Pak. J. Bot., 51(1): 65-72, 2019. DOI: 10.30848/PJB2019-1(26)

MESORHIZOBIUM CICERI-CR-39 INOCULATION TO WHEAT FOR DROUGHT TOLERANCE AT CRITICAL GROWTH STAGES

MUHAMMAD BAQIR HUSSAIN[1,2], ZAHIR AHMAD ZAHIR[1], IJAZ MEHBOOB[3], SAJID MAHMOOD[1], NIAZ AHMED[4*], TANVEER UL HAQ[2], IFTIKHAR AHMAD[5] AND MUHAMMAD IMRAN[1]

[1]Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan
[2]Department of Soil and Environmental Sciences, MNS University of Agriculture, Multan, Pakistan
[3]District Fertility Lab., Kasur, Pakistan
[4]Department of Soil Science, Bahauddin Zakariya University, Multan, Pakistan
[5]Department of Environmental Sciences, COMSATS Institute of Information Technology, Vehari, Pakistan
[*]Corresponding author's email: jamniaz@yahoo.com

Abstract

Sandy soils are known for dry spells due to low rainfall in arid-semiarid climatic regions. A study was conducted to identify the effectiveness of moderately drought tolerant and characteristically plant growth promoting bacterial strain *Mesorhizobium ciceri*-CR-39 for improving growth, physiology, nutrition and yield of wheat (cultivar: *Sahar*) under drought at critical growth stages (tillering, flowering, and grain filling) in a sandy loam. Results demonstrated significant decreases due to drought at all critical growth stages. However, inoculation with *Mesorhizobium ciceri*-CR-39 significantly reduced the impact of drought at either growth stage of the crop. Grain filling stage drought showed highest decreases in growth, physiology, yield and nutrient concentrations. Inoculation significantly improved the parameters relating to plant photosystem, growth, biomass, yield and nutrient concentrations under normal conditions followed by the plants experiencing drought at tillering stage. Conclusively, *Mesorhizobium ciceri*-CR-39 significantly demonstrates its potential as a plant growth promoter for wheat under drought due to stress tolerance, more root colonization, nutrient solubilization, and auxin and exopolysaccharides production ability. However, field oriented studies are recommended for gauging its potential.

Key words: Drought, Wheat, Rhizobia, Nutrition, Physiology.

Introduction

Wheat is a cereal crop feeding almost 50% of the World's population. It is sensitive to mild-severe drought where the severity response varies at different growth stages of the crop. Unfortunately, global warming has increased variability in rainfall patterns which may lead to severe droughts in near future (Gregersen *et al.,* 2013). Similarly, predictions for severe drought have been narrated in Anon. (2007) report whereas, Pfeiffer *et al.,* (2005) reported periodic drought in more than 50% wheat cultivated areas worldwide. Moreover, the effects of terminal drought on wheat yields are likely to increase in future (Dias de Oliveria *et al.,* 2013). Currently, around 32% of the wheat cultivated area (99 mha) in developing countries observe frequent drought (Rajaram, 2001).

It is an established fact that drought adversely affects plant growth and development and results significant losses in plant's productivity (Farooq *et al.,* 2009; Mirzaei *et al.,* 2012). However, the extent of loss depends on the intensity, length and severity of drought, type of cultivar, and growth stage of the plant where it happened (Mark and Antony, 2005; Khakwani *et al*., 2012; Abid *et al.,* 2016). On the contrary, efficient utilization of water during sensitive growth stages of wheat (such as tillering, booting, earing, anthesis, and grain development stages) under water deficits condition may result into better crop performance (Jamal *et al.,* l996; Zhang & Oweis, 1999). Also, drought may affect wheat growth during all phenological stages but the reproductive and grain-filling are the most sensitive (Pradhan *et al.,* 2012).

Wheat is the major cereal crop, grown in nearly all parts of Pakistan. The average wheat yield is reasonably low in rain-fed areas and lands at the tail end of canals, where shortage of water is frequent (Ashraf, 1998). Besides other factors, arid and semi-arid environments could result in reduction of crop yield by inducing water deficit stress during crop growth and development (Ashraf *et al.,* 1995). Drought stress conditions impedes crop production by changing physiological and biochemical responses such as alteration in antioxidant defense, exopolysaccharides and phytohormones levels; buildup of compatible organic solutes like amino acids, sugars and biochemical processes (Bartels & Sunkar, 2005). It is, therefore, a great concern to stabilize crop yields under adverse environmental conditions through developing novel ways of sustainable crop management.

Drought shock could be mitigated through the interactions between rhizosphere microorganisms and plants (both in controlled and ordinary environments) in specific soil. These interactions affect the hormonal balance and source-sink relationships of plants with a potential to alter their responsiveness to drought (Staudinger *et al.,* 2016). Plant growth promoting bacteria (PGPB) could be a bulwark against drought stress and can amplify crop productivity under such stressful environments utilizing their drought tolerance and plant growth promoting traits (Kaushal & Wani, 2016). PGPB alleviate the shock of drought stress on plants by modulating physiological and biochemical processes leading to modification in phytohormones level and anti-polyamines. Production of volatile organic compound, de-hydrins and heat shock proteins by PGPB have also been recorded to play a significant role in the attainment of tolerance against drought. Selected rhizobial strains could develop endophytic association with wheat and can enhance its production under drought stress (Yanni *et al.,* 2016). The current study was conducted to examine the capability of

rhizobium strain *Mesorhizobium ciceri*-CR-39 in ameliorating the damaging effects of drought on wheat at its various growth stages in a sandy loam soil and to get sustainable yields under water deficit stress environment.

