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INOCULATION WITH *Rhizobial consortium* FOR IMPROVING THE GROWTH, YIELD AND QUALITY OF MAIZE UNDER SALT-STRESSED CONDITIONS

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Salinity is one of the major factors which affect overall food production of the world, especially in arid and semi-arid regions. Present study was conducted to evaluate the effectiveness of four [two from chickpea (CRM-7 and CRM-9) and two from lentil (LMR-5 and LMR-10)] different strains of rhizobia individually as well as their consortium for reducing the effect of salinity on the growth and yield of maize in pot experiment under wire house conditions. Salinity stress negatively affected the growth, yield, quality parameters and chlorophyll contents of maize. However, rhizobial inoculation significantly reduced the adverse effects of salinity. Multi-strain rhizobial inoculation proved better approach than sole rhizobial inoculation. Improvement in plant height (34%), grain yield (49%), cob length (67%) and weight (25%), relative water content (57%), crude protein (34%), quality parameters N (34%), P (36%) and K (36%) in grains, and chlorophyll contents (37, 37 and 46% in chlorophyll a, b and carotenoids, respectively) was observed by consortial inoculation (LMR-5, LMR-10, CRM-7 and CRM-9) compared to unstressed un-inoculated control at 8 dS m^{-1}. Among separate inoculation, chickpea rhizobial isolates performed better than lentil rhizobial isolates in inducing salinity tolerance in maize. It can be suggested that rhizobium consortium has better potential to promote growth and yield of maize than separate use of these strains. However, site specific field evaluation is required to confirm capability of consortial inoculant to get maximum benefit in terms of better growth and yield.
Keywords: Salinity, *Zea mays*, salt tolerance, relative water content, rhizobia, non-legume

INTRODUCTION

World's growing population leads towards increasing food demand and engulfs more land for housing development. Salinization is decreasing the world's irrigated land by 1-2 % every year, affecting badly in the arid and semi-arid regions (FAO, 2008). Productivity of the food, forage and fodder is also diminishing every year due to desertification and salinization (FAO, 2009). It is estimated that over 6% of land area of the world is affected by salinity, which is approximated to 800 million ha (FAO, 2008). About 400-thousand-hectare land in Pakistan is rendered useless every year by salinity (Ghaffor *et al*., 2004. To respond to increasing demands of food, new technologies are needed to produce more from less land.

Soil salinity directly affects the soil structure by inhibiting the clump formation which makes the soil tight and impervious. It reduces the air and water holding capacity of soil. Thus, plants may not receive enough moisture and oxygen to grow (Brady and Weil, 2002; Shober, 2015). High salts concentrations in soil solution also cause toxic effects to plants that ultimately disturb various biochemical and physiological processes which causes reduction in biomass and yield of various crops (Munns, 2002). In general, salinity affects plant physiology at whole plant and in particular at cellular levels through osmotic and ionic stress (Munns, 2002; Yadav *et al.,* 2011). Above and beyond bringing about osmotic and ionic stress, salinity stress usually causes ionic imbalance that may decline the selectivity of root plasma membranes and develop potassium deficiency (Ahmad *et al*., 2006). The build up of high amounts of toxic salts in the apoplasm of leaf outcomes to dehydration and loss in the cell turgor that eventually demises leaf cells and tissues (Affenzeller *et al.,* 2009) leading to reduction in growth and yield.

Salt affected soils can be managed by adopting different ways such as by using deep tube wells which lower groundwater levels, leaching of excess soluble salts by using fresh irrigation water, flushing soils that contain salt crust at the surface, improvement by chemical application like gypsum, organic matter, acids (Murtaza *et al*., 2009; Raafat and Tharwat, 2011) and by means of biological approaches e.g. developing salt resistance cultivars, use of plant growth regulators, inoculating crop seed and seedlings with plant growth promoting rhizobacteria (PGPR) (Athar and Ashraf, 2009). However, physical and chemical means are either costly and hazardous to environment or impracticable. Among PGPR, rhizobia are the most studied plant growth promoting microorganisms which are environment friendly, economic and practicable (Tairo and Ndakidemi, 2013).

