Evaluating the Efficacy of Plant Growth-Promoting Rhizobacteria (PGPRs) in Enhancing the Chickpea (Cicer Arietinum L.) Productivity Under Different Moisture Regimes

Azhar Mahmood Aulakh[1] (Corresponding Author), **Obaid ur Rehman[2], Adeel Anwar[3], Riffat Bibi[4], Muhammad Yunas[5], Ayesha Malik[6], Safia Naureen Malik[7], Muhammad Iftikhar[8], Tariq Sultan[9], Sultan Ahmad Rizvi[10], Waqas Naseem[11], Khalid Mahmood[12], Rizwan Latif[13], Muhammad Waleej Arslan[14], Ghulam Muhammad[15], Qaiser Mahmood[16],**

[1, 4, 5, 15] Senior Scientist, Soil and Water Conservation Research Institute, Chakwal, Pakistan, Email: azuaulakh@gmail.com (CA)
[2, 6,] Chief Scientist, Soil and Water Conservation Research Institute, Chakwal, Pakistan
[3,] Assistant Professor, Department of Agronomy, PMAS Arid Agriculture University, Rawalpindi, Pakistan
[7,] Principal Scientist, Soil and Water Conservation Research Institute, Chakwal, Pakistan
[8,] Senior Scientific Officer, Land Resource Research Institute Islamabad, Pakistan
[9,] Principal Scientific Officer, Land Resource Research Institute Islamabad, Pakistan
[10,] Principal Scientist, Soil and Water Conservation Research Institute, Chakwal, Pakistan
[11,] Scientific Officer, Soil and Water Conservation Research Institute, Chakwal, Pakistan
[12,] Assistant Professor, Department of Agronomy, Poonch University Rawalakot, Pakistan
[13,] Principal Scientist, Soil and Water Testing Laboratory, Chakwal, Pakistan
[14,] Agriculture Officer, Extention Department, Hazro Pakistan
[16,] Water Management Officer, OFWM, Chakwal, Pakistan

Abstract

Prolonged and severe drought spell during growing season of field crops especially under rain fed conditions affects growth and productivity due to impact of ongoing climate change and is likely to enhance day by day directing to worldwide food insecurity. This experiment was planned to alleviate drought effects on chickpea through PGPRs to observe its productivity at different moisture regimes (50%, 70% and 90% of field capacity) in the pots keeping under glass house. Different bacterial strains were isolated from pink colored nodules, rhisospheric and rhizoplane soil of the plant roots of chickpea, those were collected from the field under stressful conditions. Six isolates were morphologically and biochemically characterized and identified through 16sRNA sequencing. Then, eight consortia were made from combination of identified six isolates and tested on chickpea plants. The consortia under treatment T3 showed best performance by giving 11.817 nodules plant-1 and grain yield 15.483 g pot-1 at moisture level 1 (50% of FC) followed by T5 which showed 22.837 nodules plant-1 and economic yield 20.607 g pot-1 at moisture level 2 (70% of FC). Similarly, consortia under treatment T2 gave 23.443 plant-1 and 20.283 g pot-1 regarding nodules and grain yield respectively at moisture level 3 (90 % FC). The experimental results indicate the key role of bacterial isolates consortium on chickpea productivity under drought stressful environment. The present study showed that the consortium T3 (Mesorhizobium ciceri SS1+Bacillus mojavensis PMCC-9 + Enterobacter cloacae PMCC-7) appears as resilient soil microbes under stressful conditions (50% of FC). Similarly, T5 (Mesorhizobium ciceri SS5+Bacillus mojavensis PMCC-9 + Enterobacter cloacae PMCC-7) and T2 (Mesorhizobium ciceri SS1+Bacillus mojavensis PMCC-9 + Bacillus subtilis PMCC-4) can be adopted in moderate moisture availability (70% of FC) and in irrigated areas (90% of FC) respectively, for maximum productivion of chickpea.