Materials and Methods

Seed inoculation: Rhizobial strain *Mesorhizobium ciceri*-CR-39 was selected on its ability to tolerate severe drought, and growth promotion of wheat under polyethylene glycol induced drought (Hussain *et al.,* 2014a). Fresh inocula was prepared in 100 mL sterilized yeast extract mannitol (YEM) broth in 250 mL conical flask. The inoculated broth was incubated at 28 ± 1°C and 100 rpm for 72 h in an orbital shaking incubator. After incubation the cells were harvested at 4°C by centrifugation at 4000*g* for 15 min. The inocula of optical density 0.5 McFarland units (0.5 OD ~ 10^8 cells mL^{-1}) with harvested cells in sterilized YEM broth was developed using densitometer (Den-1 Densitometer, McFarland, UK) (Shakeri *et al.,* 2011). Wheat (*Triticum aestivum* L.) seeds of cultivar *Sahar* were inoculated with a mixture of clay, peat, 10% sugar solution and bacterial inoculum (2:6:1:1, respectively). Control treatment (uninoculated) was also maintained by applying sterilized mixture of clay, peat, 10% sugar solution and YEM broth on wheat seeds. The seeds were dried in lab at room temperature for 6-8 h.

Experimental conditions: Sterilized soil was used in the experimentation having following characteristics [texture, sandy loam soil; EC_e, 2.17 dS m^{-1}; pH, 7.3; OM, 0.84%; saturation percentage, 37%; extractable K, 107 mg kg^{-1}; available P, 7.1 mg kg^{-1}; total N, 0.04%]. Five inoculated seeds of wheat were sown in each pot having 10 kg soil. After two weeks of germination, the seedlings were thinned to three plants per pot. Recommended doses of fertilizers (NPK, 120:90:60 kg ha^{-1}) were applied as urea, diammonium phosphate (DAP) and muriate of potash (MOP). Complete recommended dose of P, K and 1/3 N was applied before sowing whereas the remaining N was supplied in two splits at tillering and flowering stages of the crop. The pots were arranged in completely randomized design in three replications. Initially the pots were irrigated normally. Afterwards, drought stress was imposed at tillering, flowering or grain filling stages of wheat by withholding the irrigation till the symptoms of wilting appeared. A set of normal irrigated plants was also maintained as control. The plants beard drought at one stage, did not exposed to drought at other stages. The plants were managed with all recommended agronomic practices and grown up to maturity. At harvest all the growth and yield parameters were recorded.

Gas exchange measurement: Rate of photosynthesis (*A*), transpiration rate (*E*), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) was measured using a portable photosynthesis measuring system "infrared gas analyzer" [IRGA (LCA-4), Germany] early in the morning (time: 08:15 am to 10:30 am) when photon flux density was 1200 to 1400 µmol m^{-2} s^{-1} (Ben-Asher *et al.,* 2006). The measured parameters were used to derive water use efficiency (WUE) (i), intrinsic/photosynthetic water use efficiency (i/pWUE) (ii), and mesophyll conductance (MC) (iii) following the formulas given by Fischer *et al.,* (1998), Ahmadi & Siosemardeh (2005), and Ahmad *et al.,* (2013), respectively, as below.

$$WUE = (A) / (E) \quad \text{(i)}$$
$$i/pWUE = (A) / (g_s) \quad \text{(ii)}$$
$$MC = (A) / (C_i) \quad \text{(iii)}$$

Chlorophyll content: Chlorophyll contents of the flag leaf were measured using SPAD-502 chlorophyll meter (Konica-Minolta, Japan).

Nutrient analysis of plant: The dried and ground grain and straw samples were digested following the method described by Wolf (1982). Digested grain samples were analyzed for nitrogen through Kjeldahl method, phosphorus by spectrophotometer (Shimadzu, Japan), and potassium following the method of Ryan *et al.,* (2001) using Flame photometer (Jenway PFP-7, England).

Characterization of *Mesorhizobium cicero*- CR-39: The strain was characterized for various plant growth promoting and drought abiding parameters. Oxidase, chitinase, and catalase were observed following the procedures described by Steel (1961), Chernin *et al*., (1998), and MacFaddin (1980), respectively. Standard procedures were adopted to measure exopolysaccharides (Ashraf *et al*., 2004), P-solubilization (Mehta & Nautiyal, 2001), organic acid (Vincent, 1970), siderophores (Schwyn & Neilands, 1987) and auxin production (Sarwar *et al*., 1992). Procedures described by Madi & Henis (1989) and Simon *et al*., (1996) used to calculate % aggregation and root colonization ability of bacteria whereas survival in dry soil and low nutrient conditions was determined by following Fallik & okon (1996) and Tal & Okon (1985), respectively.

Statistical analysis

Data collected were analyzed statistically using analysis of variance (ANOVA; Steel *et al.,* 1997) and means were compared following least significance difference (LSD) technique. Statistix 8.1 (Analytical Software, USA) was used for statistical analysis and Microsoft Office 2013 (Excel) was used for data presentation.

Results

Rhizobium inoculation influence on the growth of wheat: Drought at tillering and flowering stage significantly reduced shoot length as compared to normal irrigated plants (Table 1). However, shoot length under normal conditions remained statistically at par with plants under drought at grain filling stage. Inoculation with CR-39 showed significantly higher shoot length over control under drought at flowering or grain filling stages or normal conditions (Table 1). Root length increased significantly under drought at grain filling stage as compared to normal irrigated plants (Table 1). Inoculation significantly increased root length under normal and drought conditions as compared to respective controls. Both drought and inoculation could not produce

significant differences in spike lengths within drought levels and between inoculation and control (Table 1). In the same way, drought at any growth stage could not cause significant reduction in number of tillers as compared to normal irrigated plants but inoculation significantly increased tillers over respective controls under normal and drought at grain filling and tillering stage of the crop (Table 1). Drought at grain filling and flowering stage significantly reduced number of spikes as compared to normal irrigated plants (Table 1). Inoculation increased number of spikes over respective controls under drought at grain filling stage and normal conditions.