The beneficial effect of Rhizobia in legumes is well known. Recently, rhizobia have also been reported to enhance the growth and yield of non-legumes e.g. wheat (Mehboob *et al.,* 2011), rice (Tan *et al.*, 2014), maize (Hadi and Bano, 2010) etc. Rhizobial isolates colonize roots of non-legumes and enhance plant growth by several direct and indirect mechanisms of action. Directly, rhizobial isolates synthesize many plant growth hormones e.g. auxins (Zahir *et al.*, 2010), cytokinins (Senthilkumar *et al.*, 2009), abscisic acid (Boiero *et al.*, 2007) and gibberellins (Boiero *et al.*, 2007) and secrete many other chemicals beneficial to plant growth such as siderophores (Huang and Erickson, 2007), exopolysaccharides (Zafar-ul-Hye *et al.,* 2013; Hussain *et al.*, 2014a), ACC-deaminase (Duan *et al.*, 2009), phosphatases (Afzal and Bano, 2008), phosphohydrolases (Gugi *et al.,* 1991), phytase (Glick, 2012), etc. Rhizobia also enhance availability to the plant by mobilizing the nutrients in the soil and improving the soil structure (Barea and Richardson, 2015). Indirectly, rhizobia improve plant health by improving the self-defense of plant through induction of systemic resistant (Reddy, 2013) against harmful insects, pathogens, diseases and viruses (Huang and Erickson, 2007).

Rhizobia have also been reported to improve the growth and yield of legumes (Ahmad *et al.,* 2012; El-Akhal *et al.*, 2013) and non-legumes (Afzal and Bano, 2008; Bano and Fatima, 2009) under salt stressed conditions. There are extensive reports of rhizobia for enhancement of number of primary roots, root proliferation, and plant growth promotion even under increased level of salinity (Naz *et al.*, 2009; El-Akhal *et al.,* 2013). Further, it has been demonstrated that co-inoculation or multi-strain consortium of rhizobia with other PGPR or mycorrhizae gave better improvement than their alone inoculation under normal as well as salt stressed conditions (Yu *et al.,* 2007; Afzal and Bano, 2008; Bano and Fatima, 2009). However, application of rhizobial consortium especially from different legumes is neglected.

Keeping in view the above discussion, the present experiment was conducted to assess application of single and multi-strain consortial inoculation using rhizobia from different legumes as a tool to induce salinity tolerance in maize under salt stressed conditions.

MATERIALS AND METHODS

***Isolation, purification and screening of rhizobia*:** Numerous pure colonies of rhizobia were isolated from the root nodules of lentil (*Lens culinaris* M.) and chickpea (*Cicer arietinum* L.) plants sampled from different salt affected fields of Faisalabad, Jhang and Shorekot following the standard procedure (Abd-Alla *et al.,* 2012; Muzzamal *et al.,* 2012). These isolates were confirmed as rhizobia by streaking on congo-red agar plate (Grams test) and by studying their colony morphology. Ten fast growing strains from each legume were selected and osmoadaptation assay of these strains was carried out as described by Vincent (1970) to assess their salinity tolerance at original (control), 4, 8, 12 and 16 dS m^{-1} salinity levels. These all twenty strains were tested for plant growth promotion under salt stressed axenic conditions using maize as test plant (data not shown). On the basis of osmoadaptation assay and plant growth promotion activity, four rhizobial isolates (LMR5, LMR10, CMR7, and CMR9) were selected for further study.

