

## **Beneficial effect of *Rhizobium* inoculation on growth and yield of chickpea (*Cicer arietinum* L.) in saline soils**

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### **Abstract**

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The beneficial combinations between chickpea (*Cicer arietinum* L.) genotypes (Flip 03-74, Xisor, Halima (Flip 1-23), Flip 06-66, Flip 06-102, CIEN-45, Flip 06-155, Flip 03-102) with different symbiotic *Rhizobium* strains (viz. R4, R6, R9, and IC53) were studied in saline soil. The field experiment was set in moderately saline soil with an electrical conductivity (EC) of 6.3 dS/m in the north-east area of Uzbekistan. Results indicated that the inoculation with the adequate strains of rhizobia increased the chickpea growth and yield, accompanied by improving the seed weight, and seed protein contents. It was observed that Flip 06-66 and Flip 06-155 genotypes surpassed local Uzbekistan-32 cultivars with and without *Rhizobium* inoculation in grain yield, a weight of 1000 seeds, seed protein, and oil contents. Among the *Rhizobium* strains, R9 and R6 strains produced the best results, particularly grain yield, seed protein, and oil content in Halima and Flip 06-66 genotypes were increased by 27.8% and 36.5%, 5.8% and 5.9%, 2.4% and 4.6% over the control, respectively. It was concluded that indigenous rhizobial strains have the characteristics of broad host range, effective stimulation, higher nodulation efficiency, greater salt tolerance, and can be considered as a bio-fertilizer for enhancing chickpea productivity in saline soils of Uzbekistan.

*Keywords:* Chickpea; *Rhizobium*; growth; nodulation; saline soil; yield

### **Introduction**

Increasing of population coupled with climate change is posing a great threat to the crop production, especially in arid regions. Uzbekistan is located in an arid region with harsh climate encountering hot summer and cold snowy winter weather. Almost 60% irrigated crop fields in Uzbekistan have a different level of soil salinity. Depleted soil with low nitrogen (N) content and salinity are the limiting factors of chickpea production in Uzbekistan. Chickpea (*Cicer arieti-*

*num* L.) is a one of the essential legume food crops which contains valuable nutrients with the high amount of protein (24%) and oil (7%) in the seed (Kantar et al., 2007). In the current state, chickpea production does not meet the demand in Uzbekistan. Lack of chickpea production led to the increase of chickpea prices in local markets 2 to 3-times higher during the last few years.

New chickpea varieties with high yield potential and salt tolerant ability have been released, however many farmers still continue to grow locally available old varieties in conjunction

with poor agronomic management practices. Furthermore, natural and anthropogenic factors such as salinity, drought, lack of nutrients availability and rhizobial strains in the soil deteriorating chickpea production in the area (Khaitov, 2016). There is an urgent need to implement new management technologies for increasing grain yield and quality of chickpea. Legume-rhizobium symbiotic N fixation is the most important biological function by providing fixed N to the plants, and also plays a critical role in improving soil N fertility (Zaidi et al., 2003). Chickpea fixes atmospheric N biologically if they form nodules with appropriate *Rhizobium* available in the soil. Previous studies have shown that when legumes are inoculated with appropriate *Rhizobia*, nodulation efficiency increases which subsequently promotes plant growth, and leads to the increase of grain yield (Mirza et al., 2007; Khaitov et al., 2016).

Soil beneficial microbes play an important role in soil-plant nutrient dynamics (Mohammadi & Sohrabi, 2012). However, some researchers have suggested that legumes are susceptible to salt stress, and are not nodulated by rhizobia in saline soils (Mudgal et al., 2009; Soussi et al., 1998). In contrast, many other researchers declared that an effective symbiosis between chickpea and *Rhizobium* strains is crucial in N-deficient saline soils (Rokhzadi et al., 2008; Elkoca et al., 2007). While, inoculation efficiency and its sensitivity to stresses can significantly differ between crops species, the inoculation effect on plant growth and yield increase mainly depends on the competition for nodulation of rhizobial strains and their nitrogen fixation capacity and efficiency (Molla et al., 2012). In order to improve the *Rhizobium* inoculation effects, it is required to select suitable rhizobial strains with good adaptation to local saline soil and strong competition ability for nodulation as well as efficient N fixation (Kahraman et al., 2015).

