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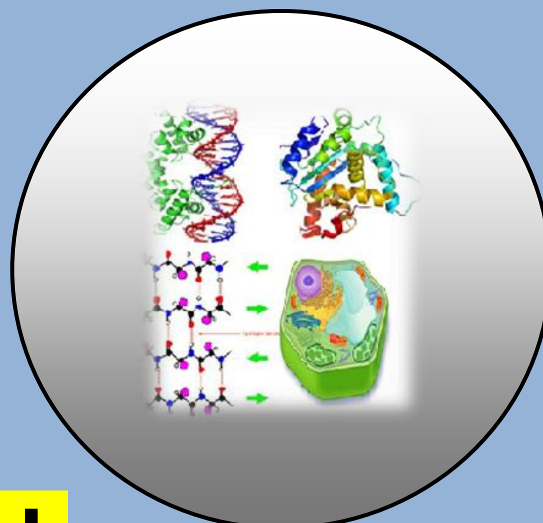

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RESEARCH PAPER

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Screening of Salt Tolerant Rhizobia for Improving Growth and Nodulation of Chickpea (*Cicer arietinum*) under Arid Soil Conditions of Uzbekistan

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ABSTRACT

The growth, development and symbiotic performance of chickpea are strongly influenced by abiotic stress factors such as drought, salinity and nutrient deficiencies. The selection for salt tolerant rhizobia may have an important effect on the successful Rhizobium-legume associations under stress conditions. The aim of this study was to screen for Mesorhizobium strains isolated from chickpea for their ability to tolerate salt stress and improve growth and symbiotic performance of chickpea under saline soil conditions. The results of this study showed that salt tolerant strains stimulated root, shoot growth and nodulation of chickpea affected by salt stress. The shoot length increased by 52%, root length by 43%, shoot dry weight by 57%, and root dry weight by 82%. Inoculation of plants with strains Mesorhizobium sp. Mc4 and Mesorhizobium sp. Mc9 significantly increased shoot, root dry matter, and nodule number by 17, 12, and 200% above the uninoculated plants, respectively. Inoculation significantly increased the pod number by 13 and yield 18% as compared to control plant. This study indicates that screening for salt tolerant rhizobial strains are essential to improve symbiotic performance of chickpea under salt stress condition.
Key words: Chickpea, Saline Soil, Rhizobia, Nodulation and Root Colonization.

INTRODUCTION

The abiotic stresses such as drought, salinity are one of the main consequences of climate change and remains as a big challenge while addressing the problem of food insecurity, hunger and malnutrition (Parvaiz and Satyawati 2008; Tuberosa 2012). Unsustainable practices of irrigation agriculture and crop residue removal in connection with a one-sided orientation of crop production on cotton monocultures have led to wide-spread soil salinity in Uzbekistan (Egamberdieva et al. 2010, 2013). The main crops grown in Uzbekistan are cotton and winter wheat (occupying 80% of the total irrigated area) but crop yields have been decreasing due to worsening soil conditions. Resource-conserving technologies, based on diversified crop rotation, are likely to provide economic benefits to the farmers that may be large enough for a fast-track adoption of sustainable land management measures. Legume cropping systems that increase carbon sequestration (CS) and concurrently enhance plant productivity and prevent erosion and desertification are of major interest in many countries of the world (Abberton 2010).Chickpea *(Cicer arietinum* L.) is a major food legume crop produced worldwide and considered as an important source of protein in many countries and also used in crop rotation to replenish nitrogen deficient soil instead of using chemicals or fertilizer (Namvar and Sharifi 2011; Egamberdieva et al. 2014). Chickpea is known as sensitive plant abiotic stresses, and together with heat and water stresses, high salinity is responsible for considerable crop losses of chickpea yield, indicating needs to increase crop yield (Molina et al. 2011). Previous studies have shown that salinity and drought stress led to a significant decline in plant biomass accumulation (root and shoot), nodule development, and nitrogenase activity in chickpea (Garg and Baher 2013; Egamberdieva et al. 2013; Hashem et al. 2014). The symbiotic interactions of chickpea and *Mesorhizobiumciceri* have been known to be susceptible to salinity (Velagaleti et al. 2002). An explanation for the reduction in symbiotic legume growth might be that the salt stress causes a failure of the infection and nodulation process. For example, according to Bouhmouch et al. (2005), salt reduces the growth of roots, root tips and root hairs, thereby decreasing sites for potential rhizobial infection and further nodule development. In other study Slattery et al. (2004) observed negative effect of low (4.5) and high (8.0) soil pH and temperature on rhizobial population in soil. The salt tolerance abilities of rhizobia may have an important effect on the successful Rhizobium-legume associations under stress conditions. In the present study, *Mesorhizobium sp.* strainsisolated from chickpea were screened for their salt tolerance abilities and evaluated for symbiotic performance with chickpea under arid saline conditions.