***Preparation of inocula and seed inoculation*:** Fresh inoculum for each isolate (LMR5, LMR10, CMR7 and CMR9) was prepared in 250 mL conical flask containing 100 mL sterilized yeast extract mannitol broth. After three days of incubation at 28±1°C and 100 rpm in orbital shaking incubator, cells were harvested by centrifugation at 10,000 *rpm* and 4°C for 15 minutes. Inocula of 0.5 optical density (10^{6-8} CFU mL^{-1}) was prepared using OD meter by adding harvested cells of each isolate in sterilized YEM broth. Maize seeds were inoculated with respective inoculum, mixed with autoclaved peat and 10% sterilized sugar solution (inoculum to peat ration 1:1 w/w). For un-inoculated control, seeds were coated with sterilized broth mixed with sterilized peat. Inoculated seeds were dried for 6-8 h in shade before sowing.

***Pot experiment*:** Four different salt tolerant rhizobial strains were evaluated for improving growth and yield of maize under pot conditions. Pots containing 12 kg of air dried, sieved and physico-chemically analyzed soil (Table 1) were arranged following completely randomized design (CRD) in factorial set with three replications. Three salinity levels (1.46, 4, and 8 dS m^{-1}) were maintained by using NaCl. Five inoculated maize seeds per pot were sown and thinned to one plant after two weeks of germination. Recommended dose of NPK was applied at 180, 140, and 90 kg ha^{-1} using urea, diammonium phosphate and muriate of potash fertilizers. The whole P and K were supplemented as basal dose whereas N was added in three splits, i.e. at sowing, at first irrigation and at knee height. Pots were irrigated with tap water having EC<0.7 dS m^{-1}. Plants were grown to maturity and at harvest (102 days after sowing), parameters related to growth and yield (plant height, cob length, cob weight and 100-grain weight) were recorded.

***Determination of crude protein and relative water content*:** Crude protein was determined by multiplying the nitrogen contents with a factor of 6.25 (Rhee, 2001). Relative water content (RWC %) was determined according to the method of Barrs and Weatherley (1962) using the following formula:

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fully turgid weight} - \text{dry weight}} \times 100$$

***Determination of chlorophyll and carotenoids contents*:** Acetone extract (80% v/v) of homogenized fresh leaves was used for the determination of chlorophyll and carotenoid contents by the spectrophotometer set at wavelengths of 663, 645, and 480 nm for chlorophyll a, b, and carotenoids contents, respectively (Arnon, 1949).

Determination of NPK: After harvest, 0.1 g oven dried and ground plant sample was digested according to the method as described by Wolf (1982). Nitrogen was determined by Kjeldhal's method (UDK-126D, Velp-Scientifica, Italy) (Jackson, 1962); potassium contents were determined with flame photometer (Model FP-410, Sherwood, UK) (Mason, 1963) and phosphorus contents were determined with spectrophotometer (e-300, Thermo Electron Corporation, USA) using the standard protocols (Quinlan and DeSesa, 1955).

Statistical analysis: The data were analyzed using analysis of variance technique by Statistix 8.1 statistical package (Statistix, USA). Mean values were compared using Tukey's (HSD) test at $p < 0.05$ (Steel *et al.*, 1997).

RESULTS

Salinity stress negatively affected the growth, yield, chlorophyll and ionic contents of maize plants grown under salt-stressed pot conditions. Rhizobial inoculation significantly improved all the attributes at all salinity levels. All rhizobial isolates responded differently at different salinity levels. Moreover, rhizobial consortium performed better than their sole application.

Data (Table 1) showed that salinity decreased the plant height, cob weight, cob length and 100-grain weight of maize and the effect was more at higher salinity levels. Inoculation with different rhizobial strains increased the plant growth and yield at all levels of salinity. However, combination of rhizobial strains (consortium) resulted in more improvement in growth and yield of maize as compared to their individual application of these strains under salinity stress. *Rhizobial consortium* (LMR-5, LMR-10, CRM-7 and CRM-9) promoted the plant height (34%), cob weight (25%), cob length (67%) and 100 grain weight (49%) at 8 dS m^{-1} salinity levels as compared to respective un-inoculated control plant.