Rhizobium inoculation impact on the physiology of wheat: Chlorophyll contents increased in plants under drought at flowering stage and decreased significantly due to drought at grain filling stage as compared to unstressed control and drought at tillering stage (Table 2). *Mesorhizobium ciceri*-CR-39 inoculation significantly increased the chlorophyll contents in normal as well as drought conditions in comparison to respective control treatments. Drought at all critical growth stages significantly decreased rate of photosynthesis, transpiration rate, stomatal conductance and water use efficiency as compared to normal irrigated plants (Table 2). Whereas a significant decrease in intrinsic water use efficiency and mesophyll conductance was observed due to drought at grain filling stage as compared to unstressed control. Similarly, flowering and grain filling stage drought significantly decreased intercellular CO_2 concentration over control. Inoculation of *Mesorhizobium ciceri*-CR-39 significantly increased rates of transpiration and photosynthesis in comparison to respective uninoculated control treatments under normal and drought conditions at tillering, flowering or grain filling stages (Table 2). Stomatal conductance was higher in inoculated plants whether irrigated normally or drought applied at tillering or flowering stages. But intercellular CO_2 concentration was improved via inoculation in plants under drought at grain filling stage with respect to respective control. Mesophyll conductance was only improved due to inoculation under normal conditions over uninoculated control. CR-39 significantly improved iWUE and WUE at tillering stage drought and normal irrigated conditions in contrast to respective control with an exception of grain filling stage drought for iWUE.

Table 1. Inoculation effect on wheat growth under drought.

Treatments		Shoot length (cm)	Root length (cm)	Spike length (cm)	No. of tillers/pot	No. of spikes/pot
Control	D0	71.50 b	21.00 e	8.33 ns	9.00 de	7.67 bc
	D1	61.50 d	23.58 de	8.33	8.33 e	6.00 cd
	D2	66.67 c	23.17 de	7.50	9.33 cde	5.33 d
	D3	71.00 b	26.50 d	8.33	9.33 cde	5.33 d
Inoculation	D0	76.50 a	44.70 c	8.33	11.67 ab	11.33 a
	D1	64.17 cd	62.50 b	8.00	10.00 cd	8.00 bc
	D2	74.33 ab	65.52 ab	7.67	10.67 bc	4.33 d
	D3	77.50 a	67.33 a	8.00	12.33 a	8.67 b
LSD		**3.9895**	**3.9252**	**1.1718**	**1.6191**	**2.0297**

D0: No drought, D1: Drought at tillering, D2: Drought at flowering, D3: Drought at grain filling, Control: No inoculation, Inoculation: *Mesorhizobium ciceri*-CR-39 inoculation, LSD: Least significant difference between treatment means, (n=3). Means sharing similar letters are statistically at par to each other at $p \leq 0.05$

Table 2. Inoculation effect on the photosynthetic parameters of wheat flag leaves under drought.

Treatments		Chl	*A*	*E*	*gs*	*Ci*	iWUE	WUE	MC
Control	D0	50.07 d	8.96 c	2.64 c	0.20 bc	296 ab	44.22 bc	3.41 b	0.030 bcd
	D1	49.17 d	5.89 e	2.01 d	0.16 de	263 bc	37.60 c	2.96 c	0.020 de
	D2	53.37 c	5.10 f	2.10 d	0.12 ef	218 c	42.50 bc	2.43 d	0.023 cde
	D3	45.20 e	1.43 h	1.29 e	0.11 f	114 d	13.16 e	1.12 e	0.013 e
Inoculation	D0	67.90 a	14.22 a	3.72 a	0.26 a	353 a	56.02 a	3.84 a	0.040 ab
	D1	58.07 b	11.68 b	3.33 ab	0.24 ab	264 bc	48.79 ab	3.51 ab	0.043 a
	D2	57.33 b	6.79 d	3.31 b	0.16 cd	240 bc	42.53 bc	2.07 d	0.033 abc
	D3	58.10 b	3.16 g	2.22 d	0.13 def	206 c	24.55 d	1.43 e	0.013 e
LSD		**1.7370**	**0.5073**	**0.4003**	**0.0417**	**67.742**	**8.5375**	**0.4116**	**0.0117**

D0: No drought, D1: Drought at tillering, D2: Drought at flowering, D3: Drought at grain filling, Control: No inoculation, Inoculation: *Mesorhizobium ciceri*-CR-39 inoculation, LSD: Least significant difference between treatment means, (n=3). Means sharing similar letters are statistically at par to each other at $p \leq 0.05$. Chl: chlorophyll content (SPAD), A: Photosynthetic rate (µmol m^{-2} s^{-1}), E: Transpiration rate (mmol m^{-2} s^{-1}), gs: Stomatal conductance (mmol m^{-2} s^{-1}), Ci: intercellular CO_2 concentration, iWUE: intrinsic water use efficiency (A/gs), WUE: water use efficiency (A/E), MC: mesophyll conductance (A/Ci)

Rhizobium inoculation impact on the yield components of wheat: Drought at all stages significantly decreased shoot fresh weight over control but tillering stage drought could not show significant decrease in shoot dry weight over control (Table 3). Except drought at flowering stage, all the drought treatments remained statistically at par with control for rood dry weight while no difference was recorded for root fresh weight between drought treatments and control (Table 3). A significant decrease in the number of grains per spike and thousand grain weight over control was observed where drought at tillering and flowering stage was applied whereas drought at grain filling stage remained at par with control (Table 3). Grain yield was significantly decreased due to drought at tillering, flowering and grain filling stage as compared to control (Table 3). The plants inoculated with *Mesorhizobium ciceri*-CR-39 showed significant increases in root/shoot fresh/dry weights, grains per spike, thousand grain weight and grain yield as compared to respective uninoculated control treatments whether under drought at tillering, flowering or grain filling stages or normal conditions (Table 3).

