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RHIZOBIAL INOCULATION FOR IMPROVING GROWTH PHYSIOLOGY, NUTRITION AND YIELD OF MAIZE UNDER DROUGHT STRESS CONDITIONS

MUHAMMAD BAQIR HUSSAIN[1,2,3], SAJID MAHMOOD[3,4], NIAZ AHMED[2] AND HAMID NAWAZ[5]

[1]*Department of Soil and Environmental Sciences, Faculty of Agriculture and Environmental Sciences, Muhammad Nawaz Shareef University of Agriculture, Multan, (60000), Pakistan*
[2]*Department of Soil Science, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, (60000), Pakistan*
[3]*Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, (38040), Pakistan*
[4]*Department of Arid Land Agriculture, King Abdulaziz University, Makka 21454, Saudi Arabia*
[5]*Department of Agronomy, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, (60000), Pakistan*
[*]*Corresponding author's email:* jamniaz@yahoo.com

Abstract

Water scarcity is a potential effector restraining crop yield and economic returns to the farmers. This situation may be rescued by inoculating drought tolerant rhizobia owing to their plant growth promotion attributes and high root colonization efficiency. A pot experiment was conducted in sandy clay loam soil to assess the potential of four drought tolerant rhizobial strains *Rhizobium phaseoli*-RS-1, *R. phaseoli*-RS-3, *Mesorhizobium ciceri*-RS-8, and *M. ciceri*-RS-12 for ameliorating drought impact in maize. The stress was applied at five leave (vegetative) and silking (reproductive) stages. Rhizobial strain RS-8 significantly ($p \leq 0.05$) decreased electrolyte leakage and increased root/shoot dry biomass, 100-grain weight and ultimately the grain yield in normal and drought condition compared to respective uninoculated control. Differential response of rhizobial inoculation was observed on physiological characters and plant nutrient uptake under normal and drought condition, however, RS-1 and RS-8 was more promising for improving chlorophyll contents, photosynthetic/transpiration rate, intrinsic water use efficiency, stomatal/mesophyll conductance, relative water contents and nutrient uptake in maize subjected to normal and drought conditions. Moreover, root colonization capability of RS-1 (5.52×10^7 CFU cm^{-1} root) and RS-8 (5.89×10^7 CFU cm^{-1} root) was higher than control (2.3×10^4 CFU cm^{-1} root) and other strains under normal irrigation. Similarly, higher root colonization efficiency of RS-1 and RS-8 was observed under drought stress which seemed to improve plant yield by modulating physiological characters and nutrient uptake in stress conditions. Thus, the results are encouraging in the utilization of rhizobial strains (RS-1, RS-8) as biofertilizer for sustainable production of maize under water deficit conditions.

Key words: Maize, physiology, *Mesorhizobium* spp., *Rhizobium* spp., Water deficit, Root colonization.

Introduction

Crop production has come across a variety of abiotic stresses due to global warming and climate change incidents worldwide. These stresses include heat, salinity, low rainfall and water shortage as a prominent hazard to crop productivity among the stresses (Saleem *et al*., 2007; Anjum *et al*., 2011). In this climate change scenario, the world's fresh water resources are diminishing swiftly, whereas the food demand for the rapidly bourgeoning human population is projecting to further aggravate the adversity of depleted water resource. According to the Climate Change Vulnerability Index report (Anonymous, 2010), Pakistan comes under "high risk" country category for frequent events of inundation and severe drought.

Drought is a stress which has predominantly affected morphology, physiology and biochemistry of crop plants which lead to reduced yields in many crops (Menconi *et al*., 1995; Anjum *et al*., 2011). Keeping in view the problem, various researchers have tried different physical, chemical and biological strategies to ameliorate or reduce the impact of drought on plant growth and yield (Hussain *et al*., 2011). The inoculation with plant beneficial bacteria remained advantageous over chemical or physical treatments whether in terms of economic returns or environmental sustainability. *Rhizobium* is a large group of nodule forming bacteria in symbiotic association with legumes. In these nodules like structures they fix atmospheric di-nitrogen using nitrogenases. Initially Rhizobium were strictly believed to be beneficial for legumes only but now many researchers have demonstrated their ability to improve the growth of non-legumes through several plant growth promoting attributes (Hussain *et al*., 2009; Mehboob *et al*., 2012; Hussain *et al*., 2014a). Increased population of plant beneficial rhizobia in association with plant may bring some physiological changes leading towards induced systemic tolerance (IST) and ultimately improve tolerance to different abiotic stresses (Yang *et al*., 2009; Hussain *et al*., 2014a). Rhizobia may have multiple plant beneficial characteristics for improving plant growth under stress. These characteristics may include phytohormones production (Chandra *et al*., 2007), increased bioavailability and enhanced uptake of nutrients (Afzal & Bano, 2008; Hassan *et al*., 2017), regulation of ethylene reducing enzyme ACC-deaminase (Duan *et al*., 2009), Fe chelating siderophores complex production (Arora *et al*., 2001; Saidi *et al*., 2013), up-regulation of enzymatic/ non-enzymatic antioxidant activity (Berjak, 2006) and biosynthesis of plant compatible solutes/ osmolytes (Grover *et al*., 2010) etc.