The results revealed that salinity stress significantly decreased the relative water content, crude protein, chlorophyll "a" and "b", and carotenoids contents of maize plants when compared with un-inoculated unstressed control (Fig.1-2; Table 2). Rhizobial inoculation significantly improved these attributes at all salinity levels. Multi-strain rhizobial combination improved the relative water content (57%), crude protein (34%), chlorophyll content "a" and "b" (37 and 38%, respectively) and carotenoids contents (46%) at 8 dS m^{-1} salinity levels as compared to respective un-inoculated control plant.

Results regarding N, P and K contents are presented in Table 3. It is clearly evident from the data that the increasing level of salinity caused a decrease in N, P, and K concentrations compared with un-inoculated and unstressed control. Improvement in N, P, and K concentration was improved by the rhizobial inoculation. Different rhizobial isolates behaved differently at different salinity levels. *Rhizobial consortium* gave maximum increase (19, 28 and 42% compared to respective un-inoculated control) in nitrogen percent in straw at original salinity level, 4 and 8 dS m^{-1}, respectively.Further, maximum promotion in nitrogen percent in grain (34%) was observed in plants inoculated with *Rhizobial consortium* at original salinity level. While maximum increase in

Table 1. Growth and yield parameters of maize influenced by rhizobial inoculation under salt stressed pot conditions.

Strains	Plant height (cm)			100-grain weight (g)		
	original	4 dS m^{-1}	8 dS m^{-1}	original	4 dS m^{-1}	8 dS m^{-1}
Control	141.33 c†	113.07 g	96.22 h	23.00 d-g	19.40 h	15.40 k
LMR-5	151.97 b	128.47 d-f	123.48 ef	24.70 cd	22.50 fg	18.97 hi
LMR-10	152.93 b	126.30 d-f	121.02 fg	24.97 c	23.83 c-f	17.67 ij
CRM-7	153.87 b	129.28 de	120.60 fg	24.27 c-e	21.50 g	16.57 jk
CRM-9	155.90 ab	130.65 de	123.92 ef	26.83 b	22.23 fg	19.30 hi
Combination	163.47 a	131.98 d	128.46 d-f	31.80 a	27.57 b	22.90 e-g
LSD		7.9556			1.7332	
	Cob length (cm)			Cob weight (g)		
Control	13.70 f-h	11.54 i	9.01 j	131.40 fg	121.60 ij	114.50 k
LMR-5	16.90 b-d	15.30 d-f	12.73 hi	143.13 cd	144.50 bc	125.60 hi
LMR-10	16.63 b-e	15.83 c-e	13.27 hi	143.13 cd	144.33 bc	119.27 jk
CRM-7	17.37 bc	16.40 c-e	12.67 hi	148.30 ab	138.43 de	123.03 ij
CRM-9	16.63 b-e	16.03 c-e	13.40 gh	142.33 cd	135.70 ef	128.43 gh
Combination	19.93 a	18.30 ab	15.07 e-g	153.03 a	148.17 ab	142.83 cd
LSD		1.7156			4.8917	

†Means sharing similar letters are statistically similar to each other at p=0.05. HSD shows honestly significant difference.

Table 2. Effect of rhizobial inoculation on chlorophyll and carotenoids contents of maize under salt stressed pot conditions.

Strains	Chlorophyll 'a' (mg g^{-1} fresh weight)			Chlorophyll 'b' (mg g^{-1} fresh weight)			Carotenoids contents (mg g^{-1} f.w)		
	original	4 dS m^{-1}	8 dS m^{-1}	original	4 dS m^{-1}	8 dS m^{-1}	original	4 dS m^{-1}	8 dS m^{-1}
Control	2.81 c-e	2.44 f	1.87 h	1.46 ef	1.28 ij	1.04 k	0.49 d-f	0.39 h-j	0.28 k
LMR-5	2.96 ab	2.71 e	2.15 g	1.54 cd	1.47 d-f	1.27 ij	0.54 b-d	0.43 f-h	0.37 ij
LMR-10	2.95 a-c	2.72 e	2.27 g	1.58 a-c	1.53 c-e	1.26 j	0.55 bc	0.49 c-e	0.33 jk
CRM-7	2.94 a-c	2.78 de	2.23 g	1.53 c-e	1.51 c-f	1.34 hi	0.55 b	0.46 e-g	0.34 j
CRM-9	2.87 b-d	2.78 de	2.26 g	1.56 bc	1.55 bc	1.37 gh	0.55 bc	0.45 e-h	0.35 j
Combination	3.08 a	2.96 ab	2.56 f	1.65 a	1.62 ab	1.43 fg	0.63 a	0.54 b-d	0.41 g-i
HSD		0.1428			0.0783			0.0584	