Keywords: Plant Growth Promoting Rhizobacteria, moisture regimes, drought, chickpea, Pothwar

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Introduction

In the present scenario, the climate change has considered worldwide burning issue due to its direct and indirect effects on global warming, increases the drought severity and abrupt change in temperature in many parts of the world, which adversely impacts on crop productivity and farmer's income. So, the severity of moisture stress and extreme temperature reduces moisture in soil profile and altering physiological conditions of the rainfed crops leading to low economic yield. Thus, sustaining worldwide food security, it is required to develop a strategy to alleviate drought harshness. Growth and net return of field crops affected by many biotic and abiotic factors; however, the drought play main role among all the stressors throughout the globe (Naveed et al., 2014).

On other side, the human population is increasing day by day and it is expected to touch 9 billion by 2050, thus, the food requirements is also running parallel to the world population, which depends upon agricultural crops and needs to adopt a strategy to minimize the obstacle (agricultural drought) against crop production towards fulfilling food security for world population under available limited arable land resources (Gatehouse et al., 2011; Alexanratos and Bruinsma, 2012; Mancosu et al., 2015). Thus, drought is multifunctional stress, which influences quantitatively and qualitatively on plant growth attributes and decreasing net productivity of field crops. Therefore, it is necessary to overcome drought stress effects for enhancement of productivity and ultimately to availability of food.

In this regard, the most efficient PGPRs and Rhizobium soil microbes reduce occurrence of drought spell (Sati et al., 2023; Barnard et al., 2013). An illustration of how interactions between plant roots and PGPR can help plant growth under stressful environment is through the reduction of water loss via improved diffusive resistance or the improvement of water and nutrient accommodation via increased nutrient acquisition and availability (Singh et al., 2022; Chieb et al., 2023). Therefore, in semi-arid and arid areas where water insufficiency is the most substantial hindrance to crop growth and productivity (Danish et al., 2020; Patial et al., 2024), PGPR can rise crop yield and improve drought stress (Reddy et al., 2024). A complete knowledge of the mechanisms and processes through which they ensure and impact sustainable agriculture is vital for practical application in the agricultural sector (Mohanty et al., 2021; Patel et al., 2023). Rhizobium strains those have ability to indorse growth of host plants entitled plant growth promoting rhizobium (PGPR), (Patel et al., 2023). The microorganisms found in the Rhizoplane and Rhizospheric soil perform a vital role in plants life cycle as well as they improve and maintain concerned soil fertility (Aulakh et al., 2020). 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. PGPRs play a vital role to minimize the stressful effects of drought on the rainfed crops having the ability to produce 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). Soil microorganisms cope to drought stress through adaptations of several physiological mechanisms such as acquisition of congenial osmolytes like proline, glycine and betain as well as secretion of most effective plant growth promoting phytohormonal enzymes protecting cellular protein from denaturing and supports to maintain integrity of cell membrane (Sharma et al., 2019; Bérard et al., 2015). Prolongation of drought stress spell in the soil profile intended bacteria to store ribosome in high quantities helping cell protein synthesis vary rapidly (Placella et al., 2012). Occasionally bacteria under stressed environment release extracellular polymeric substances (EPS) securing itself as well as habitat in which it is located. So, bacteria can survive and shows activity under drought stress conditions by adopting up-mentioned mechanisms (Rossi et al., 2012).

However, chickpea is an important pulse crop growing in Thal desert and rainfed areas of the Pothwar, Punjab, Pakistan, facing the drought stress (Khan et al., 2019), and the present scheme of experiments was designed to evaluate the resilient soil microbes for maximum production of chickpea in Thal Desert under drought conditions as well as in rainfed areas of Pothwar. It was hypothesized that isolates in consortium may be helpful to resist drought stress with the help of producing some biochemical components such as Indole Acidic Acid (ABA), exopolysaccharides, producing ACC-deaminase and having powerful enzymatic characteristics to boost up the chickpea growth attributes under stress environment. Similarly, *rhizobium* bacteria have the ability to fix the environmental nitrogen with in the nodules through the process of biological nitrogen fixation (BNF) to supply the nitrogen to enhance the chickpea crop production. Therefore, this experiment was planned to evaluate the efficacy of PGPRs in enhancing the productivity of chickpea under different moisture regimes.