Rhizobium inoculation impact on the nutritional quality of wheat straw and grain: Drought at critical growth stages significantly increased the grain and straw nitrogen concentration as compared to well-watered plants however, inoculation with *Mesorhizobium ciceri*-CR-39 further increased nitrogen concentration in shoot and grains as compared to respective uninoculated treatments (Table 4). No difference for phosphorus and potassium concentration was observed in grain and straw samples whether plants were irrigated normally or drought imposed at tillering, flowering or grain filling stages. Inoculation under grain filling stage drought showed a significant increase in straw P concentration as compared to uninoculated and normally irrigated ones. However, increases in grain and straw potassium concentrations were recorded due to inoculation with *Mesorhizobium ciceri*-CR-39 under normal conditions and drought at flowering stages in contrast to respective uninoculated controls (Table 4). Grain phosphorus concentration increased by *Mesorhizobium ciceri*-CR-39 inoculation under normal condition and drought at flowering stage while significantly high straw phosphorus concentration was observed in inoculated plants under drought at grain filling stage over uninoculated control (Table 4).

Table 3. Effect of inoculation on yield parameters of wheat under drought.

Treatments		SFW	SDW	RFW	RDW	Grains/ Spike	TGW	GY
Control	D0	20.33 b	12.07 de	11.50 ef	4.17 d	30.33 de	44.33 b	5.97 b
	D1	15.67 c	10.68 ef	10.60 f	7.13 cd	23.33 f	32.00 d	2.77 e
	D2	16.67 c	7.30 g	14.33 de	13.77 b	23.00 f	40.33 c	3.15 e
	D3	12.67 d	9.78 f	9.70 f	6.07 d	27.33 e	42.33 bc	4.21 d
Inoculation	D0	24.00 a	15.49 a	16.33 cd	9.33 c	39.67 a	53.33 a	7.64 a
	D1	21.33 ab	15.32 ab	19.07 bc	13.57 b	36.67 ab	43.67 bc	4.59 cd
	D2	20.00 b	13.81 bc	21.13 b	18.50 a	32.33 cd	50.33 a	5.12 c
	D3	20.67 b	13.47 cd	25.13 a	20.80 a	35.00 bc	52.67 a	5.11 c
LSD		**2.8704**	**1.5643**	**3.0233**	**3.0945**	**3.9185**	**3.5332**	**0.8338**

D0: No drought, D1: Drought at tillering, D2: Drought at flowering, D3: Drought at grain filling, Control: No inoculation, Inoculation: *Mesorhizobium ciceri*-CR-39 inoculation, LSD: Least significant difference between treatment means, (n=3). Means sharing similar letters are statistically at par to each other at $p \leq 0.05$. SFW: shoot fresh weight (g pot^{-1}), SDW: shoot dry weight (g pot^{-1}), RFW: root fresh weight (g pot^{-1}), RDW: root dry weight (g pot^{-1}), TGW: thousand grain weight (g), GY: grain yield (g pot^{-1})

Table 4. Influence of inoculation on nutrient concentrations of wheat grain and straw under drought.

Treatments		Grain concentration (%)			Straw concentration (%)		
		Nitrogen	Phosphorus	Potassium	Nitrogen	Phosphorus	Potassium
Control	D0	1.58 f	0.21 d	0.08 c	1.01 c	0.15 c	0.14 b
	D1	1.73 e	0.24 bcd	0.08 c	0.86 d	0.15 bc	0.13 b
	D2	2.17 d	0.22 cd	0.08 c	0.49 e	0.16 bc	0.16 b
	D3	2.39 c	0.23 cd	0.08 c	0.37 f	0.16 abc	0.13 b
Inoculation	D0	2.15 d	0.29 b	0.13 b	1.29 a	0.16 abc	0.25 a
	D1	2.46 bc	0.25 bcd	0.08 c	1.23 ab	0.17 abc	0.14 b
	D2	2.56 ab	0.38 a	0.16 a	1.18 b	0.17 ab	0.27 a
	D3	2.64 a	0.27 bc	0.09 c	1.06 c	0.18 a	0.16 b
LSD		**0.1011**	**0.0540**	**0.0289**	**0.0883**	**0.0183**	**0.0606**

D0: No drought, D1: Drought at tillering, D2: Drought at flowering, D3: Drought at grain filling, Control: No inoculation, Inoculation: *Mesorhizobium ciceri*-CR-39 inoculation, LSD: Least significant difference between treatment means, (n=3). Means sharing similar letters are statistically at par to each other at $p \leq 0.05$

Table 5. Plant growth promoting characteristics of *Mesorhizobium ciceri*- CR-39.