†Means sharing similar letters are statistically similar to each other at p=0.05. HSD shows honestly significant difference.

Table 3. Effect of rhizobial inoculation on nitrogen, phosphorus and potassium concentration in straw and grain of maize under salt stressed pot conditions.

Strains	Nitrogen in straw (%)			Nitrogen in grain (%)			Phosphorus in maize straw (%)		
	original	4 dS m^{-1}	8 dS m^{-1}	original	4 dS m^{-1}	8 dS m^{-1}	original	4 dS m^{-1}	8 dS m^{-1}
Control	1.05 e†	0.95 f	0.59 j	1.44 f	1.55 e	1.30 g	0.23 de	0.18 g	0.11 i
LMR-5	1.15 cd	1.13 d	0.68 i	1.73 cd	1.66 d	1.38 f	0.26 bc	0.20 fg	0.12 hi
LMR-10	1.18 bc	1.11 d	0.76 h	1.74 c	1.74 c	1.46 f	0.28 b	0.22 ef	0.12 i
CRM-7	1.15 cd	1.15 cd	0.77 h	1.75 c	1.79 bc	1.44 f	0.26 bc	0.22 ef	0.13 hi
CRM-9	1.21 b	1.14 d	0.76 h	1.75 c	1.79 bc	1.55 e	0.26 bc	0.22 d-f	0.13 hi
Combination	1.25 a	1.22 ab	0.84 g	1.99 b	1.86 a	1.74 c	0.31 a	0.24 cd	0.15 h
HSD		0.0394			0.0775			0.0263	
	Phosphorus in grain (%)			Potassium ion in leaves (%)			Potassium in grain (%)		
Control	0.45 bc	0.35 cd	0.22 e	2.59 ef	2.27 g	1.94 h	1.56 c-f	1.49 d-g	1.20 h
LMR-5	0.51 ab	0.47 b	0.24 de	2.78 bc	2.56 ef	2.34 g	1.63 b-e	1.64 b-d	1.46 fg
LMR-10	0.54 ab	0.45 bc	0.25 de	2.83 ab	2.62 de	2.53 f	1.74 b	1.67 bc	1.51 c-g
CRM-7	0.48 ab	0.49 ab	0.24 de	2.78 bc	2.57 ef	2.55 ef	1.75 b	1.66 bc	1.36 gh
CRM-9	0.49 ab	0.46 bc	0.26 de	2.77 bc	2.56 ef	2.59 ef	1.77 b	1.65 bc	1.47 e-g
Combination	0.59 a	0.47 b	0.3 de	2.87 a	2.82 ab	2.7 cd	1.96 a	1.76 b	1.63 b-e
HSD		0.1136			0.0767			0.1601	

†Means sharing similar letters are statistically similar to each other at p=0.05. HSD shows honestly significant difference.

phosphorus percent in grain (36%), potassium ion concentration in leaves (39%) and grain (36%) was recorded in plants treated with multi-strain rhizobial combination at 8 dS m^{-1} when compared with plants grown at same salinity level without inoculation.were identified by Biolog® as *Rhizobium leguminosarum* (LMR5 and LMR10) and *Mesorhizobium ciceri* (CMR7 and CMR9).