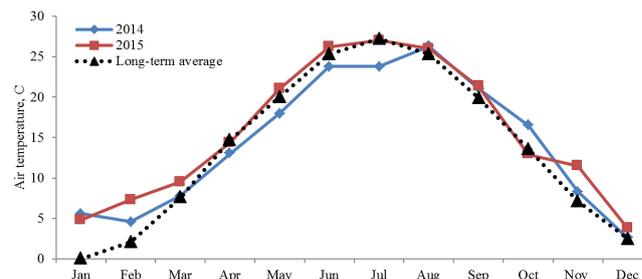
In Uzbekistan, the existence of indigenous rhizobial strains in most soils is very low due to lack of legume cultivation, harsh soil-climatic condition and nutrient depleted poor soils. Therefore, it is very essential to select effective rhizobial strains which are well adapted to saline soils for promoting chickpea production in order to solve the chickpea supply and demand crisis in Uzbekistan. The objective of this field study was to evaluate the effect of locally isolated rhizobial strains on growth and yield of chickpea genotypes grown in nutrient deficient saline soil.

## Material and Methods

### Experimental site

The field experiment was carried out on a typical sierozem soil with no history of chickpea cultivation at the Experimental Station of Tashkent State Agrarian University, Tashkent,

Uzbekistan during the spring-summer seasons in 2014-2015. This region's climate is classified as arid continental, with a relatively low annual precipitation of about 200±45 mm. A typical characteristic of the summer temperature is hot and often surpasses 40°C, however, winter temperatures average about -2°C, but sometimes may fall up to -25°C. The mean temperature of the growing season in 2014 and 2015 was 14–20° (April–May) and 25–27° (June–July) (Figure 1).



**Fig. 1. Weather data on air temperature, Tashkent State Agrarian University, Tashkent, Uzbekistan (2014 to 2015 growing years and long-term data)**

### Experiment layout

There were 36 treatments containing 9 chickpea genotypes and 4 *Rhizobium* treatments. The experiment was set up in a randomized complete block design in a split-plot arrangement with three replicates. Plots were 2.4 m × 10 m size with row spacing of 60 cm, whereas the distance between plants in a row was 15 cm maintained by thinning and a space of 1 m was allowed between plots.

Agronomic cultivation practices were kept uniform in all treatments throughout the growing season. Chickpea was irrigated 3 times around 600-700 m<sup>3</sup> per hectare in each time by surface furrow irrigation method. Fertilization rate was N-100 kg ha<sup>-1</sup>, P<sub>2</sub>O<sub>5</sub>-150 kg ha<sup>-1</sup> and K-100 kg ha<sup>-1</sup>. The total amount of K and half of P in the form of potassium chloride and single superphosphate, respectively were applied during land preparation, while the N in the form of ammonium nitrate and the remaining P were split and applied before first and second irrigation.

Two months after planting, nodules weight and number per plant formed in chickpea root were determined. The nodules were dried at 70°C in an air oven for 72 h and dry weight was measured. At the end of the growing season, plant parameters like a dry weight of shoot and root, plant height, number and weight of pods were recorded using standard procedures. The crop was harvested in the second week of July to determine chickpea seed yield as well as protein and oil content in seeds.

### Chickpea seeds and bacterial inoculants

Chickpea seeds (*Cicer arietinum* L.) cv Flip 03-74, Xisor, Halima (Flip 1-23), Flip 06-66, Flip 06-102, CIEN-45, Flip 06-155, Flip 03-102 were provided by the International Center for Agricultural Research in the Dry Areas (ICARDA), these genotypes originated in India and local variety Uzbekistan-32 as a control were chosen for the study.

*Rhizobium leguminosarum* strains R4(Rhizobium-4), R6(Rhizobium-6),and R9 (Rhizobium-9)used in this study were previously isolated from the chickpea roots rhizosphere in salinated soil of Uzbekistan (Khaitov et al., 2016) and kept at the Biotechnology department for long-term storage in nutrient broth (NB) with 15% glycerol at  $-80^{\circ}\text{C}$ . *Mezorhizobium ciceri* strain IC53 was obtained from the culture collection of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). The strains were grown in yeast mannitol (YEM broth) at  $28^{\circ}\text{C}$ . Before starting the seed inoculation, all bacterial strains were grown in NB at  $28^{\circ}\text{C}$  and the suspension was adjusted to the final concentration of approximately  $10^8$  CFU mL $^{-1}$ .

