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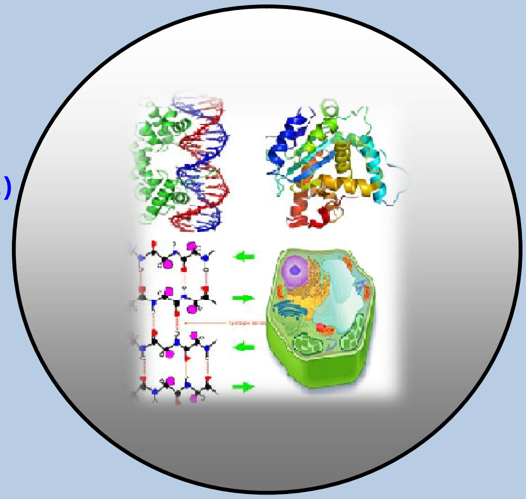

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RESEARCH PAPER

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Growth and Symbiotic Performance of Chickpea (*Cicer arietinum*) Cultivars under Saline Soil Conditions

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ABSTRACT

The present study was conducted to investigate growth and symbiotic performance of selected salt tolerant chickpea (Cicer arietinum) cultivars under arid saline soil conditions. Plants were grown in saline soil of Syrdarya province, Uzbekistan. The results showed that the growth and nodulation of chickpea cultivars Uzbekiston-32, Xalima, Miroz, Flip 1-33 and CIEW 45 were much better under saline conditions compared other chickpea cultivars. Inoculation of salt tolerant chickpea cultivars with M. ciceri IC53 significantly increased numbers of nodules on the roots (300%), shoot and root dry weight (27%), pod numbers (28%) and yield (23%). The above results revealed that for achieving the highest symbiotic effectiveness under salinity conditions plant cultivars have to be taken into account. It could be suggested that cultivation of salt tolerant cultivars with its symbiotic partners could be an effective selection technology to overcome the problem of soil salinity.
Key words: Chickpea, Cultivars, Salinity, Mezorhizobium ciceri, and Nodulation.

INTRODUCTION

Salinization is recognized as the main threat to environmental resources in many countries and affects almost 1 billion hectares worldwide (Vincent, et al. 2006; FAO Land and Nutrition Management Service, 2008).

Uzbekistan, located in Central Asia, is an example of a country in which soil salinity is a major concern in that it results in degradation of agricultural land (Shirokova, et al. 2000; Egamberdiyeva, et al. 2007). Salinization of irrigated lands resulting from over irrigation and poor drainage as well as the wind transport of salts from the exposed sea bed of Aral Sea is a major constraint to crop production in the region. Novel agricultural technologies are required to improve food production in saline and dry soils (Wherheim and Martius, 2008; Egamberdieva and Lugtenberg, 2014).

Most legumes are sensitive to salinity. Soil salinity reduces growth, nodulation, and nitrogenase activity of several legumes such as soybean (*Glycine max*) (Jabborova et al., 2013), common bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*) (Rabie, et al. 2005) and goats rue (Egamberdieva et al., 2013). Further, salt reduces the growth of roots, and root hairs, thereby decreasing sites for potential rhizobial infection and further nodule development (Bouhmouch, et al. 2005).

Chickpea *(Cicer arietinum* L.) is a major food legume crop and an important source of protein in many countries including Uzbekistan with cultivation of up to 25,000 hectares and its production is limited by soil salinization. Chickpea is known as salt sensitive plant and several reports indicated that germination and seedling growth of chickpea is reduced in saline soils with varying responses due to cultivars (Dua, 1992; Gandour, 2002; Al-Mutawa, 2003). Elsheikh and Wood (1990) reported that nodulation and N_2 fixation in chickpea was more sensitive to salinity than plant growth. Other studies also indicated disturbance of interactions between *Mesorhizobium ciceri* and chickpea by salinity stress (Rao and Sharma, 1995; Rao, et al. 2002; Tejera, et al. 2006). The symbiotic performance of chickpea under salt stress has been used as an indicator to select chickpea cultivars grown in Indian soils (Garg and Singla, 2004). Authors suggest that in salt-affected soils salt tolerant cultivars have more efficient nodulation and support higher rates of symbiotic nitrogen fixation than the sensitive cultivars. In this context, the selection of salt tolerant chickpea cultivars based on symbiotic performance will be helpful to enhance the productivity of the chickpea in areas adversely affected by salt stress. The present study was conducted to investigate growth and symbiotic performance of selected salt tolerant chickpea cultivars in search for the best *Rhizobium*/chickpea combinations for arid saline soil conditions.