MATERIAL AND METHODS

Plant and microorganisms

Seeds of the chickpea “Uzbekistan” were obtained from International Centre for Agricultural Research in the Dry Areas (ICARDA). Bacterial strains used are listed in Table 1. All bacterial isolates were obtained from the Culture Collection of the National University of Uzbekistan(CCNUU). The strains were previously isolated from the rhizosphere of chickpea grown in salinated soil of Uzbekistan.

Salt tolerance of bacterial isolates

In order to determine the optimum salt concentration for growth, bacterial strains were cultured in YEM medium supplemented with different amounts of NaCl: 2%, 3%, 4%, and 5% NaCl (w/v). The growth rate of bacteria isolates was determined with spectrophotometer after 24, 48, 72 hours.

Germination of seeds

The seeds were first sorted to eliminate broken, small seeds and then they were surface-sterilized in 10% v/v NaOCl for 1 min and rinsed five times with sterile, distilled water. Surface-sterilized seeds were transferred on paper tissue towels soaked in 0.5 mM $CaSO_4$ and germinated for seven days in a dark room at 25°C.

Plant growth promotion in pots

For the seed inoculation, *Mesorhizobium* strains were grown overnight in TY broth. One ml of bacterial culture was pelleted by centrifugation and cell pellets were washed with 1 ml phosphate buffered saline (PBS; 20 mM sodium phosphate, 150 mMNaCl, pH 7.4) and re-suspended into PBS. The suspension used for the inoculation was adjusted to the final concentration of approximately 10^7 CFU mL^1. Uniform seedlings were first placed with sterile forceps into bacterial suspension for 15 minutes and were then transplanted into pots filled with salinated soil (500 g each pot). Two chickpea seedlings were transplanted into each pot, but later one seedling was removed. Soil for pot experiment were sampled from an irrigated agricultural site located in Syrdarya Province (41°00'N, 64°00'E,) in north-eastern Uzbekistan. According to the WRB-FAO (2006) classification, the soils of selected fields were identified as Calcisol (silt loam serozem). The surface soil horizon was calcareous saline whereas the deeper soil horizons were only mildly alkaline (Egamberdiyeva *et al.* 2007). In these soils, cotton has been grown for the last 50 to 60 years under a continuous monoculture production system and under flood irrigation without proper drainage facilities but using a natural flow system. In general, high concentration of Ca^{2+}, K^+, and Na^+ are associated with $CO_3^{2-}$and Cl^- ions, reflecting the dominance of carbonates and chlorides in saline soil. On average, the two kinds of soils was taken contained 42 ± 9 g of sand kg^{-1}, 708 ± 12 g of silt kg^{-1}, and 250 ± 13 g clay kg^{-1} (Egamberdieva and Kucharova 2009). The main chemical soil properties are: organic matter 0.79 %; Ct 2.39 %; Nt 0.07 %; CO_3^{2-}-C 1.59%, Ca^{2+} 54.3 g/kg; Mg^{2+} 26.1 g/kg; K^+ 6.7 g/kg; P 1.2 g/kg; Cl^- 0.1 g/kg; Na^+ 0.8 g/kg; pH 8.0. The high concentration of Ca^{2+}, K^+, and Na^+ are associated with $CO_3^{2-}$and Cl^- reflecting the dominance of carbonate and chloride in saline soil. The pot experiment had two treatments: seeds without bacterial inoculation and seeds inoculated with bacteria. Plants were grown at 20 - 26°C during the day and 17 - 18°C at night and after six weeks the shoot and root length and dry matter of chickpea were measured.

Survival of bacterial strains

Spontaneous and stable rifampicin (200 µg/ml) resistant mutants of the wild type strains were used for the colonization studies. Mutants of *Mesorhizobium sp.* strainsthat were marked with antibiotic resistance were obtained by plating the parental strain onto TY agar amended with 200 µg/ml rifampicin. After incubation, isolates were selected based on similarities in colony morphology and growth rate with the parent strain, and were recultured on medium containing rifampicin to ensure stability of the antibiotic resistance marker.

Plants were grown in plastic pots containing salinated soil. Chickpea seedlings were coated with bacteria by dipping the seedlings in bacterial suspensions that resulted in 10^8 CFU ml^{-1} seeds. Plants were grown at 24 - 26°C during the day and 16 - 18°C at night and after two months plants were harvested and the adhering soil was removed from chickpea roots and 1 g roots were shaken in 9 ml sterile PBS.

The resulting suspensions were evaluated for colony forming units (CFU) according to the dilution-plate method in TY agar with addition of 200 µg/ml rifampicin. After incubation for 2-3 days at 28°C the reisolated, rifampicin resistant strains were identified for their colony characteristics.

Statistical procedures

Data were tested for statistical significance using the analysis of variance package included in Microsoft Excel 97. Comparisons were done using Student's *t*-test. Mean comparisons were conducted using a least significant difference (LSD) test (P=0.05).

RESULTS AND DISCUSSION

Forty bacterial strains of *Mesorhizobium* sp. previously isolated from chickpea root were screened for their salt tolerance abilities. Most of strains where able to growth under 3% NaCl, and only 20 strains were able to tolerate up to 5% NaCl (data not shown). Those 20 strains where taken for further studies on their effect on growth and symbiotic performance of chickpea under salt stress condition. The results of study showed that salt tolerant rhizobial strains stimulated root, shoot growth and nodulation of chickpea affected by salt stress (Table 1).