Material and Methods

Sample Collection

Chickpea plants along with rhizospheric and rhizoplane soil attached on roots were taken from major chickpea producing area of Thal desert, as well as Pothwar region, Punjab, Pakistan under stressful environment during 2018-19.

Isolation of PGPRs

The strains were isolated from nodules, rhizospheric and rhizoplane soil of collected samples and these isolates were grown on Yeast Mannitol Agar (YMA) plates (Aung et al., 2020), Pikovskaya agar and Luria Bertani medium (Kumar, 2022) to check its growth after incubation with in the incubator (New Brunswick Scientific Innova 4230, manufactured by Eppendorf, a German-based life sciences company)

Morpho-Physiological and Biochemical Characterization of Isolates

The developed colonies were analyzed for their shape, margin and color along with gram (Dimri et al., 2020, Hussain et al; 1919, London et al; 2011, Kumar et al; 2012, Qurashi et al; 2012, Etesami et al; 2014, Subair, 2015).

Molecularly and quantitatively assessing the Isolates for ACC-Deaminase, EPS, IAA and P Solubilization

The isolates were molecularly characterized by amplification and sequencing of 16sRNA gene. This process was done by Polymerase Chain Reaction (PCR) apparatus (Thermal Cycler PCR PEQSTAR, 96X Standard, Munich, Germany). The amplified PCR products

were sent to Macrogen, Seoul Korea, for sequencing, and strains were identified using the EzBioCloud server Macrogen, Seoul Korea. Accession number of identified isolates were allotted after sequencing submission in NCBI, gene bank.

Experiment under Glass House at different moisture Levels

The experiment was laid out in pots keeping under glass house at Plant Genomic Research Institute (PGRI), NARC Islamabad to assess the effectiveness of characterized Rhizobium and PGPRs on productivity of chickpea in contrast to the uninoculated control. The pots with equal sizes (40 × 25 cm height and diameter, respectively) were filled with 8 kg autoclaved soil which was sieved by 2mm diameter and three moisture levels (50%, 70% and 90% of field capacity) were developed. The required moisture level in each pot was sustained by an electric moisture meter which is called Time Domain Reflectometer (TDR) during this experiment (Bache et al; 2008, NASA, 1968). Chickpea varietal seeds of Bhakker-2011 were sterilized and sown in respective pots according to the designed experimental treatments (Table 1).

Table 1. Treatments used during experiment on chickpea under controlled conditions.

Treatments	Identified Bacterial Isolates
T1	Mesorhizobium ciceri SS1 + Bacillus mojavensis PMCC-9 + Providencia vermicola PMCC-8
T2	Mesorhizobium ciceri SS1 + Bacillus mojavensis PMCC-9 + Bacillus subtilis PMCC-4
T3	Mesorhizobium ciceri SS1 + Bacillus mojavensis PMCC-9 + Enterobacter cloacae PMCC-7
T4	Mesorhizobium ciceri SS5 + Bacillus mojavensis PMCC-9 + Providencia vermicola PMCC-8
T5	Mesorhizobium ciceri SS5 + Bacillus mojavensis PMCC-9 + Enterobacter cloacae PMCC-7
T6	Mesorhizobium ciceri SS5 + Bacillus mojavensis PMCC-9 + Bacillus subtilis PMCC-4
T7	Mesorhizobium ciceri SS1 + Enterobacter cloacae PMCC-7 + Bacillus subtilis PMCC-4
T8	Mesorhizobium ciceri SS5 + Enterobacter cloacae PMCC-7 + Bacillus subtilis PMCC-4
T9	Control

Soil Properties

The soil used in the pots was analyzed before sowing (Aulakh et al., 2020).

Table 2. Pre-sowing analysis of soil used in the glass house experiment.