MATERIAL AND METHODS

Plant and Microorganisms

The experiments were carried out in the laboratory of the Department of Microbiology and Biotechnology, Faculty of Biology and Soil Sciences, National University of Uzbekistan. Twenty nine chickpea (*Cicer arietinum* L.) cultivars, five locally grown cultivars, two cultivars from Tajikistan and from International Centre for Agricultural Research in the Dry Areas (ICARDA) were used in the study (Table 1). Salt tolerant *Mezorhizobium ciceri* strain IC53 was obtained from the culture collection of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). The strain was grown on tryptone yeast extract agar (TY) at 28°C.

Plant growth in pots

The soil used for pot experiments was selected from a deep tillage (0-40 cm) irrigated agricultural field affected by salinity from the Sayhunobod district ($41^0$00'N, $64^0$00'E), Syr-Darya Province, in the North-East of Uzbekistan. The field had an EC value of 6,6 dS m^{-1} soil. The soil surface horizon was calcareous saline, and the deeper levels are mild alkaline in nature.

Table 1. Shoot and root dry weight and nodule number recorded five weeks after see seeding of chickpea genotypes grown in saline soil.

Name	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Nodule number (plant^{-1})
Jaxongir	0.497	0.437	1.8
Uzbekiston-32	0.710	0.773	9.5
Lazzat	0.453	0.520	4.3
Zimistoni	0.327	0.293	1.3
Xalima	0.690	0.623	13.8
Miroz	0.653	0.747	5.3
Muqtadir	0.607	0.610	0.0
Xisor-32	0.313	0.310	0.8
Sino	0.430	0.617	2.0
Flip 1-01	0.550	0.550	0.0
Flip 1-04	0.507	0.463	4.3
Flip 1-05	0.613	0.587	2.5
Flip 1-19	0.293	0.343	0.3
Flip 1-21	0.567	0.703	11.5
Flip 1-22	0.517	0.473	6.3
Flip 1-29	0.377	0.293	2.8
Flip 1-31	0.427	0.460	5.5
Flip 1-33	0.640	0.747	12.8
Flip 03-102c	0.563	0.443	13.3
CIEW-45	0.650	0.567	16.8
Flip 05-69 c	0.343	0.310	0.0
Flip 03-74c	0.297	0.290	0.0
Flip 06-102c	0.370	0.300	4.5
Flip 06-66	0.477	0.357	9.8
Flip 05-65	0.463	0.503	0.5
Flip 06-124c	0.270	0.273	0.0
Flip 06-80c	0.453	0.497	12.0
Flip 03-27c	0.367	0.320	2.0
Flip 06-155c	0.493	0.657	0.0
Mean	0.480	0.485	4.9
LSD (5%)	0.0705	0.0692	1.99
CV (%)	10	10	29

The soil contained 43±9 g sand kg^{-1}, 708±12 g silt kg^{-1}, and 25±13 g clay kg^{-1}. A high concentration of Ca, K, and Na associated with CO_3 and Cl reflects a dominance of carbonate- and chloride-associated salts. The salts that moved towards the surface evidently have higher Na, CO_3, and Cl contents, thereby increasing the salinity of the soil. The organic matter content of the soil was 0.69 %; total C, 2.5%; total N, 0.09 %; Ca, 63.5 g/kg; Mg, 20.7 g/kg; K, 6.2 g/kg; P, 1.2 g/kg; Cl, 0.1 g/kg; Na, 0.7 g/kg, and the pH is 8.0.

Chickpea seeds were sorted to eliminate broken and small seeds. They were surface-sterilized for 5 min with 1% sodium hypochlorite solution followed by 70% ethanol for 3 min, and rinsed five times with sterile distilled water. One seed was sown per plastic pot (15 cm diameter; 20 cm deep), each containing 500 g of saline soil, at a depth of approximately 1.5 cm. Each treatment contained of twelve plants. The plants were grown under open natural conditions at 28-32°C and were watered when necessary. After six weeks of growth the shoot, root lengths, dry weight and nodulation were determined.

Table 2. Analysis of variance for various traits recorded on seven chickpea genotypes inoculated with *M. ciceri* IC53 (The study was conducted at Experimental station of the Tashkent State University of Agriculture in 2012).