Uniform chickpea seeds were selected visually and surface-sterilized with 0.1% HgCl $_2$  solution for 10 min, rinsed thoroughly with distilled water. For inoculum, the seeds were inoculated with bacterial suspensions by adopting a standard procedure for 30 min before planting. Uninoculated chickpea seeds of each variety were used as controls.

Chickpea seeds were sown manually to the soil depth of 4-5 cm at a rate of 50 kg ha $^{-1}$  at the beginning of March on a well-prepared seedbed during both years. Soil moisture was enough for seed germination and no irrigation provided during planting period.

### Soil characteristics and analyses

The soil of the experimental field is classified as calcisol silt loam sierozem and it is moderately saline with an electrical conductivity (EC) of 6.3 dS/m and a pH of 7.9. The soil contains: 1% organic matter; total N 0.75 g kg $^{-1}$ ; extractable K 153 mg kg $^{-1}$ ; extractable P 19.6 mg kg $^{-1}$  and total P, 0.94 g kg $^{-1}$ . The soil is characterized by low nutrient content after long years of frequent ploughing and continuous cotton mono-cropping (Table 1). Application of excessive amount of chemical fertilizers had a major impact on the nutrient

balance and contents of soil. In this region, the underground water is located in 3-4 m depth, saline and contains 5-7 g saltL $^{-1}$ .

The Kjeldahl method (Keeney & Nelson, 1982) was used to measure the total nitrogen content (N $_{\text{tot}}$ ) and the Nelson Somers method was applied to determine the total carbon content (Nelson and Somers, 1975). Total phosphorus content (P $_{\text{tot}}$ ) was measured using the molybdenum blue method and the flame photometric method (Riehm, 1985) employed to determine the potassium in the soil. An atomic absorption spectrophotometer method was applied to measure calcium chloride (CaCl $_2$ ) and extractable magnesium (Schachtschabel & Heinemann, 1974). Matriz-I laser electron equipment was employed to determine the protein and oil content in the chickpea seeds at the Crop Science Department Lab of the University of Natural Resources and Life Sciences, Tulln, Austria.

### Statistical analyse

The collected data were analyzed using ANOVA (CROPSTAT 2007.2)analysis of variance technique and the least significant difference by using the Student Newman test ( $P < 0.05$ ). There was no significant difference in any observed parameters between years and agronomic data of the two consecutive years (2014-2015) were pooled and statistically analysed.

## Results

### Effect of inoculation with rhizobial strains on chickpea growth

Results showed that rhizobial inoculation significantly ( $P < 0.05$ ) improved the shoot and root biomass of all tested nine chickpea genotypes compared with uninoculated control (Table 2 and 4). Root dry weights of Xisor, Halima and Flip 06-155 genotypes were increased by 35.1%, 28.4%, and 30.7%, respectively the following inoculation with R6 over the control treatment. Similar results were obtained at the R9 inoculation with Flip 06-66, Uzbekistan-32, Flip 06-102, and CIEN genotypes and the increase were estimated to be 39.2%, 23.3%, 47.4%, and 23.9%, respectively. The efficiency of rhizobial strains on the dry weight of chickpea differed among genotypes. For example, the highest root dry weight was observed by the inoculation with R6 in Xisor,

**Table 1. Chemical properties of soil layer**

Soil horizons, cm	The total content, %				Mobile forms, mg/kg		
	Humus	N	P	K	N-NO $_3$	P $_2$ O $_5$	K $_2$ O
0-30	0.905	0.071	0.123	0.230	4.8	41.7	180.5
30-50	0.810	0.056	0.109	0.180	3.2	15.0	141.0

Halima and Flip 06-155 genotypes, and the inoculation with R9 exhibited the best results in Flip 06-66, Uzbekistan-32, Flip 06-102, and CIEN genotypes. Flip 03-74, Halima and Flip 06-155 genotypes were most sensitive to inoculation than that of Uzbekistan-32 and Flip 03-102 in regards to the root biomass.