Characteristics		*Mesorhizobium ciceri*- CR-39
Oxidase		+
Chitinase		+
Catalase		++
Exopolysaccharides		++
P-solubilization		++
Organic acid		++
Siderophores		-
Survival in soil ($\times10^5$ CFU g^{-1})		4.12
Starvation test ($\times10^5$ CFU mL^{-1})		4.20
Root colonization ($\times10^5$ CFU g^{-1})		6.78
Aggregation (%)		6.89
IAA production (mg L^{-1})	Without L-TRP	3.86
	With L-TRP	35.15

Plus sign (+) represents the presence and minus sign (-) shows the absence of the characteristics. All characters are means of three replications

Plant growth promoting characteristics of *Mesorhizobium ciceri*- CR-39: The rhizobial strain *Mesorhizobium ciceri- CR-39* was capable to produce oxidase, chitinase and catalase enzymes, produce exopolysaccharides, organic acids and phosphate solubilization (Table 5). The strain was incapable to produce siderophores. The strain had a great potential to survive in bulk soil (4.12×10^5 CFU g^{-1}) and starved conditions (4.20×10^5 CFU mL^{-1}). Root colonization was 6.78×10^5 CFU g^{-1} and aggregation potential was 6.89% (Table 5). The strain had a great ability to produce indole acetic acid in the presence (35.15 mg L^{-1}) or absence (3.86 mg L^{-1}) of L-tryptophan (Table 5).

Discussion

Drought is a serious environmental apprehension in the era of Global warming particularly in arid-semiarid regions. Whereas, increased population, reduction of cultivable land and diminution of useable irrigation water are major concerns for sustainable crop production and food security. Therefore, it is imperative to establish a biotechnology which may manipulate the rhizosphere ecology of cereal crops for sustainable crop production and food security through environment friendly and economical way. In the present study, moderately drought tolerant rhizobial strain *Mesorhizobium ciceri*-CR-39 (Hussain *et al.,* 2014a) was supplied into the rhizosphere of wheat crop through seed coating, to improve nutrition, growth physiology, production and survival under drought at different growth stages (tillering, flowering, grain filling) in a sandy loam soil. The strain *M. ciceri*-CR-39 was capable to survive under drought due to catalase and exopolysaccharides production activity (Hussain *et al.,* 2014a). Rhizobia are advantageous inoculants as they benefit the wheat crop from germination to grain maturity (Yanni *et al.,* 2016) and response to the severity of drought varies with respect to developmental growth stage of the crop (Gupta *et al.,* 2001). The results in the study witness the significance of *M. ciceri*-CR-39 inoculation to wheat crop under drought, as it significantly improved tillering, root/ shoot length, fresh/ dry biomass and number of spikes (Tables 1, 3) whether under drought or normal conditions.

These increases in the growth parameters could be due to higher root colonization ability of inoculated rhizobia (Table 5) (Hussain *et al.,* 2014a; Jiménez-Gómez *et al.,* 2016) and auxin producing ability of the strains (Hussain *et al.,* 2014a) which might have increased the root growth and ultimately the area of exploration (Jiménez-Gómez *et al.,* 2016; Menéndez *et al.,* 2016) to combat drought like situation (Leinhos & Bergmann, 1995). Rashad *et al.,* (2002) inoculated sorghum with auxin and cytokinin producing *Bradyrhizobium* or *Rhizobium* sp. under drought and observed significant increases in the root/ shoot dry biomass. Moreover, the phosphate solubilization and siderophores production ability of the inoculants (Table 5) might have improved nutrient uptake in the plants (Jiménez-Gómez *et al.,* 2016; Menéndez *et al.,* 2016). Fitouri *et al.,* (2012) recorded a significant increase in plant dry biomass due to the inoculation of moderately drought tolerant *R. sultae* under drought stress condition which was isolated from the semi-arid region of Tunisia.

Drought impaired the physiological functioning of plants by reducing stomatal conductance, denaturation of chlorophyll containing cells and ultimately destroyed the photosynthesis system (Wang *et al.,* 2003; Naveed *et al.,* 2014). However, *M. ciceri*-CR-39 inoculation in this study showed significant increases in chlorophyll contents, photosynthesis rate, and transpiration rate, stomatal and intercellular CO_2 concentration under normal as well as drought conditions (Table 2). Various researchers have demonstrated increased photosynthesis system of crops due to the inoculation of different plant beneficial bacteria under drought like conditions (Sandhya *et al.,* 2010; Vardharajula *et al.,* 2011; Yandigeri *et al.,* 2012; Naveed *et al.,* 2014). Drought usually triggers the development of reactive oxygen species in plant photosystem which increase losses and denature membranes or macromolecules in cells (Mittler, 2002). The impact of these reactive oxygen species is diluted by the production of enzymatic or non-enzymatic antioxidants by the plant (Wang *et al.,* 2003). So, the inoculants might have triggered the accelerated production of antioxidants inside the plant body to improve the efficiency of photosynthesis system. Chakraborty *et al.,* (2013) have reported that the bacterized plants produce more antioxidant to dilute the impact of reactive oxygen species under drought stress condition.

The ability of plant to maintain more water relations and leak less cell electrolytes under drought stress is considered a property of drought tolerance (Pereyra *et al.,* 2006). In the present study, *M. ciceri*-CR-39 inoculation improved the plant-water relations (water use efficiency and intrinsic water use efficiency and mesophyll conductance) in the crop under drought conditions (Table 2). The inoculated bacteria might have improved the root surface area through the production of excess IAA (Table 5) in the rhizosphere to harvest more amount of water (Zahir *et al.,* 2008; Hussain *et al.,* 2014a). Menéndez *et*

al., (2016) demonstrated the redirection of plant roots in response to rhizobial inoculation. The exopolysaccharides activity of the inoculants (Hussain *et al.,* 2014a) might have developed a biofilm around the roots which enhanced the water and nutrient holding capacity of rhizosphere. Fujishige *et al.,* (2006) described that the rhizobial strains are characteristically destined to develop biofilms around roots and aggregates in the interlayer spaces of roots and root emergence sites.