Source of variation		Yield		No. of pod		Node number		RDW[a]		SDW[b]	
Inoculation	1	10025.9	**	1104.06	**	14833.73	**	3.30057	**	424.301	**
Rep/Inoculation	8	20.9		7.46		29.5		0.04691		2.92	
Genotype	6	928.48	**	1590.27	**	423.36	**	1.78233	**	109.162	**
Genotype*Inoculation	6	33.87		16.92		496.23	**	0.03296		6.43	
Error	48	17.46		14.11		10.91		0.08772		3.565	
CV (%)		3.1		8.5		10.6		12.0		7	

** Significant at 0.01 probability level.

[a] Root dry weight

[b] Shoot dry weight

Field experiments

The field trial was conducted at the Experimental Station of Tashkent State University of Agriculture in April and July 2012. The climate of the area is continental with an annual average rainfall of 200±36 mm and more than 90 percent of the total rain falling between October and May. The average minimum monthly air temperature is 0°C in January, the maximum of 37°C in July, and the soil temperature ranges between -2 to 35°C. The average highest relative humidity is slightly more than 80% in January and the minimum is less than 45% in June. The soil in the experimental field had an EC value of 560 mS m^{-1}. It was characterized as calcareous serozem with 2.4% organic matter, N 0.1%, P 1.34%, K 7.1%. The pH is 7.8. The experimental site was divided in plots, each 5 m by 2 m (10 m^2) containing four rows planted 0.5 m apart. Seven chickpea cultivars (FLIP 06-80, CIEN-45, FLIP 03-102, FLIP 06-66, Uzbekiston – 32, FLIP 1-33, Xalima) which showed good symbiotic performance in pot experiments with saline soil were chosen for field experiments . The experiment was arranged in a randomized block design with six replicates. No fertilization was applied to the soil. The two treatments were seeds inoculated with *M. ciceri* strain IC53, and uninoculated control. Bacterial inoculant *M. ciceri* was grown overnight in TY broth.

One ml of culture was pelleted by centrifugation and the supernatant was discarded. Cell pellets were washed with 1 ml phosphate buffered saline (PBS, 20 mM sodium phosphate, 150 mM NaCl, pH 7.4) and suspended in PBS. The bacterial suspension was added aseptically to trays containing 50 g of peat and mixed. The population of bacteria in formulation was checked, by plating dilutions on the corresponding TY medium, which was approximately 10^8 CFU g^{-1} peat. For uninoculated control, equal volume PBS was added to peat.

The seeds of chickpea were surface-sterilized by immersion for 5 min in concentrated sulphuric acid followed by 3 min in 70% ethanol, and rinsed five times with sterile, distilled water. Sterilised seeds were coated with peat (5g peat for 1 kg seeds) inocula with *M. ciceri* IC53. Chickpea seeds were planted by hand in each plot in the beginning April and irrigated by furrow irrigation. Control plots included non-inoculated chickpea plants. The mean temperature of growing season in 2012 was 17–19^{o}C (April, May) and 32–34 ^{o}C (June July). Ten weeks after sowing, plant shoots were separated from roots, and roots were washed. Shoots and roots of each individual plant were dried to constant weight at 100°C and weighed. The number of pods and nodules per plant root was determined. Seed yields, taken from the two central rows of each plot (m^2 per plot), was determined at maturity (17 weeks after sowing).

Statistical analysis

The data were subjected to Analysis of Variance (ANOVA) (GenStat 10.1 version 2007, Lawes Agricultural Trust, Rothamsted Experimental Station) to evaluate the efficiency of the PGP agents. Significance of differences between the treatment means were tested at P = 0.001, 0.01 and 0.05.

Table 3. **Comparative performance of seven chickpea genotypes under control and inoculation with *M. ciceri* IC53 in a field condition. Study conducted at Experimental station of the Tashkent State University of Agriculture in 2012.**

Genotypes	Yield (g/ m^2)		Increase (%)	Pod number		Increase (%)	Nodule number		Increase (%)	Root dry weight (g/plant)		Increase (%)	Shoot dry weight (g/plant)		Increase (%)
	Control	IC53		Control	IC53		Control	IC53		Control	IC53		Control	IC53	
Flip 06-80	115	140*	22	31	37*	18	16	49*	202	2.1	2.6*	25	25	30*	22
CIEN-45	123	144*	18	64	74*	16	14	47*	236	1.9	2.2*	18	25	27	11
Flip 03-102	134	160*	19	44	53*	22	20	39*	98	2.3	2.7	16	27	33*	22
Flip 06-66	116	139*	20	31	36*	16	13	31*	139	1.8	2.1	15	22	26	18
Uzbekiston-32	118	140*	19	34	42*	24	18	68*	276	2.4	2.9	21	26	32*	22
Flip1-33	124	143*	15	36	41	16	22	32*	45	2.5	2.9	18	20	23*	18
Xalima	136	167*	23	41	53*	28	13	54*	323	2.9	3.5	21	26	34*	27
Mean	124	147	19	40	48	20	16	46	177	2.3	2.7	19	24	29	20
$LSD_{0.05}$	4.8	6.0		4.9	4.9		3.5	5.0		0.4	0.4		2.6	2.3	
CV (%)	3.0	3.1		9.3	7.9		16.1	8.4		12.4	11.5		8.2	6.0	

* Statistically significant at 0.05.