The data showed that the dry shoot weight of chickpea genotypes differed significantly with respect to the inoculation. Significantly more shoot dry weight per plant was recorded in Halima (35.6 g), Flip 06-66 (26.3 g) and Flip 06-155 (26.2 g) as compared to local variety Uzbekistan-32, which showed a shoot weight of 21.7 g. Also, shoot dry weight of local chickpea variety Uzbekistan-32 were increased by 23.3% following the *Rhizobium* inoculation. These values remark the high potential of nodulation and N<sub>2</sub> fixation in chickpea when suitable symbiotic partners are met. In general, *Rhizobium* inoculation increased the average biomass of the tested nine chickpea genotypes by 3.3% -33.9% relative to the uninoculated control; however, chickpea genotype Halima produced maximum biomass following the inoculation with strain R6. The lowest shoot weight was recorded without *Rhizobium* inoculation (control) in all tested chickpea genotypes. As shown in Tables 2 and 3, inoculation with all tested rhizobial strains significantly increased the root and shoot biomass of chickpea genotypes to some extent, but R6 and R9 were found to be more efficient compared to other strains.

The abundance of beneficial *Rhizobium* bacteria in this region is very low because of the lack of legume cultivation and harsh soil-climatic condition. It turned out that the isolated rhizobial strains differ in their adaptability to host plant and efficiency in saline soil condition. The plant growth in chickpea tended to increase in *Rhizobium* inoculated treatments compared to the control. This might be attributed to the quick release of available N synthesized by the rhizobia infected roots to the plant root rhizosphere during the vegetative period (Poonia & Pithia, 2014).

The inoculations of the seeds with *Rhizobium* strains in the present investigation improved the root and shoot biomass of all chickpea genotypes and were found statistically superior over the control.

#### **Effect of inoculation with rhizobial strains on chickpea nodulation**

Inoculation with rhizobial strains significantly increased nodulation in all nine chickpea genotypes (Table 4). Results showed that two rhizobial strains R6 and R9 had very strong nodulation activities in the three chickpea genotypes tested, namely, Flip 06-66, Flip 06-155, and Halima. Amount of

root nodules per plant in these genotypes were 66.1, 72.4 and 58.6 piece per plant (data not shown), and the dry weight of nodules was about 1.13, 1.43 and 1.34 g/plant, respectively. The nodulation rates in all the three inoculated chickpea genotypes were 100%, and the nodule numbers per plant were 10 to 82 on average. According to the efficiency of *Rhizobium* chickpea interactions, the dry weight of nodules was 0.56-1.43 g/plant.

The significance of *Rhizobium* bacteria in improving nodulation and protection plant roots from various pathogens in legumes has been extensively reported (Afrasayab et al., 2010; Mhadhbi et al., 2004). However, if nutrient and water imbalances are observed in plants, legumes meet severeness to form nodules in saline soils (Mirza et al., 2007). Favourable effects of inoculation with rhizotrophic microorganisms in N<sub>2</sub> fixing and a significant increase in nodulation, growth, and yield of legume crops under harsh soil-climatic conditions have been declared by many researchers (Qurashi & Sabri, 2012). The effective indigenous rhizobial strains in this study had the characters of stimulatory ability, broad host range, high nodulation efficiency, and great salt tolerance. The results of the present experiment are in agreement with previous studies where bacterial inoculation stimulated the plant root growth in salt stress condition (Mohammadi and Sohrabi, 2012). Also, few studies reported that high yielding chickpea cultivars usually have an extensive root system, high nodulation ability, and strong and tall stem with a relatively higher number of pods and grains per pod (Kasole et al., 2005; Singh et al., 2003). Also in this study, there were significant genotypic differences in terms of plant responses to inoculation.

Moreover, salt tolerant *Rhizobium* strains have the ability to produce phytohormones which promote root growth and nodulation formation (Sohrabi et al., 2008). The inoculation effect on yield increase in legumes mainly depends on the competition of rhizobial strains for nodulation and the efficiency of nitrogen fixation (Lugtenberg & Kamilova, 2009).