Many researcher have demonstrated the beneficial effects of rhizobial inoculation to cereals in terms of improved growth and yield including rice (Hussain *et al*., 2009), wheat (Bajgiran *et al*., 2008; Hussain *et al*., 2014b), and maize (Mehboob *et al*., 2012; Hussain *et al*., 2016). However, the role of rhizobia for improving the

productivity of cereals like maize under drought like situations is less explored. Therefore, the present experiment was designed and conducted to reconnoiter the rhizobial potential (utilizing their drought abiding and higher root colonization ability) for improving physiological, nutritional, water and yield related trait of maize under water deficit stress at vegetative or reproductive growth stages in glasshouse conditions.

Materials and Methods

Collection of rhizobia: About fifty (50) rhizobia were isolated from the root nodules of *Vigna radiata* (L.) Wilczek (mung bean) and *Cicer arietinum* L. (chickpea). These isolates were tested for plant growth promoting activity in gnotobiotic conditions on maize under drought and four prominent isolates as RS-1, RS-3, RS-8 and RS-12 were selected for further evaluation (Hussain *et al*., 2014a). Present pot trial was devised to test the potential of selected rhizobial isolates for improving growth, nutrition and yield of maize under various water deficit regimes.

Identification and functional characterization of rhizobial isolates: The isolates RS-1 and RS-3 were identified as *Rhizobium phaseoli* whereas RS-8 and RS-12 were identified as *Mesorhizobium ciceri* using BIOLOG® identification system (Model: Microlog System release 4.2, Biolog Inc., USA). Re-inoculation of these isolates to respective hosts caused nodulation, which is the main criteria to confirm genus rhizobia. The isolates were characterized for various plant growth promoting traits like catalase, oxidase, siderophores, exopolysaccharides, organic acid production, P solubilization, and auxin production as given in Hussain *et al.* (2014a). In this study these isolates were further characterized for desiccation tolerance (Falik & Okon, 1996) and chitinase activity (Chernin *et al*., 1998).

Preparation of inocula and inoculation: Sterilized broth media (200 mL) of yeast extract mannitol (YEM) was prepared for each isolate. Each rhizobia was inoculated to separate media and incubated at 28±1 ºC and 180 rpm on an orbital shaking incubator for 48 h (Firstek Scientific, Tokyo, Japan). The culture was centrifuged at 4000*g* and 4 ºC for 10 min to harvest bacterial cells. The inoculum of optical density (OD) 0.5 McFarland units (i.e., 10^8 cells mL^{-1}) was developed by using densitometer (Den-1 Densitometer, McFarland, UK) in sterilized YEM liquid medium (Shakeri *et al*., 2011). Un-inoculated control medium was maintained to subtract its OD from the OD of the inoculum. Seeds of maize cv. *Neelam* were surface sterilized by dipping in 70% ethanol (2 min) and 5% sodium hypochlorite (NaOCl) solution (5 min) and washed (2-3 times) using autoclaved DI water. Seed inoculation with each rhizobia was done by coating a slurry of clay, peat, 10% sugar solution and respective bacterial culture at 1:2:1:1 ratio. Peat was calculated weight to weight basis with seed as 1:1.25. Uninoculated control was maintained using slurry prepared by mixing sterilized YEM broth with other constituents (peat, clay, sugar solution).

Experimental setup: A pot experiment was carried out to evaluate the inoculation of preselected isolates of rhizobia for improving growth and yield of maize under drought in glasshouse conditions of the Institute of Soil and Environmental Sciences (ISES), University of Agriculture, Faisalabad (UAF), Pakistan (latitude 30°31.5 N, longitude 73°74 E). The experimental soil was analyzed for textural class and other physico-chemical properties. It was sandy clay loam, alkaline (pH 7.7), non-saline (EC_e 2.5 dS m^{-1}), low in organic matter (0.93%), and had low concentrations of total nitrogen (N), available phosphorus (P) and extractable potassium (K) (0.07%, 7.8 mg kg^{-1}, 107 mg kg^{-1}, respectively). The pots (height of 30 cm, inside diameter of 23 cm) were filled with 12 kg of dried and sieved sandy clay loam soil. Inoculated seeds of maize were sown (three seeds per pot) and thinned to maintain one plant per pot after one week of germination. The experiment was arranged following completely randomized design (CRD), maintaining triplet of each treatment. Fertilizers urea, di-ammonium phosphate (DAP) and muriate of potash (MOP) were used to apply 180-140-90 kg ha^{-1} (Recommended doses) of N, P and K, respectively, in each pot. The pots were irrigated with canal water according to the crop requirement. Drought was imposed by delaying irrigation at five leaves (vegetative) and silking (reproductive) stages of maize. The stress was continued till the appearance of wilting symptoms in plants. Plants showed wilting symptoms by delaying irrigation for 9 days at vegetative and 7 days at reproductive stage. Plants were grown up to maturity and harvested after 96 days from sowing. Growth, yield and physiological parameters were measured at harvest. Nutrient (N, P and K) analysis of grain and stover samples was performed following the procedures of Ryan *et al.* (2001).