Characteristics	Value	Characteristics	Value
Sand (%)	62.7	Electrical Conductivity (dSm^{-1})	0.44
Silt (%)	17	Available P (mg/kg)	3.45
Clay (%)	20.3	Available K (mg/kg)	72
Texture	Sandy Loam	Organic Matter (%)	0.25
Ph	8.7	Nitrogen (%)	0.011

Statistical Analysis

The growth and yield attributes of chickpea were analyzed statistically by using statistix 8.1 adopting a Complete Randomized Design (CRD). The Least Significance Difference (LSD) was used to compare the means of the treatments their separation among each other's (Steel et al., 1997).

Results and Discussion

Morphological Characterization of PGPRs isolates

Primarily, 25 strains were isolated from the samples and among those selected 6 best colony forming isolates for further characterization by Phase Contrast Microscope (Phase contrast 2, Nikon, Japan) for the colony morphology. Isolates (PMCC-7, PMCC-8 & PMCC-9) were adopted irregular shape with convex elevation and three others i.e PMCC-4, SS1 and SS5 were found in round form with raised elevation. Our results are justified with the finding of Aulakh et al., 2020, those narrated that during their study on chickpea, some soil bacteria showed irregular and round shape with convex and raised elevation. In addition, the PMCC-7 showed yellow color with undulate margin of colony, however, other strains were found in yellowish and white in colony color with entire margin which were observed by scanning electron microscope (Table 3).

Table 3. Morphological characterization of PGPRs isolates.

Isolates	Bacteria	Accession No.	Gram Reaction	Bacterial Colony			
				Shape	Elevation	Color	Margin
PMCC-7	Enterobacter cloacae	MN420824	+ve	Irregular	Convex	yellow	Undulate
PMCC-4	Bacillus subtilis	MN420813	-ve	Round	Raised	Yellowish	Entire
PMCC-8	Providencia vermicola	MN420820	+ve	Irregular	Convex	Yellowish	Entire
PMCC-9	Bacillus mojavensis	MN420832	-ve	Irregular	Convex	white	Undulate
SS1	Mesorhizobium cicero	MN420828	-ve	Round	Raised	White	Undulate
SS5	Mesorhizobium ciceri	MN262094	+ve	Round	Raised	White	Entire

Biochemical Characterization of PGPRs

Biochemical characterization was analyzed and four isolates PMCC-7, PMCC-4, PMCC-8 and PMCC-9 were obtained positive for IAA and EPS production. The isolate PMCC-7 was appeared as phosphate solubilizers. Similarly, maximum phosphate solubilizing ability was found by *Enterobacter* sp., (Thakur and Putatunda, 2017). Similarly, ACC-Deaminase as well as phosphate solubilization were also showed by first two and 4th listed isolates. Two isolates SS-1 and SS-5 evaluated as ammonia producers, whereas, others resulted as non-producer for this test. Similar results were shown by Naseem *et al*; 2018, who elaborated that some soil bacterial species release ammonia and increase crop productivity. The amylase and catalase enzymes produced by PMCC-4 and PMCC-8 (also +ve for protease) as given in Table 4.

Table 4. Biochemical Characterization of PGPRs.

Isolates	IAA test	Phosphate solubilization	Ammonia Test	Amylase Test	Protease Test	Catalase Test	ACC-Deaminase	EPS
PMCC-7	++	+	-	-	+	-	+	+
PMCC-4	+	-	-	+	-	+	+	+
PMCC-8	+	-	-	+	+	+	-	+
PMCC-9	++	-	-	+	+	-	+	+
SS-1	-	-	+	-	-	-	-	-
SS-5	-	-	+	-	-	-	-	-

IAA = Indole-3-acetic acid; ACC-deaminase = 1-aminocyclopropane-1-carboxylate; EPS = Exopolysaccharide.