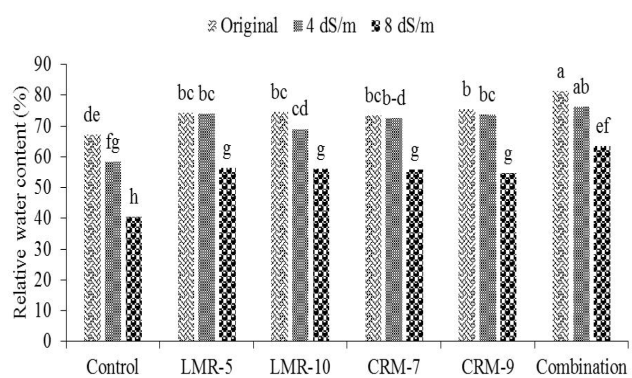

Figure 1. Effect of single and multi-strain inoculation on relative water contents (%) of maize under salt-stressed pot conditions (average of three replicates).

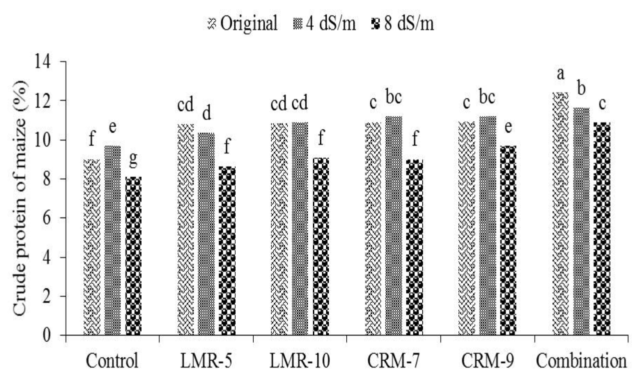

Figure 2. Effect of single and multi-strain inoculation on crude protein (%) of maize under salt-stressed pot conditions (average of three replicates).
Bars sharing same letters are statistically at par at 5% level of probability.

Further confirmation was carried out through re-inoculation of lentil and chickpea seedlings with these strains. When lentil and chickpea seedlings were re-inoculated with their respective strains, they produced nodules in seedlings under axenic conditions. So, it was confirmed that these are lentil and chickpea nodulating strains i.e. *Rhizobium leguminosarum* and *Mesorhizobium ciceri,* respectively.
ncentrations compared with un-inoculated and unstressed
Identification of rhizobial isolates: The selected strains

DISCUSSION

Salinity negatively affects the growth, physiology and ionic balance of crop plants and is one of the major contributing factors to lower yield. The use of bacteria for improving crop growth and productivity under stress environments has been well documented Ahmad *et al.*, 2011, 2012, 2013;. Results of the present study revealed that salinity stress negatively affected all the attributes of maize plants. However, inoculation with rhizobial strains isolated from the root nodule of chickpea and lentil significantly reduced the drastic effects of salinity. Chickpea isolates showed more promising results than lentil isolates. Moreover, multi-strain rhizobial consortium of all four strains gave better improvement in growth, yield and quality parameters of maize than their individual application. The improvement in growth, yield, chlorophyll and nutrient contents of maize in response to rhizobial inoculation might be attributed to production of auxins (Zahir *et al.*, 2010), exopolysaccharides (Zafar-ul-Hye *et al.*, 2013; Hussain *et al.*, 2014a), phosphate solubilization (Afzal and Bano, 2008), siderophore (Huang and Erickson, 2007) the solubilization, mobilization, availability and uptake of nitrogen, phosphorus and potassium by the plants (Pal *et al.*, 2000; Zahir *et al.*, 2009). Moreover, inoculation also induces systemic resistance in plants against viruses and other harmful pathogens (Huang and Erickson, 2007) and improved root respiration (Volpin and Phillips, 1998) by improving plant physiology. Therefore, improvement in growth, yield and other attributes might be due to possession of more than one mechanism of action other than N_2 fixation.