Measurement of physiological traits: Plant photosynthesis system was measured using portable infra-red gas analyzer [IRGA (LCA-4) Germany] between 10:00 *a.m* and 02:00 *p.m* using photosynthetic photon flux density at 1200-1400 µmol m^{-2} s^{-1}). Four parameter including rate of photosynthesis (*A*), rate of transpiration (*E*), sub-stomatal CO_2 concentration (*Ci*) and stomatal conductance (g_s) were recorded. For measurement, a fully developed and expanded leaf in the top part of the plant was selected. Using the data of transpiration (*E*) and photosynthesis rates (*A*), water use efficiency was calculated (Ahmad *et al*., 2013). Carbon dioxide conductance in the mesophyll cells was derived using *A* and C_i data (Fischer *et al*., 1998). Division of *A* with g_s gave intrinsic water use efficiency (Ahmadi *et al*., 2005). Formulas used are as following.

$$\text{Water use efficiency} = \frac{\text{Rate of photosynthesis } (A)}{\text{Rate of transpiration } (E)}$$

$$\text{Intrinsic water use efficiency} = \frac{\text{Rate of photosynthesis } (A)}{\text{Stomatal conductance } (g_s)]}$$

$$\text{Mesophyll conductance} = \frac{\text{Rate of photosynthesis } (A)}{\text{Sub-stomatal conductance } (C_i)}$$

Chlorophyll contents: Chlorophyll contents were measured using SPAD-502 (Konica-Minolta, Japan) chlorophyll meter. The data was recorded in triplicate from each leaf.

Electrolyte leakage and relative water content: Flag leaves were sampled to measure the electrolyte leakage (EL) and relative water content (RWC).

For measuring the electrolyte leakage (EL), leaf discs were cut and dipped in deionized (DI) water (10 mL) contained in a test tube. The tubes were incubated at 28±1 °C and 100 rpm in an orbital shaking incubator for 4 h. After incubation, first reading (R1) of electrical conductivity (EC) was recorded using Jenway Conductivity Meter (Model 4070). Second reading (R2) of electrical conductivity was measured after autoclaving the samples (121°C and 15 psi for 20 min). The final value of electrolyte leakage was calculated using the formula given below (Jambunathan, 2010).

$$\% \text{ Electrolyte leakage} = \frac{\text{R1 (EC before autoclaving)}}{\text{R2 (EC after autoclaving)}} \times 100$$

The relative water contents of maize leaves were measured using the fresh weight and turgid weight after 16-18 h incubation at 4 °C in DI water. The dry weight was obtained by drying the leaves in oven at 72 °C for 24 h. Following formula was used to determine the relative water contents of leaves (Mayak *et al*., 2004).

$$\% \text{ Electrolyte leakage} = \frac{\text{R1 (EC before autoclaving)}}{\text{R2 (EC after autoclaving)}} \times 100$$

$$\text{Relative water content} = \frac{\text{Fresh leaf weight} - \text{Dry leaf weight}}{\text{Turgid leaf weight} - \text{Dry leaf weight}}$$

Nutrient analysis: Dried plant and grain samples were ground and digested using 0.1 g of sample in di-acid mixture (conc. H_2SO_4 and H_2O_2 in 2:1 ratio) following the protocol as described by Wolf (1982). Nitrogen content in the samples was measured by distillation in Kjeldahl apparatus and following titration with 0.01 N H_2SO_4 (Jackson, 1962). Phosphorus content was determined by mixing the sample (5 mL) in Barton reagent (10 mL) and measuring absorbance at 420 nm on spectrophotometer and a standard curve (Ryan *et al*., 2001). Flame photometer (model: Jenway PFP-7, England) was used to determine potassium content in the samples (Ryan *et al*., 2001).

Root colonization assay: At harvesting, microbial root colonization of maize roots was determined according to Simon *et al.*, (1996). For the isolation of microbes from maize rhizosphere, roots were harvested in a 1% HCl solution from uninoculated control and inoculated plants either under drought stress or normal. After rinsing in sterilized DI water, about 1 cm root tip was cut and suspended in 1 mL saline (0.9%). Samples were mixed and serial dilutions were made of each suspension in 0.9% saline. To count the average bacterial population (colony forming units, CFU mL^{-1}), 1 mL from each dilution was spread on autoclaved minimal salt agar plate and counted using colony counter (Ahmad *et al*., 2015).

Statistical analysis: The data was analyzed statistically using analysis of variance (ANOVA) following completely randomized design (Steel *et al*., 1997). The statistical software Statistix v8.1 was used. The means were compared following Least Significance Difference (LSD) test at $p \leq 0.05$ probability.