Drought condition reduced the transport/uptake of nutrient in the rhizosphere because low moisture eliminated the diffusion process. Therefore, decreased the NPK concentrations in straw and grain samples under drought. Whereas, *M. ciceri*-CR39 inoculation increased the nutrient concentration in wheat straw and grains whether in normal or drought condition. However, *M. ciceri*-CR-39 showed significant increases in NPK contents of straw and grain samples as compared to un-inoculated (Table 4). Those increases might be due to higher root growth and more exploratory area under the influence of roots. San-Francisco *et al.,* (2005) demonstrated that auxin producing bacteria inoculation in peppers increased the mineral nutrition even under nutrient stress condition. Inoculation to sunflower with exopolysaccharides producing rhizobium strain- YAS34 significantly improved the plant dry mass, water uptake and nitrogen concentration (Alami *et al.,* 2000). Soil physical condition (aggregation) is improved due to exopolysaccharides producing rhizobia (strain KYGT207) which is helping in drought abiding ability of crops under stress (Kaci *et al.,* 2005).

Adversity of drought lead to yield losses in the crops by influencing the development of reproductive organs and ultimately the grain yield (Gupta *et al.,* 2001; Kettlewell *et al.,* 2010; Naveed *et al.,* 2014). In this study, drought reduced the yield parameters where applied at tillering and flowering stage as compared to drought at grain filling stage. However, rhizobial inoculation significantly improved yield parameters (grain number per spike, 1000-grain weight and grain yield) under drought and normal conditions (Table 3). These increases in the yield could be attributed to the improved nutrition, root growth, water relations and photosynthesis of the inoculated plants under drought in sandy loam soil. As the improvements in these plant growth parameters lead towards better yields. Hussain *et al.,* (2014b) recorded more sensitivity of reproductive growth stage to drought like situation in maize plant and significant improvement in growth and yield parameters due to rhizobial inoculation under drought. Vardharajula *et al.,* (2011) and Yandigeri *et al.,* (2012) have also demonstrated similar results in non-legumes with the inoculation of plant growth promoting bacteria under drought stress condition.

From the whole discussion of results it can be inferred that *M. ciceri*-CR-39 (a nodule forming bacteria) has immense potential as a plant growth promoter for non-legumes whether in normal or stress condition like drought. Wheat crop suffered the most due to drought at tillering and flowering stages but tillering stage was most responsive to *M. ciceri*-CR-39 inoculation under drought condition. The beneficial interaction might be due to the specificity of *M. ciceri*-CR-39 to wheat cv. *Sahar* in a sandy loam soil under drought condition. However, there is a need for further exploration to gauge the potential of that strain under field conditions.

Acknowledgements

The authors are thankful to the Higher Education Commission of Pakistan for supporting this research under Indigenous 5000 PhD Fellowship Program.

References

Abid, M., Z. Tian, S.T. Ata-Ul-Karim, Y. Liu, Y. Cui, R. Zahoor, D. Jiang and T. Dai. 2016. Improved tolerance to post-anthesis drought stress by pre-drought priming at vegetative stages in drought-tolerant and-sensitive wheat cultivars. *Plant Physiol. Biochem*., 106: 218-227.

Ahmad, M., Z.A. Zahir, M. Khalid, F. Nazli and M. Arshad. 2013. Efficacy of Rhizobium and Pseudomonas strains to improve physiology, ionic balance and quality of mung bean under salt-affected conditions on farmer's fields. *Plant Physiol. Biochem*., 63: 170-176.

Ahmadi, A. and A. Siosemardeh. 2005. Investigation on the physiological basis of grain yield and drought resistance in wheat: leaf photosynthetic rate, stomatal conductance and non-stomatal limitation. *Int. J. Agric. Biol*., 7: 807-811.

Alami, Y., W. Achouak, C. Marol and T. Heulin. 2000. Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharides producing *Rhizobium* sp. strain isolated from sunflower roots. *Appl. Environ. Microbiol*., 66: 3393-3398.

Anonymous. 2007. Synthesis Report; Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change; IPCC, 2007; Geneva, Switzerland: p. 104.

Ashraf, M., S.H. Berge and O.T. Mahmood. 2004. Inoculating wheat seedling with exopolysaccharides producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. *Biol. Fertil. Soils*, 40: 57-162.

Ashraf, M.Y. 1998. Yield and yield components response of wheat (*Triticum aestivum* L.) genotypes tinder different soil water deficit conditions. *Acta Agron. Hung*., 46: 45-51.

Ashraf, M.Y. and S.S.M. Naqvi. 1995. Studies on water uptake, germination and seedling growth of wheat genotypes under PEG-6000 induce water stress. *Pak. J. Sci. Ind. Res*., 38: 103-133.

Bartels, D. and R. Sunkar. 2005. Drought and salt tolerance in plants. *Criti. Rev. Plant Sci*., 24: 23-58.

Ben-Asher, J., I. Tsuyuki, B.A. Bravdo and M. Sagih. 2006. Irrigation of grape vines with saline water, II. Mathematical simulation of vine growth and yield. *Agric. Water Manag*., 83: 22-29.

Chakraborty, U., B.N. Chakraborty, A.P. Chakraborty and P.L. Dey. 2013. Water stress amelioration and plant growth promotion in wheat plants by osmotic stress tolerant bacteria. *World J. Microbiol. Biotechnol*., 29: 789-803.

Chernin, L.S., M.K. Winson, J.M. Thompson, S. Haran, B.W. Bycroft, I. Chet, P. Williams and G.S.A.B. Stewart. 1998. Chitinolytic activity in *Chromobacterium violaceum*: substrate analysis and regulation by Quorum sensing. *J. Bacteriol*., 180: 4435-4441.