RESULTS AND DISCUSSION

The results showed that the chickpea cultivars showed in a wide range of growth under saline soil conditions. The cultivars Flip 1-19, Flip 1-29, Muqtadir, Xisor-32, Flip 05-69c, Flip 03-74c, Flip 05-65, Flip 03-27c, Flip 06-155c and Sino were the most salt-sensitive chickpea cultivars. Their shoot, root growth and nodulation inhibited by salt stress (Table 1). 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, thereby decreasing sites for potential rhizobial infection and further nodule development. Among 29 chickpea cultivars Uzbekiston-32, Xalima, Miroz, Flip 1-33 and CIEW 45 were found to be salt-tolerant and form nodules which indicate cultivars variation in chickpea to salt stress. Highest shoot and root dry weight was recorded for cultivar Uzbekiston (0.710/0.773 g plant $^{-1}$), Xalima (690/623 g plant $^{-1}$), Flip 1-33 (0.640/0.747 g plant $^{-1}$), CIEW 45 (0.650/0.567 g plant $^{-1}$) respectively. It is already reported that salt stress inhibits growth, development, nodulation and nitrogen fixation of chickpea with varying responses due to cultivars (Soussi et al. 1998, Gandour 2002, Al-Mutawa 2003, Abdelmajid 2009) and under salt stress the host tolerance could be the determining factor for the symbiosis (Cordovilla et al. 1995; Soussi et al. 1999; Tejera et al. 2006). Franzini et al. (2010) observed that legume responses to rhizobia are influenced by the compatibility of the interactions between plant and symbiont.

The cultivars Flip 06-80, CIEN-45, Flip 03-102, Flip 06-66, Uzbekiston – 32, Flip1-33 and Xalima which showed good performance in nodulation in pot experiments were inoculated with *M. cieri* and grown in the field. Response to inoculation as evaluated by total number of nodules, shoot and root dry matter, number of pods and yield of inoculated plants over uninoculated ones depended on the plant cultivars.

The numbers of nodules varied between 13 - 20 for uninoculated plants and 31-68 for inoculated plant with *M. ciceri*. Inoculation significantly induced ($p<0.05$) higher numbers of nodules on the roots of cultivars Flip 06-80, CIEN-45, Uzbekiston – 32 and Xalima than on those of Flip 03-102, Flip 06-66 and Flip1-33 (Table 2, 3). The nodule number showed a high correlation with shoot and root weights, which is an indication of the connection between the nodules and plant growth. Inoculation of plants with *M. ciceri* IC53 was significantly increased shoot and root dry matter ($P < 0.05$) by an average of 20% above the uninoculated plant. Analysis of variance showed no significant difference between the cultivars for all characters studied (Table 2). The interesting results from this research are that growth parameters resulting from inoculation with *M. ciceri* IC53 were greater for local cultivars Uzbekiston-32 and Xalima. This result confirmed those of Raovelagaleti and Marsh (1989) and Sattar et al. (1995) who observed that cultivar selection may have an important effect on the successful Rhizobium-legume associations under stress conditions. Those results underline the importance for local screening of salt tolerant cultivars in order to improve rhizobium-symbioses in chickpea.

Inoculation significantly increased the pod number and yield for all cultivars as shown in Table 3. The number of pods ranged from 16 to 18 for Flip 06-80, CIEN-45, Flip 06-66, Flip 1-33 and between 22 -28 for Flip 03-102, Uzbekiston – 32, Xalima.

The mean grain yield of all cultivars associated with *M. ciceri* IC53 was 147 g/m^2 as compared to control plant 124 g/m^2. The percentage of yield increase due to inoculation was higher in Xalima (23%) than Flip 06-80 (22%). The shoot and root dry weight also followed the trends similar to yield. In general, under field conditions, plant growth provided by efficient *Rhizobium*/chickpea combinations led to almost 300% of nodule number, 27% plant growth, 28% pod number and 23% yield recorded with *M. ciceri* IC53 treatment. These values remark the high potential of nodulation and N_2 fixation in chickpea when suitable symbiotic partners are met. Similar observation obtained by Mhadhbi et al. (2004) and Sadiki and Rabih (2001) where yield potential of chickpea depends on the rhizobia association and plant cultivar which together influencing the symbiotic performance. The above results revealed that for achieving the highest symbiotic effectiveness under salinity conditions plant cultivars have to be taken into account. It could be suggested that cultivation of salt tolerant cultivars with its symbiotic partners could be an effective selection technology to overcome the problem of soil salinity.

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