Results

Biomass and yield parameters: Drought stress severely decreased the root and shoot dry mass of maize plants, but the inoculation of rhizobia significantly ($p \leq 0.05$) increased the parameters under both normal and drought stress conditions as compared to respective un-inoculated controls (Table 1). Under normal conditions, RS-8 produced maximum shoot (up to 26%), whereas RS-12 caused higher root (up to 89%) dry biomass as compared to uninoculated control. However, rhizobial strain RS-8 was distinct in improving shoot and root dry biomass under drought stress at vegetative (up to 35 and 66%, respectively) and reproductive stage (up to 30 and 95%, respectively) as compared to respective control. All the other strains produced significant results, but the same rhizobial strain (RS-8) was found more efficient in producing grain yield pot^{-1} and 100-grain weight in normal (up to 43 and 57%, respectively) and in drought stress conditions (up to 56 and 40%, respectively, at vegetative while 83 and 37%, respectively, at reproductive stage) in comparison to respective control (Table 1).

Table 1. Effect of rhizobial inoculation on dry biomass and yield of maize under drought stress, (n=3).

	Shoot dry weight (g pot^{-1})			Root dry weight (g pot^{-1})		
Strains	**D0**	**D1**	**D2**	**D0**	**D1**	**D2**
Control	54 d	46 d	40 e	13.3 d	9.2 d	6.3 d
RS-1	64 b	56 b	46 c	13.0 d	11.3 c	7.3 c
RS-3	63 c	56 b	47 b	17.3 c	13.2 b	11.3 b
RS-8	68 a	62 a	52 a	19.2 b	15.3 a	12.3 a
RS-12	64 b	55 c	44 d	25.2 a	11.3 c	7.4 c
	100 Grains weight (g)			**Grain yield (g pot^{-1})**		
Control	11.7 e	10.0 e	9.0 d	37.7 d	31.0 d	22.0 c
RS-1	17.0 b	13.0 b	10.7 b	48.0 b	41.7 b	37.7 a
RS-3	15.3 c	12.0 d	9.7 c	43.0 c	36.0 c	29.3 b
RS-8	18.4 a	14.0 a	12.3 a	54.0 a	48.3 a	40.3 a
RS-12	15.0 d	12.5 c	9.0 d	47.7 b	42.3 b	31.7 b

D0 indicates no drought whereas D1 and D2 indicate drought at vegetative and reproductive stages, respectively. (n=3)
Note: Mean sharing similar letter(s) are statistically similar to each other at $p<0.05$

Table 2. Effect of rhizobial inoculation on physiology (stomatal conductance, water use efficiency, photosynthesis and transpiration rate) of maize under drought stress, (n=3).

	Photosynthesis rate (mmol m^{-2} S^{-1})			Transpiration rate (mmol m^{-2} S^{-1})		
Strains	**D0**	**D1**	**D2**	**D0**	**D1**	**D2**
Control	3.53 e	1.59 e	1.50 e	1.26 e	0.85 e	0.82 d
RS-1	6.53 b	6.12 a	3.97 a	1.97 a	1.89 a	1.38 a
RS-3	7.16 a	4.55 c	2.41 c	1.75 b	1.33 b	1.15 b
RS-8	6.00 c	4.74 b	3.53 b	1.49 c	1.26 c	1.02 c
RS-12	4.04 d	2.24 d	1.86 d	1.32 d	1.05 d	0.99 c
	Water use efficiency (WUE=A/E)			**Stomatal conductance (mmol m^{-2} S^{-1})**		
Control	2.81 d	1.88 e	1.82 d	0.03 d	0.025 d	0.010 d
RS-1	3.32 b	3.24 c	2.88 b	0.056 a	0.050 a	0.041 a
RS-3	4.10 a	3.43 b	2.09 c	0.051 b	0.040 b	0.030 b
RS-8	4.02 a	3.75 a	3.48 a	0.042 c	0.031 c	0.030 b
RS-12	3.06 c	2.12 d	1.89 d	0.031 d	0.025 d	0.024 c

D0 indicates no drought whereas D1 and D2 indicate drought at vegetative and reproductive stages, respectively. (n=3)
Note: Mean sharing similar letters are statistically similar to each other at $p<0.05$

Table 3. Effect of rhizobial inoculation on physiology (relative water content, electrolyte leakage, mesophyll conductance, intrinsic water use efficiency) of maize under drought stress, (n=3).

	Relative water content (%)			Electrolyte leakage (%)		
Strains	**D0**	**D1**	**D2**	**D0**	**D1**	**D2**
Control	63.91 d	45.43 e	46.43 e	79.53 a	93.60 a	99.00 a
RS-1	91.31 a	66.96 a	62.73 b	68.13 c	77.43 d	92.33 b
RS-3	71.93 c	55.16 c	59.30 c	65.87 d	81.43 c	92.43 b
RS-8	75.98 b	62.33 b	68.56 a	63.33 e	75.13 e	91.73 b
RS-12	75.61 b	53.22 d	56.00 d	70.57 b	83.73 b	93.17 b
	Mesophyll conductance (MC=A/Ci)			**Intrinsic WUE (iWUE=A/g_s)**		
Control	0.020 c	0.012 d	0.014 d	118 c	65 e	144 a
RS-1	0.027 a	0.036 a	0.035 a	117 c	121 b	98 c
RS-3	0.026 b	0.028 c	0.018 c	139 a	114 c	80 d
RS-8	0.026 b	0.033 b	0.027 b	144 a	153 a	118 b
RS-12	0.014 d	0.011 d	0.012 e	129 b	91 d	77 d

D0 indicates no drought whereas D1 and D2 indicate drought at vegetative and reproductive stages, respectively. (n=3)
Note: Mean sharing similar letters are statistically similar to each other at $p<0.05$

Table 4. Root colonization, desiccation tolerance and chitinase activity of rhizobial isolates, (n=3).