Quantitatively assessing the Isolates for IAA, P Solubilization, ACC-Deaminase and EPS

The two isolates (PMCC-4 and PMCC-9) found most promising in IAA (71 ± 2, 68 ± 2 µg/mL) P Solubilization (11.4 ± 1, 10.2 ± 1 ug/L), ACC-Deaminase (0.65 ± 0.02, 0.67 ± 0.02 µM/mg Protein/h) and EPS (0.74 ± 0.02, 0.61 ± 0.02 mg/mL) as shown in Table 5. Similarly, the isolates PMCC-7 and PMCC-8 were showed positive in IAA as well as ACCD. The results are in agreement with the findings of Khan *et al*; 2018, and Kumar *et al*; 2016, who studied the role of soil microbes to exhibit drought resistance in cereal and pulses by secreting indole acetic acid (IAA) and ACC-deaminase to decrease ethylene presence in the roots. However, all parameters were not detected by SS-1 and SS-5

Table 5. Quantitatively assessing the Isolates for IAA, P Solubilization, ACC-Deaminase and EPS

Isolates	IAA Production (µg/mL)	Phosphate Solubilization (ug/L)	ACC-Deaminase (µM/mg Protein/h)	Exopolysaccharide (mg/mL)

PMC C-7	63 ± 2	ND*	0.63 ± 0.02	ND
PMC C-4	71 ± 2	11.4 ± 1	0.65 ± 0.02	0.74 ± 0.02
PMC C-8	59 ± 2	ND	0.58 ± 0.02	ND
PMC C-9	68 ± 2	10.2 ± 1	0.67 ± 0.02	0.61 ± 0.02
SS-1	ND	ND	ND	ND
SS-5	ND	ND	ND	ND

* ND = Not Detected

Experiment under Glass House at different moisture Levels

The different growth and economic return parameters of chickpea presented in the following table, (Table 6) show that the consortium under treatment T3 showed highest number of nodules per plant (11.817), plant height (35.063 cm), number of pods (26.267), biological yield g per pot (41.683) and grain yield g per pot (15.483) as compared to untreated control (1.473, 26.640 cm, 16.270, 30.783 g pot^{-1}, 8.667 g pot^{-1} respectively, at moisture level 1 (50 % FC). Our results coincide with the findings of Singh et al., 2022 and Chieb et al., 2023, who observed during their study that some soil bacteria induce drought tolerance in the crop plants under stressful environment to help in potential crop productivity. Similarly, T5 gave best results regarding all parameters (22.837, 36.693 cm, 26.377, 55.623 g pot^{-1}, 20.607 g pot^{-1}) at moisture level 2 (70 % FC) and T2 at moisture level 3 (90 % FC). *Mesorhizobium ciceri* inoculation played its role to develop the symbiotic relationship in favor of chickpea plants nodulation (Romdhane *et al*; 2007, khan and Bano, 2019, Dey *et al*; 2004). The results were supported that growth and yield attributes were increased by inoculation with PGPRs which producing IAA, EPS and ACCD as well as phosphate solubilizing isolates in comparison with the un-inoculated treatment (Verma *et al*; 2012).

Table 6. Effect of isolates consortium on growth and yield attributes of chickpea at different moisture levels.