Dias de Oliveria, E., H. Bramley, K.H.M. Siddique, S. Henty, J. Berger and J.A. Palta. 2013. Can elevated CO_2 combined with high temperature ameliorate the effect of terminal drought in wheat? *Func. Plant Biol*., 40: 160-171.

Fallik, E. and Y. Okon. 1996. Inoculants of *Azospirillum brasilense*: biomass production, survival and growth promotion of *Setaria italic* and *Zea mays*. *Soil Biol. Biochem*., 28: 123-126.

Farooq, M., A. Wahid, N. Kobayashi, D. Fujita and S.M.A. Basra. 2009. Plant drought stress: effects, mechanisms and management. *Agron. Sustain. Dev*., 29: 185-212.

Fischer, R.A., D. Rees, K.D. Sayre, Z.M. Lu, A.G. Candon and A.L. Saaverda. 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. *Crop Sci*., 38: 1467-1475.

Fitouri, S.D., D. Trabelsi, S. Saïdi, K. Zribi, F.B. Jeddi and R. Mhamdi. 2012. Diversity of rhizobia nodulating sulla (*Hedysarum coronarium* L.) and selection of inoculant strains for semi-arid Tunisia. *Ann. Microbiol*., 62: 77-84.

Fujishige, N.A., N.N. Kapadia, P.L. DeHoff and A.M. Hirsch. 2006. Investigations of Rhizobium biofilm formation. *FEMS Microbiol. Ecol*., 56: 195-206.

Gregersen, P.L., A. Culetic, L. Boschian and K. Krupinska. 2013. Plant senescence and crop productivity. *Plant Mol. Biol*., 82: 603-622.

Gupta, N.K., S. Gupta and S. Kumar. 2001. Effect of water stress on physiological attributes and their relationship with growth and yield of wheat cultivars at different stages. *J. Agron. Crop Sci*., 186: 55-62.

Hussain, M.B., Z.A. Zahir, H.N. Asghar and M. Asgher. 2014a. Can catalase and EPS producing rhizobia ameliorate drought in wheat. *Int. J. Agric. Biol*., 16: 3-13.

Hussain, M.B., Z.A. Zahir, H.N. Asghar and S. Mahmood. 2014b. Scrutinizing rhizobia to rescue maize growth under reduced water conditions. *Soil Sci. Soc. Am. J*., 78: 538-545.

Jamal, M., M.S. Nazir, S.H. Shah and A. Nazir. 1996. Varietals response of wheat to water stress at different growth stages and effect on grain yield, straw yield, harvest index and protein content in grains. *Rachis*, 2: 38-45.

Jiménez-Gómez, A., E. Menéndez, J.D. Flores-Félix, P. García-Fraile, P.F. Mateos and R. Rivas. 2016. Effective colonization of spinach root surface by Rhizobium. In: (Eds.): González-Andrés, F., E. James. Biological nitrogen fixation and beneficial plant–microbe interactions, Springer International Publishing Switzerland, pp. 109-122.

Kaci, Y., A. Heyraud, M. Barakat and T. Heulin. 2005. Isolation and identification of an EPS-producing Rhizobium strain from arid soil (Algeria): characterization of its EPS and the effect of inoculation on wheat rhizosphere soil structure. *Res Microbiol*., 156: 522-531.

Kaushal, M. and S.P. Wani. 2016. Rhizobacterial-plant interactions: Strategies ensuring plant growth promotion under drought and salinity stress. *Agric. Ecosys. Environ*., 231: 68-78.

Kettlewell, P.S., W.L. Heath and I.M. Haigh. 2010. Yield enhancement of droughted wheat by film antitranspirant application: rationale and evidence. *Agric. Sci*., 1: 143-147.

Khakwani, A.A., M.D. Dennett, M. Munir and M. Abid. 2012. Growth and yield response of wheat varieties to water stress at booting and anthesis stages of development. *Pak. J. Bot*., 44: 879-886.

Leinhos, V. and H. Bergmann. 1995. Changes in yield, lignin content and protein pattern of barley (*Hordeum vulgare* cv. Alexis) induced by drought stress. *J. Appl. Bot.,* 69: 206-210.

MacFaddin, J.F. 1980. Biochemical tests for identification of medical bacteria. Williams and Wilkins, Baltimore, USA.

Madi, L. and Y. Henis. 1989. Aggregation in *Azospirillum brasilense* Cd: conditions and factors involved in cell-to-cell adhesion. *Plant Soil*, 115: 89-98.

Mark, T. and B. Antony. 2005. Abiotic stress tolerance in grasses from model plants to crop plants. *Plant Physiol*., 137: 791-793.

Mehta, S. and C.S. Nautiyal. 2001. An efficient method for qualitative screening of phosphate solubilizing bacteria. *Curr. Microbiol*., 43: 57-58.

Menéndez, E.V.E., F. Sánchez-Juanes, N.V. Daza, J.P. Yépez, M.L. Barrios, R.P. Galdona, A. Garrido and J.M.G. Buitrago. 2016. Identification of human pathogenic bacteria in plant Roots by using MALDI-TOF MS Methodology. In: (Eds.): González-Andrés, F., E. James. Biological nitrogen fixation and beneficial plant–microbe interactions, Springer International Publishing Switzerland, pp. 3-12.

Mirzaei, M., D. Pascovici, B.J. Atwell and P.A. Haynes. 2012. Differential regulation of aquaporins, small GTPases and V-ATPases proteins in rice leaves subjected to drought stress and recovery. *Proteomics*, 12: 864-877.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci*., 7: 405-410.