***Rhizobium* strains**	**Root colonization (CFU cm^{-1} root)**			**Chitinase activity**	**Desiccation tolerance (10^5 CFU g^{-1})**
	Normal (10^7)	**Drought at vegetative stage (10^6)**	**Drought at reproductive stage (10^4)**		
R. phaseoli (RS1)	4.28	5.10	5.56	-	3.25
R. phaseoli (RS3)	5.52	3.89	4.18	-	4.10
M. ciceri (RS8)	5.89	6.12	7.57	+	3.91
M. ciceri (RS12)	2.68	4.98	3.64	+	5.13
†Control	2.3×10^4	5.72×10^2	6.2×10^1		

†control treatment did not receive any exogenous rhizobial inoculation, however, data shows colonization of indigenous rhizobia
Note: Positive sign shows the presence while negative sign shows the absence of the character

Plant physiological parameters: The imposed drought stress down regulated all the physiological machinery, which was up regulated upon rhizobial inoculation (Tables 2, 3). The rhizobial strains RS-1 and RS-8 improved maize physiology not only under normal but also under drought conditions. Under normal conditions, differential response of rhizobial strains was observed i.e. RS-1 caused maximum increase in SPAD chlorophyll contents (Fig. 1), transpiration rate, stomatal and mesophyll conductance (up to 27, 56, 87 and 35%, respectively), RS-3 caused maximum photosynthetic rate and WUE (up to 1.03 fold and 46%, respectively), RS-8 caused maximum intrinsic WUE (up to 22%). Under drought stress, inoculation with RS-1 showed maximum improvement in photosynthetic rate (up to 2.85 fold at vegetative, while 1.65 fold at reproductive stage), transpiration rate (up to 1.22 fold at vegetative while 68% at reproductive stage), stomatal (up to 1 fold at vegetative while 3 fold at reproductive stage), and mesophyll conductance (up to 2 fold at vegetative while 1.5 fold at reproductive stage) compared to un-inoculated control. However, RS-12 inoculation showed significantly high sub-stomatal CO2 concentration (Fig. 1) in the mesophyll cells of maize leaves as compared to uninoculated control whether normal conditions or under drought stress at vegetative or reproductive stage.

