011) Selection of effective indigenous rhizobium strain for seed inoculation of chickpea (*Cicer aritenium* L.) production. *Bacteriology Journal*, **1**, 24-30.

Yang, J., Kloepper, J.W., Ryu, C.M. (2009) Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Science*, **14**(1), 1-4.