Naveed, M., M.B. Hussain, Z.A. Zahir, B. Mitter and A. Sessitsch. 2014. Drought stress amelioration in wheat through inoculation with *Burkholderia phytofirmans* strain PsJN. *Plant Growth Regul*., 73: 121-131.

Pereyra, M.A., C.A. Zalazar and C.A. Barassi. 2006. Root phospholipids in *Azospirillum* inoculated wheat seedlings exposed to water stress. *Plant Physiol. Biochem*., 44: 873-879.

Pfeiffer, W.H., R.M. Trethowan, M. van Ginkel, M.I. Ortiz and S. Rajaram. 2005. Breeding for abiotic stress tolerance in wheat. In: Ashraf M, Harris PJC, editors. Abiotic Stresses: Plant Resistance through Breeding and Molecular Approaches, Haworth Press, New York, pp. 401-489.

Pradhan, G.P., P.V.V. Prasad, A.K. Fritz, M.B. Kirkham and B.S. Gill. 2012. Effects of drought and high temperature stress on synthetic hexaploid wheat. *Func. Plant Biol*., 39: 190-198.

Rajaram, S. 2001. Prospects and promise of wheat breeding in the 21st Century. *Euphytica*, 119: 3-15.

Rashad, M., A. Ragab and S. Salem. 2002. The influence of some *Bradyrhizobium* and *Rhizobium* strains as plant growth promoting rhizobacteria on the growth and yield of sorghum (*Sorghum bicolor* L.) plants under drought stress. *J. Plant Nutri*., 92: 664-665.

Ryan, J., G. Estefan and A. Rashid. 2001. Soil and Plant Analysis Laboratory Manual, Jointly published by the International Centre Agricultural Research in Dry Areas (ICARDA), Aleppo, Syria and National Agric. Res. Centre (NARC), Islamabad.

Sandhya, V., Sk.Z. Ali, M. Grover, G. Reddy and B. Venkateswarlu. 2010. Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. *Plant Growth Regul*., 62: 21-30.

San-Francisco, S., F. Houdusse, A.M. Zamarreno, M. Garnica, E. Casanova and J.M. García-Mina. 2005. Effects of IAA and IAA precursors on the development, mineral nutrition, IAA content and free polyamine content of pepper plants cultivated in hydroponic conditions. *Sci. Hortic*., 106: 38-52.

Sarwar, M., M. Arshad, D.A. Martens and W.T. Frankenberger, Jr. 1992. Tryptophan dependent biosynthesis of auxins in soil. *Plant Soil*, 147: 207-215.

Schwyn, B. and J.B. Neilands. 1987. Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem*. 160: 47-56.

Shakeri, S., R. Roghanian and G. Emtiazi. 2011. Comparison of intracellular polyhydroxy butyrate granules formation between different bacterial cell subpopulations by flow cytometry. *Jundishapur J. Microbiol*., 4: 229-238.

Simon, M., A. van der Bij, I. Brand, L.A. de Weger, C.A. Wijffelman and B.J.J. Laugtenberg. 1996. Gnotobiotic system for studying rhizosphere colonization by plant growth-promoting *Pseudomonas* bacteria. *Mol. Plant-Microbe Inter*., 9: 600-607.

Staudinger, C., V. Mehmeti-Tershani, E. Gil-Quintana, E.M. Gonzalez, F. Hofhansl, G. Bachmann and S. Wienkoop. 2016. Evidence for a rhizobia-induced drought stress response strategy in *Medicago truncatula*. *J. Proteom*., 136: 202-213.

Steel, K.J. 1961. The oxidase reaction as a toxic tool. *J. Gen. Microbiol*., 25: 297-306.

Steel, R.G.D., J.H. Torrie and D.A. Dicky. 1997. Principles and Procedures of Statistics- A Biometrical Approach. 2nd ed. McGraw Hill Book International Co, Singapore, pp. 204-227.

Tal, S. and Y. Okon. 1985. Production of the reserve material poly- beta-hydroxybutyrate and its function in *Azospirillum brasilense* Cd. *Can. J. Microbiol*., 31: 608-613.

Vardharajula, S., S.Z. Ali, M. Grover, G. Reddy and V. Bandi. 2011. Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. *J. Plant Interact*, 6: 1-14.

Vincent, J.M. 1970. A Manual for the Practical Study of Root Nodule Bacteria, firsted, Oxford Publication for the International Biological Program, p. 164.

Wang, W., B. Vinocur and A. Altman. 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta,* 218: 1-14.

Wolf, B. 1982. The comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. *Comm. Soil Sci. Plant Anal*., 13: 1035-1059.

Yandigeri, M.S., K.K. Meena, D. Singh, N. Malviya, D.P. Singh, M.K. Solanki, A.K. Yadav and D.K. Arora. 2012. Drought-tolerant endophytic actinobacteria promote growth of wheat (*Triticum aestivum*) under water stress conditions. *Plant Growth Regul*., 68: 411-420.

Yanni, Y.G., F.B. Dazzo, A. Squartini, M. Zanardo, M.I. Zidan and A.E.Y. Elsadany. 2016. Assessment of the natural endophytic association between Rhizobium and wheat and its ability to increase wheat production in the Nile delta. *Plant Soil*, 407: 367-383.

Zahir, Z.A., A. Munir, H.N. Asghar, M. Arshad and B. Shaharoona. 2008. Effectiveness of rhizobacteria containing ACC-deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. *J. Microbiol. Biotechnol*., 18: 958-963.

Zhang, H. and T. Oweis. 1999. Water-yield relations and optimal irrigation scheduling of wheat in the Mediterranean region. *Agri. Water Manag*., 38: 195-211.

(Received for publication 18 November 2017)