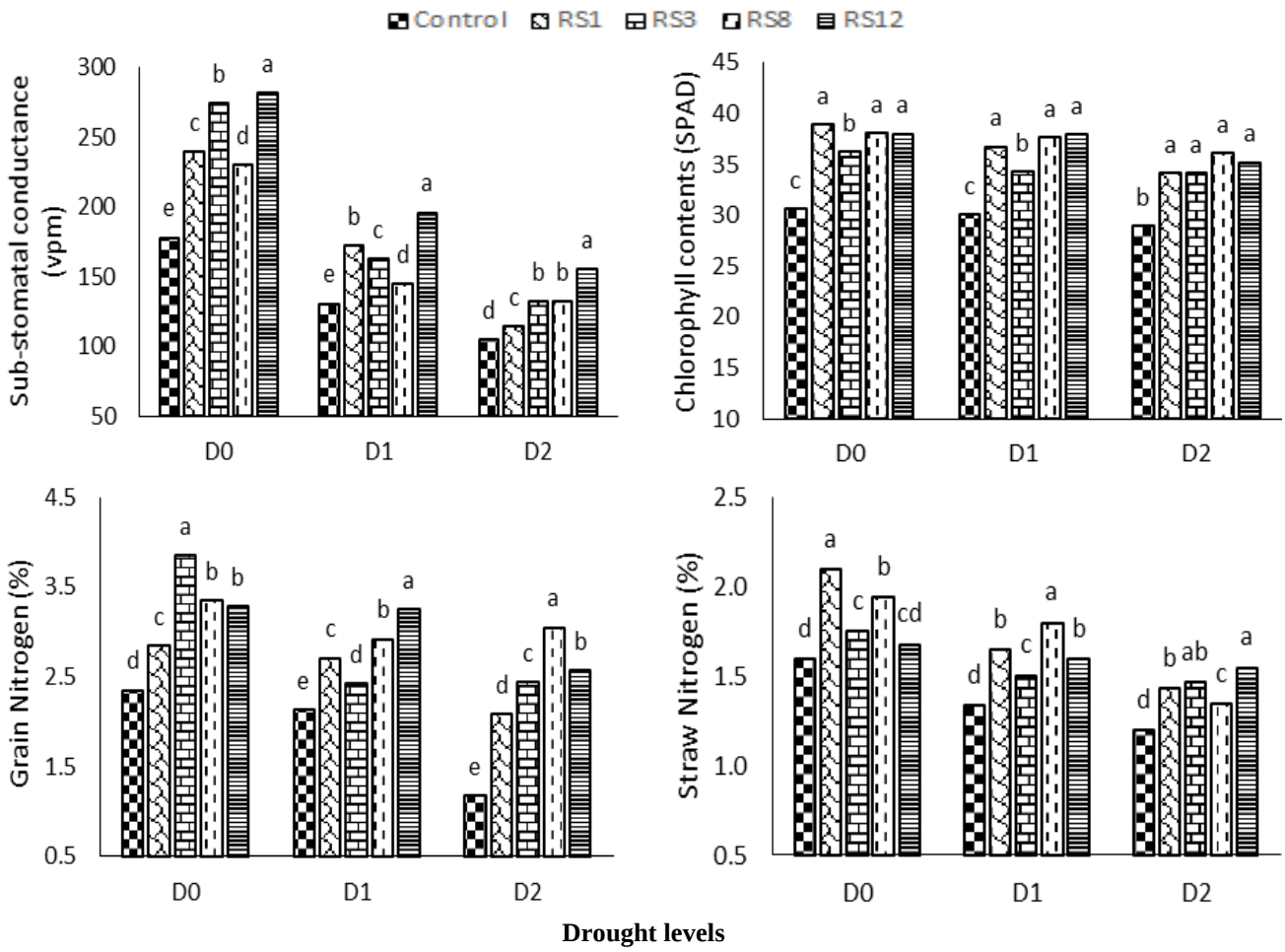

Fig. 1. Effect of rhizobial inoculation on sub-stomatal conductance, chlorophyll and nitrogen (N) contents of maize under drought stress. D0 indicates no drought whereas D1 and D2 indicate drought at vegetative and reproductive stages, respectively. Asterisk shows significant (p<0.05) differences at each drought level as compared to their respective control. (n=3)

Water deficit stress at vegetative or reproductive growth stage of maize caused significant reduction in leaf relative water contents as compared to unstressed plants. But inoculation with rhizobia including *R. phaseoli*-RS-1, *R. phaseoli*-RS-3, *M. ciceri*-RS-8, and *M. ciceri*-RS-12 significantly improved RWC of maize leaves both in normal as well as drought conditions as compared to respective un-inoculated controls (Table 3). Drought at vegetative or reproductive stage of the crop decreased RWC up to 41 and 38%, respectively, in un-inoculated plants over unstressed control. Whereas RS-1 inoculation showed highest increase among the isolates in RWC of maize leaves up to 43 and 47% in unstressed or drought conditions at vegetative stage, respectively, as compared to the respective un-inoculated controls. Similarly, about 48% increase (highest increase among the isolates) in RWC of maize leaves was recorded due to the inoculation of *M. ciceri*-RS-8 over respective un-inoculated control where drought was applied at reproductive growth stage. With decreasing RWC of maize leaves, electrolyte leakage (EL) increased significantly due to drought stress at reproductive and vegetative growth stage in comparison to normally irrigated plants. The inoculation with rhizobial isolates significantly reduced the EL over respective un-inoculated controls in normal and water deficit conditions at vegetative or reproductive growth stages of maize. Isolate RS-8 showed significantly higher reduction (up to 20, 7 and 20%, respectively) in EL of maize leaves among the isolates as compared to respective uninoculated controls whether stressed (at vegetative and reproductive stage) or not.

Mineral nutrient status: Plants inoculated with rhizobial strains showed higher NPK contents in shoot and grain both under normal and water deficit condition over un-inoculated control plants (Figs. 1, 2). Under well-watered conditions, *R. phaseoli*-RS-1 inoculation demonstrated highest increases in NPK contents of shoot samples (up to 31, 33 and 27%, respectively), while *R. phaseoli*-RS-3 gave maximum N (up to 64%) and RS-8 gave maximum PK accumulation in grain (up to 39 and 56%, respectively), as compared to their respective control. Under drought stress, rhizobial strain RS-8 caused maximum accumulation of shoot N (up to 34% at vegetative stage), grain N (up to 1.61 at reproductive stage), grain P (up to 31% at vegetative while 27% at reproductive stage), Similarly, RS-12 caused maximum accumulation of shoot N (up to 29% at reproductive stage), grain N (up to 52% at vegetative stage), shoot P (up to 40% at vegetative while 56% at reproductive stage), shoot K (up to 19% at vegetative while 32% at reproductive stage) and grain K (up to 51% at vegetative, while 32% at reproductive stage), as compared to their respective control.