Treatments	Moisture Levels	Nodules Plant^{-1}	Plant Height (cm)	Pods Plant^{-1}	Biological Yield pot^{-1}	Grain Yield pot^{-1}
T1	1	4.753 m	23.853 fgh	18.713 d-g	34.420 no	11.187 L
	2	7.183 j	30.287 def	21.160 c-f	49.293 fg	13.860 I
	3	8.993 h	37.187 a-d	20.827 c-f	53.463 a-d	16.510 EF
T2	1	6.333 k	31.317 c-f	23.267 abc	40.763 jk	13.660 I
	2	18.653 d	35.787 a-d	25.713 ab	50.093 ef	18.053 BC
	3	23.443 a	42.693 a	22.827 a-d	53.010 bcd	20.283 A
T3	1	11.817 f	35.063 a-e	26.267 a	41.683 j	15.483 GH
	2	22.577 b	34.057 a-e	26.153 ab	47.763 gh	18.463 B
	3	21.827 c	40.250 abc	19.713 c-g	50.123 ef	18.153 BC
T4	1	3.817 n	31.710 b-f	18.267 efg	34.353 no	12.013 K
	2	6.487 k	31.080 def	20.380 c-g	46.753 hi	16.433 EF
	3	7.937 i	36.860 a-d	19.600 c-g	51.583 def	17.537 CD
T5	1	5.487 l	30.600 def	25.933 ab	37.953 lm	12.737 J
	2	22.837 b	36.693 a-d	26.377 a	55.623 a	20.607 A
	3	13.937 e	40.743 ab	19.487 c-g	52.993 bcd	16.987 DE
T6	1	3.157 o	30.080 d-g	18.153 efg	33.537 o	10.643 L
	2	5.487 l	29.897 d-g	18.820 d-g	49.963 fg	16.043 FG
	3	6.937 j	35.187 a-e	18.380 efg	54.093 abc	15.043 H
T7	1	4.623 m	30.873 def	21.933 b-e	38.663 kl	11.843 K

	2	11.513 fg	35.620 a-e	21.380 c-f	49.293 fg	16.943 DE
	3	11.373 g	36.033 a-d	18.160 efg	52.293 cde	16.773 E
T8	1	3.933 n	28.770 d-h	18.493 efg	36.293 mn	11.213 L
	2	6.483 k	30.393 def	18.933 d-g	50.423 ef	16.047 FG
	3	8.033 i	36.630 a-d	17.267 fg	55.123 ab	15.047 H
T9	1	1.473 p	26.640 e-h	16.270 g	30.783 p	8.667 M
	2	1.273 p	21.063 gh	16.380 g	45.023 i	13.847 I
	3	1.233 p	20.737 h	17.377 fg	46.663 hi	13.547 I
LSD		*0.320*	*9.103*	*4.327*	*2.298*	*0.621*

All the treatments sharing common letter are similar; otherwise, they differ significantly at $p \leq 0.05$, T1 = SS1 + PMCC-9 + PMCC-8, T2 = SS1 + PMCC-9 + PMCC-7, T3 = SS1 + PMCC-9 + PMCC-4, T4 = SS5 + PMCC-9 + PMCC-8, T5 = SS5 + PMCC-9 + PMCC-7,T6 = SS5 + PMCC-9 + PMCC-4, T7 = SS1 + PMCC-7 + PMCC-4, T8 = SS5 + PMCC-7 + PMCC-4, T9 = control, where PMCC-7 = Enterobacter cloacae, PMCC-4 = Bacillus subtilis, PMCC-8 = Providencia vermicola, PMCC-9 = Bacillus mojavensis, SS1 = *Mesorhizobium ciceri*, SS5 = *Mesorhizobium ciceri*, Moisture level 1 = 50% of Field Capacity, Moisture level 2 = 70% of Field Capacity, Moisture level 3 = 90% of Field Capacity. Means followed by the same letter within a column are not significantly different at $p = 0.05$.

Conclusions

Evaluation and utilization of efficient soil microbes to manage with the issue of abiotic stress through experimentation on this crop under different moisture regimes. Here, the experimental results revealed that inoculation of soil microbes on chickpea seed increased the growth and yield attributes even under stressful conditions. The present study showed that the consortium T_3 (Mesorhizobium ciceri SS1+Bacillus mojavensis PMCC-9 + Enterobacter cloacae PMCC-7) can perform best in drought conditions (50% of FC). Similarly, T_5 (Mesorhizobium ciceri SS5+Bacillus mojavensis PMCC-9 + Enterobacter cloacae PMCC-7) and T_2 (Mesorhizobium ciceri SS1+Bacillus mojavensis PMCC-9 + Bacillus subtilis PMCC-4) can be adopted in moderate moisture availability (70% of FC) and in irrigated areas (90% of FC) respectively for maximum productivity of chickpea.

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Conflicts of Interest

The authors declare no conflict of interest.

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