In the present study, it was observed that increase in salinity level caused a significant reduction in the plant height, cob length, cob weight and 100-grain weight. Reduction in growth and yield of crops under salinity stress has extensively been reported in the literature (Zahir *et al.*, 2009; Bano and Fatima, 2009; Naz *et al.*, 2013). Salinity stress can result in osmotic stress and toxicity of specific ions in plants causing a decrease in growth and ultimately in yield . So, reduction in growth and yield of maize observed in present experiment might be attributed to the high salt accumulation in leaves. Salts may have dehydrated the cells by accumulating in the apoplast, they may have suppressed enzyme synthesis involved in carbohydrate metabolism by storing in the cytoplasm, or they may directly have caused toxic effects on photosynthesis process by amassing in the chloroplast (Munns and Tester, 2008). However, rhizobial inoculation significantly improved growth and yield parameters compared to unstressed maize plants. Improvement in growth and yield parameters under salt stressed conditions has been reported in previous studies (Zahir *et al.*, 2009; Naz *et al.*, 2013). Moreover, plant height might be attributed to the production of auxins by applied rhizobia (Zahir *et al.*, 2010) that might have enhanced the cell division and cell elongation in the seedlings (Perrot-Rechenmann, 2010) leading to improved height of inoculated plants. Improvement in grain yield and yield related parameters due to consortium application might be due to the increased combined microbial activity that might have increased the photosynthesis and physiology of inoculated plants (Bano and Fatima, 2009) leading to increased yield.

Leaf water content depicts the capability of plants to maintain water status, so it is often used as criterion to study the impact of salinity on plants grown under salt stress.. In the study, decrease in relative water content was observed in plants subjected to salt stress. Many other researchers also witnessed the decrease in relative water content of plants exposed to salinity (Srivastava *et al.*, 1998; Kaya *et al.*, 2003; Ahmad *et al.*, 2013). Reduction in relative water content due to salinity might be due to the little absorption of water under increased saline conditions (Heidari *et al.*, 2011; Amirjani, 2011). It might also be possible that high salts concentration have slackened the sap flux flow which have lowered the root hydraulic conductivity with a possible consequence of reduced relative water content (Vysotskaya *et al.*, 2010). Improvement in RWC in response to single/multi-strain inoculation has been reported in many studies (Bano and Fatima, 2009; Ahmad *et al.*, 2012).

Findings of present study also indicated the increase in RWC by rhizobial inoculation. Better results were observed with multi-strain rhizobial inoculation compared to single strain inoculation. Combined activity might have benefitted the plants with additional rhizobial hormones that might have lessened the effect of salinity. Consortium might have enhanced the root hairs and volume (Glick, 2012) that engorged the roots with more water uptake under exacting situation of salinity (Yu *et al.*, 2007).

Salinity stress caused a significant reduction in protein content in the present study. This decrease may be correlated with high induction of cellular rise of destructive reactive oxygen species, which can destroy membrane vital components such as lipids, nucleic acids and proteins (Mittler, 2002). However, inoculation improved the protein content of maize grain which could be due to lessened effect of salinity by rhizobial strains (Mohamed and Gomaa, 2012). Compared to single strain inoculated plants, consortium significantly increased protein content of grain that might be attributed to the enhanced N uptake of plant which is an important constituent of protein, lessened effect of salinity and increase in antioxidant activity that saved the damaging of protein and amino acid. Further, consortia have multi-traits compared to single strain which resulted in better improvement of the growth of maize plants.