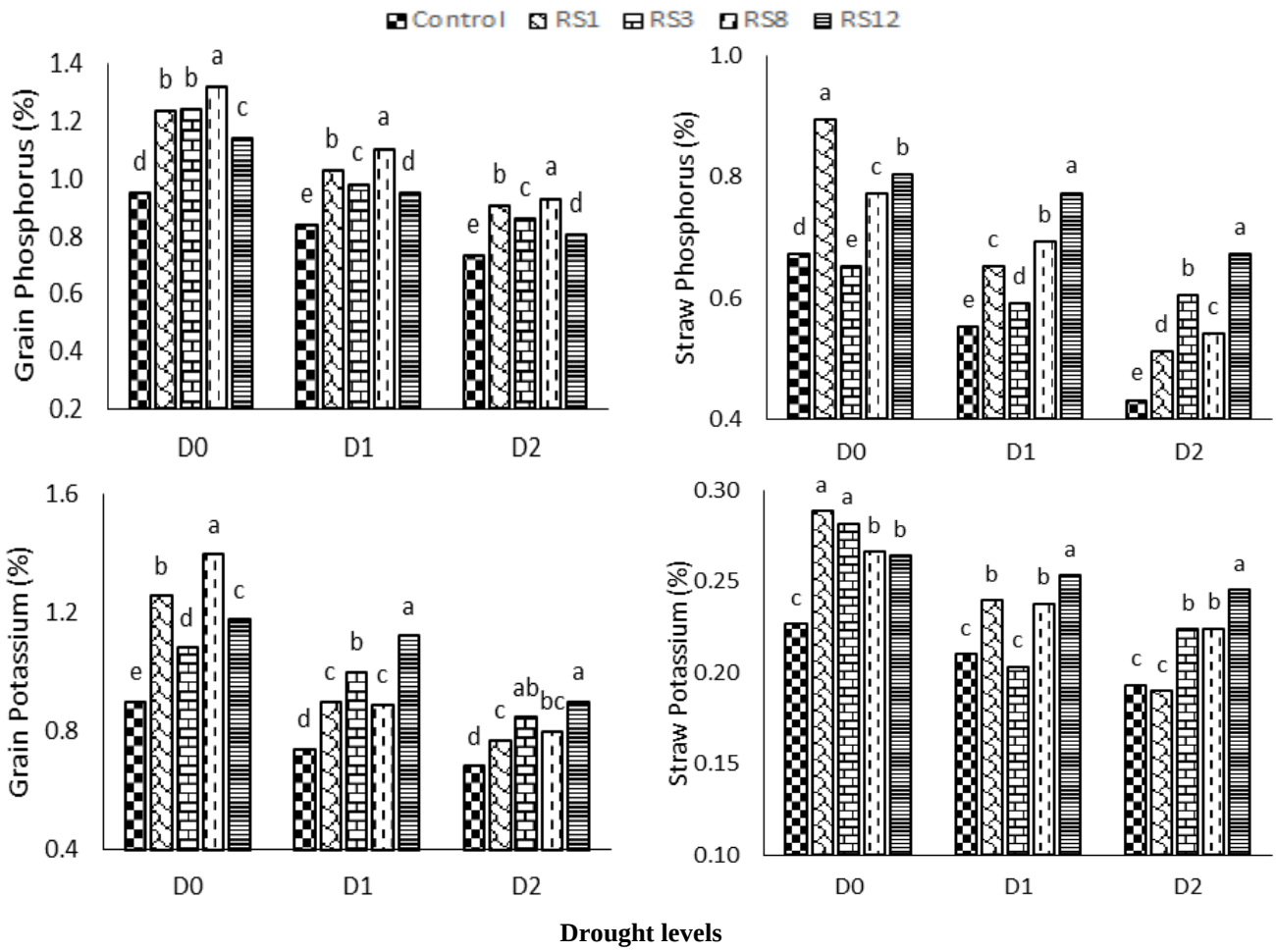

Fig. 2. Effect of rhizobial inoculation on phosphorus (P) and potassium (K) contents of maize under drought stress. D0 indicates no drought whereas D1 and D2 indicate drought at vegetative and reproductive stages, respectively. Asterisk shows significant (p<0.05) differences at each drought level as compared to their respective control. (n=3)

Root colonization, desiccation and characterization assay: Root colonization assay showed highest colonization ability of RS-8, followed by RS-3, RS-1, RS-12 in normal, whereas RS-8 showed maximum root colonization in drought stress at vegetative as well as reproductive stage followed by RS-1, RS-12 and RS-3 (Table 4). In the roots of uninoculated control plants, some kind of bacteria were observed but their colonization ability was far less as compared to inoculated strains. However, in vitro desiccation assay showed maximum tolerance of *M. ciceri*-RS-12, followed by *R. phaseoli*-RS-3, *M. ciceri*-RS-8 and *R. phaseoli*-RS-1.

Discussion

Duration and intensity of drought determines its severity and survivability of the plant, however, its incidence at any growth stage of the crop causes yield reduction (Farooq *et al*., 2009). Several approaches have been tried by the researchers to help crop plants for abiding adverse conditions but inoculation of plant beneficial bacteria may prominently rescue plant growth through improving drought tolerance in arid and semi-arid climatic conditions (Marulanda *et al*., 2007; Hussain *et al*., 2014a). Rhizobia have shown their capability to survive under hostile environments, a property which can be harnessed to prolong rhizobial association with crop plants and could lead to increased plant stress tolerance and crop productivity (Alexandre & Oliveira, 2013). In the present work, drought like situation by delaying irrigation up till wilting symptoms in plants drastically decreased maize growth, yield and gaseous exchange system but rhizobial inoculation rescued the plants and demonstrated positive results. A significant increase in the biomass and yield of maize was recorded due to inoculation with respect to un-inoculated control (Table 1). These improvements in plant's growth and yield are claimed as the common responses to inoculation of plant beneficial bacteria under drought like situations (Lucy *et al*., 2004; Naveed *et al*., 2014). Hussain *et al.* (2014a) has described certain plant growth promoting attributes in these bacteria which could play a role in improving maize plant's survival under drought. They are catalase and exopolysaccharides producers, efficient root colonizers, auxin producers and nutrient mobilizers. Moreover, rhizobia would have induced modifications in root morphology, thereby improving the nutrient and water uptake with increase in root surface area. Such modifications might have posed a positive change in the overall performance of maize plants (Hussain *et al*., 2014a). Similar findings were observed by the inoculation of *R. leguminosarum* bv. *Trifolii* (excessive producer of auxin and cytokinin) to maize and wheat crops (Hoflich, 2000). Kaci *et al.* (2005) described beneficial modifications in the physical