Table 1. The effect of *Mesorhizobium* sp. strains on the root, shoot growth and nodule number of chickpea grown under saline soil condition.

Bacterial strains	Nodules number	Shoot length (cm)	Root length (cm)	Shoot dry mass (g)	Root dry mass (g)
Control	0	17.7	13.4	0.213	0.083
Mc-1	12	18.9	14.5	0.224	0.095
Mc-2	11	19.2	14.2	0.236	0.093
Mc-3	11	18.6	13.9	0.223	0.092
Mc-4	22	26.3*	19.2*	0.336*	0.152*
Mc-5	12	19.5	14.9	0.233	0.094
Mc-6	15	22.3*	15.7*	0.253*	0.116*
Mc-7	13	19.3	14.9	0.236	0.095
Mc-8	10	18.2	13.9	0.217	0.089
Mc-9	16	21.8*	15.4*	0.244*	0.115*
Mc-10	14	19.9	15.3	0.234	0.097
Mc-11	12	18.5	14.8	0.223	0.092
Mc-12	13	20.3*	15.1	0.236	0.093
Mc-13	10	18.7	14.9	0.221	0.092
Mc-14	12	19.6	14.7	0.224	0.095
Mc-15	9	18.1	13.7	0.218	0.092
Mc-16	13	20.5*	14.8	0.235	0.089
Mc-17	11	18.6	14.3	0.236*	0.095
Mc-18	12	19.7	14.5	0.233	0.094
Mc-19	9	18.3	14.1	0.221	0.092
Mc-20	8	18.6	14.2	0.235	0.093

* Significantly different from untreated control plants at P<0.05

The shoot length increased by 52%, root length by 43%, shoot dry weight by 57%, and root dry weight by 82%. According Mhadhbi et al. (2004) and Sadiki and Rabih (2001) yield potential of chickpea depends on the rhizobia association and plant genotype which together influencing the symbiotic performance. Selected salt tolerant rhizobia had good symbiotic association with chickpea variety Uzbekistan, which previously selected as salt tolerant cultivar (Egamberdieva et al. 2014). Bano et al. (2010) reported that *Bradyrhizobium japonicum* adapted to drought stress was effective in the root-nodule symbiosis and also alleviated decreased growth and yield of chickpea imposed by drought stress. The three best, effective strains Mc4, Mc6, and Mc9 were selected for field experiments. Inoculation of plants with strains Mc4 and Mc9 significantly increased shoot, root dry matter, and nodule number by 17, 12, and 200% above the uninoculated plants, respectively. Inoculation significantly increased the pod number and yield by 13 and 18% as compared to control plant (Table 2).

Similar observations reported in other studies where inoculation of chickpea with rhizobia increased plant growth, ground dry matter, number of pods, seed yield, and nitrogen fixation under various climatic conditions (Karadavut and Ozdemir 2001; Fatima et al. 2008).

Table 2. The effect selected salt tolerant effective *Mesorhizobium* sp. on the growth and yield of chickpea under field condition.

Treatments	Nodule number	Shoot dry weight, g/plant	Root dry weight, g/plant	Pods number	Yield (dt/ha)
Control	22±4.0	21.0±0.6	1.7±0.2	36±5.2	17.84±3.1
Mc-4	67±5.2*	24.4±2.2*	1.9±0.4	41±5.3*	21.19±2.9*
Mc-6	38±4.2*	23.4±2.3	1.9±0.4	39±5.1	19.21±3.3
Mc-9	41±4.1*	24.6±0.9*	1.8±0.2	39±4.4	20.17±2.1*

* Significantly different from untreated control plants at $P<0.05$

The colonization of root associated beneficial microbes in the rhizosphere is important for their beneficial effect on plant growth, especially under stress soil conditions (Devliegher et al. 1995; Egamberdieva 2010, 2011). It has been also observed that the survival of rhizobia in the plant root and soil is affected by nutrient deficiency, salinity, drought, acidity, and soil temperature (Slattery et al. 2001). In earlier report Singleton et al. (1982) reported that salinity inhibited survival and proliferation of *Rhizobium* spp. in the soil and rhizosphere, and infection process. We have studied survival of salt tolerant Mc4, Mc6 and Mc9 strains in the rhizosphere of chickpea grown under saline soil condition. The results showed that rifampicin resistant mutants obtained from bacterial strains was able to survive in the root of chickpea and the root colonization was $5.2 \times 10^3 \pm 0.2$ for strain Mc4, $3.6 \times 10^3 \pm 0.1$ for strain Mc6 and $6.5 \times 10^3 \pm 0.4$ (CFU/g of fresh root) for strain Mc9. This study indicates that screening for salt tolerant rhizobial strains are essential to improve symbiotic performance of chickpea under salt stress condition. They are able to stimulate plant growth, alleviate salt stress and survive in the rhizosphere of plant under extreme soil conditions.

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