Data regarding chlorophyll a, b, and carotenoid contents, obviously showed that salinity stress significantly caused a reduction in chlorophyll a, b, and carotenoid contents compared to unstressed control (Bano and Fatima, 2009). Decrease in chlorophyll content might be due to high osmotic stress that might have caused the reduction in the uptake and assimilation of essential minerals (Soliman *et al.*, 2012). Deficiency of these minerals especially N, may cause inhibition in the formation of chlorophyll molecules under increased salinity stress (Huang *et al.*, 2004). Moreover, high concentration of salts in the leaves might have caused overproduction of reactive oxygen species (ROS) and forced the chloroplast to destroy all protein component of chloroplast (Muneer *et al.*, 2014). However, rhizobial inoculation resulted in improved chlorophyll contents a, b, and carotenoids compared to respective un-inoculated controls. Among all the inoculation treatments, multi-strain rhizobial combination exhibited significant improvement in chlorophyll “a”, “b”, and carotenoid contents compared to other rhizobial isolates and un-inoculated control. Isolates of chickpea gave better performance compared to isolates of lentil. Similarly, rhizobium inoculation alone and in combination with *Pseudomonas* improved the chlorophyll contents a, b and carotenoids of maize subjected to different salinity levels (Bano and Fatima, 2009). Our results are also supported by findings of Hussain *et al.* (2014b) who investigated the effect of rhizobial inoculation on maize under stressed conditions and found the variable response of maize plants to isolates of different species of rhizobium. More chlorophyll content due to the application of multi-strain rhizobial consortium might be due to enhanced solubilization and mobilization of minerals (Pal *et al.*, 2000; Zahir *et al.*, 2009) especially N, which is an essential constituent of chlorophyll (Swan, 1971), that ultimately resulted in the high chlorophyll contents (Bojovic and Markovic, 2009).

Salinity stress resulted in the significant decrease in N, P and K contents in straw and in grain of un-inoculated maize plants compared to un-inoculated unstressed control. Decrease in N, P and K content in un-inoculated plants might be due to the tendency of plants to take up more Na^+ causing the reduction in K^+ accumulation under increased salt stress. The Na^+ ions can compete with K^+ for binding sites critical for various cellular functions. While, P ions form complex molecules with Ca, Mg and Zn ions under high salt concentration and become unavailable to plants. Soil salinity also inhibits N acquisition and utilization by plants through affecting different processes of N-metabolism, like nitrate (NO_3) uptake and reduction, and amino acid synthesis (Soliman *et al.*, 2012). Moreover, the presence of toxic ions such as Na^+ and Cl^- in the root zone can decrease the uptake of nutrients (such as K^+ and NO_3) by binding with transporters in the root plasma membrane (Tester and Davenport, 2003). However, significant increase ($P<0.05$) in the N, P and K concentration in straw and grain of maize plant was observed after inoculation with rhizobial isolates either alone or in a combination compared to un-inoculated control under salt stressed condition. It might be attributed to the expression of multi-trait consortium activity that enhanced production of organic acids and thus solubilization of phosphates and better root system for acquisition of nutrients (Mehboob *et al.*, 2011).

In the study, it is observed that rhizobial consortium performed better than their sole application. It might be due to the combined action of their variable traits that might have accelerated their growth and colonization by allowing them to compete with inefficient native soil bacteria (Yadav, 2006). Further, variable response of a host plant (maize) to the isolates of different species was observed in the previous results (Mehboob *et al.,* 2012; Hussain *et al.*, 2014b). Our results also showed the superiority of the isolates of *Mesorhizobium ciceri* (CRM-7 and CRM-9) over the isolates of *Rhizobium leguminosarum* (LRM-5 and LRM-10). It is well known that capability of rhizobia for producing beneficial secretions, hormones and enzymes, either quantitatively or qualitatively, varies from species to species and even strain to strain (Mehboob *et al.*, 2008). It might be possible that some isolates have more potential to produce beneficial hormones and secretions in larger quantities compared to other which enhanced their ability for improving the growth and yield of maize.

***Conclusion*:** Improvement in the growth and yield of salt-stressed maize can be obtained by using rhizobia as bioinoculant. Chickpea rhizobial isolates are more efficient than lentil rhizobial isolates. Further, use of multi-strain rhizobial consortium could be a better strategy in improving the growth and yield of crops. Moreover, selected isolates need to be evaluated under salt-affected field conditions for improving growth and yield of maize before their recommendation for large scale application.

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