properties of wheat rhizosphere soil with the inoculation of rhizobial strain-KYGT207. A significant increase in the root and shoot dry mass, and root adhering soil has been observed in this study which might be due to higher exopolysaccharides production of inoculated rhizobia ascribed to increase in rhizosphere soil aggregation, stabilization and prolonged water and nutrient retention. In the present study, drought has markedly reduced 100 grains weight and ultimately the grain yield of maize (Table 1). Under drought like situation the moisture layer on soil surface becomes so thin that diffusion of nutrients becomes impossible, affecting net rate of photosynthesis and grain yield of the crop. However, inoculated plants with rhizobia gained their yield over un-inoculated control. As the inoculant microbes may have produced auxin and cytokinin to increase root growth which can further explore the soil for nutrient and water uptake. Whereas, catalase produced by these bacteria might have counter the harmful impacts of reactive oxygen species in the photosystem and increased maize yield under drought (Ilyas *et al*., 2008). Marked increases in grain yield of maize can also be attributed to rhizobia induced change in nutrient uptake such as higher siderophores production (Arora *et al*., 2001) and phosphate solubilization (Afzal & Bano, 2008). Similar increases in the grain yield of wheat and rice were recorded by Mehboob *et al*., (2011) and Hussain *et al*., (2009) due to rhizobial inoculation over un-inoculated control.

Chlorophyll contents, gas exchange attributes, and relative water contents (RWC) of leaf are anticipated as gauges of plant physiological response towards stress (Golding and Johnson, 2003; Hura *et al*., 2007; Maccaferri *et al*., 2011; Bürling *et al*., 2013). In this study, drought has negatively influenced the maize physiology (Tables 2, 3 & Fig. 1). All the gas exchange characteristics of maize were drastically impaired due to drought, which might be ascribed to premature leaf senescence, drastic decrease in leaf surface area, reactive oxygen species induced chloroplast lipids oxidation and damage to photosynthetic machinery and changes in the ultrastructure of proteins and pigments (Menconi *et al*., 1995). The drastic impact of drought seems to be mitigated in inoculated maize plants, which might be due to production of auxin producing rhizobia responsible for greater biomass, and nutrient uptake key to sustain in an adverse conditions. Similar to these findings, rhizobial inoculation have also been reported to improve rate of photosynthesis and transpiration, stomatal conductance and WUE in rice and mung bean (Chi *et al*., 2005; Ahmad *et al*., 2013). Moreover, in this study, all the rhizobial strains increased plant physiology, especially RS-1 and RS-8, which were also shown to increase maize biomass and yield (Tables 1, 2, 3). Thus, we could say that these strains actually modulate plant physiology in normal and drought stress, which in turn produced better maize yield. In this study, imposed drought stress at different growth stages of maize, experienced no significant effects on RWC and EL whether inoculated or uninoculated, it implied that stress damaged chlorophyll of maize, which impaired normal photosynthesis process. Inoculated rhizobial strains recovered maize plant under drought stress, repaired chlorophyll by acquisition of nutrients (Figs. 1, 2) and improved plant physiology (Tables 2, 3).

Among the four rhizobial isolates tested in this experiment, *R. phaseoli*-RS-1 and *M. ciceri*-RS-8 performed relatively better. Both of the strains were shown to increase maize biomass, yield, nutrient uptake and plant physiology in normal and drought stress. These are efficient colonizer of roots (Table 4), thereby may have extracted more nutrients from soil even in drought stress, used to repair plant physiology leading to better maize production. Apart from root colonization, these strains showed several plant growth promoting traits like oxidase, catalase, exopolysaccharide and auxin production, which might be involved in better plant performance, but as these activities were also observed in other rhizobial strains as well, thereby these may or may not be responsible for greater plant production under stress. In vitro desiccation tolerance of rhizobial strains was also could not correlate with their activity in pot culture for example, RS-12 showed maximum desiccation tolerance *in vitro* (Table 4) but it is not as efficient as RS-1 and RS-8 in improving plant physiology and yield in pot culture (Tables 1, 2, 3).

In this study, it was found that root colonization was a key factor for better plant production and mitigation of stress through enhanced nutrient extraction and improved plant physiology. However, how these strains influence accumulation of solutes (proline, phenolics and antioxidants) are still needed to be investigated for better understanding of mechanism. These results indicate that rhizobial strains RS-1 and RS-8 could be used as biofertilizer to induce drought resilience in maize.

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