Among all the treatments, the performance of the most chickpea genotypes was superior at the inoculation with R6 and R9 in comparison to other inoculants. The results of the field experiments specified that the selected rhizobial strains had a good nodulation effect and a wide host range in saline soils.

#### **Chickpea yield and seed quality improvement inoculated with rhizobial strains**

Inoculation with *Rhizobium* strains not only have many nodules formed on chickpea genotypes, but also increased chickpea plant growth and yield, and improved seed protein and oil content (Tables 5 and 6).

**Table 2. Dry weight of root of chickpea genotypes, (g per plant), (averaged across 2014 and 2015 growing seasons)**

<i>Rhizobium</i> strains	Flip 03-74	Xisor	HALIMA	FLIP 06-66	Uzbekis-ton-32	FLIP 06-102	CIEN	FLIP 06-155	FLIP 03-102
Control	1.63d	1.54d	2.11d	1.61c	1.65d	1.78e	1.97d	1.89e	1.56d
Rhizobium-9	1.73cd	1.78cd	2.58b	2.12a	2.27a	2.26a	2.44a	2.25c	1.67cd
RhizobiumIC-53	1.93ab	1.87bc	2.58b	1.83b	1.77d	2.08bc	2.33bc	2.39ab	1.77c
Rhizobium-4	2.0a	1.95ab	2.48c	1.83b	2.01c	1.98d	2.35ab	2.13d	2.06a
Rhizobium-6	1.81bc	2.08a	2.71a	1.39d	2.11b	2.11b	2.05d	2.47a	1.94b
LSD 0.05	0.09	0.11	0.09	0.09	0.07	0.08	0.11	0.11	0.10
CV(%)	2.6	3.0	2.0	2.7	1.9	2.0	2.6	2.7	2.8

Data are means of 3 replicates/plot analysed. Values in the same column at the same index followed by different letters are significantly different according to the LSD test ( $P \leq 0.05$ )

**Table 3. Dry weight of shoot of chickpea genotypes, (g per plant), (averaged across 2014 and 2015 growing seasons)**

<i>Rhizobium</i> strains	Flip 03-74	Xisor	HALIMA	FLIP 06-66	Uzbekis-ton-32	FLIP 06-102	CIEN	FLIP 06-155	FLIP 03-102
Control	18.4d	11.8e	25.6e	18.9e	17.6e	17.1e	20.1e	19.5e	16.4d
Rhizobium-9	21.8c	14.6cd	32.4c	26.3a	21.7a	24.4a	24.9a	23.2c	17.0d
RhizobiumIC-53	23.4b	14.8c	34.6ab	20.9cd	20.3cd	19.3d	22.8c	25.6ab	22.4a
Rhizobium-4	26.4a	16.2b	30.3d	21.3bc	20.4bc	21.2c	23.6ab	21.4d	20.8b
Rhizobium-6	19.5d	17.8a	35.6a	22.3b	21.4ab	23.2ab	20.9d	26.2a	18.9c
LSD 0.05	1.29	0.53	1.66	1.40	1.09	1.67	0.67	1.4	1.44
CV(%)	3.1	1.9	2.8	3.4	2.8	4.2	1.6	3.2	4.0

Data are means of 3 replicates/plot analysed. Values in the same column at the same index followed by different letters are significantly different according to the LSD test ( $P \leq 0.05$ )

**Table 4. Dry weight of nodules, (g per plant), (averaged across 2014 and 2015 growing seasons)**

<i>Rhizobium</i> strains	Flip 03-74	Xisor	HALIMA	FLIP 06-66	Uzbek is-ton-32	FLIP 06-102	CIEN	FLIP 06-155	FLIP 03-102
Control	0.12e	0.12d	0.08e	0.12e	0.15e	0.17e	0.10e	0.07e	0.24e
Rhizobium-9	0.75b	0.60b	0.56d	1.12ab	0.88a	0.76a	1.35a	0.43cd	0.56cd
RhizobiumIC-53	0.44cd	0.60b	1.02b	0.71c	0.64b	0.76a	1.09b	1.12b	0.78b
Rhizobium-4	0.51c	0.46bc	0.62c	1.13a	0.43cd	0.69b	0.99bc	0.46c	1.13a
Rhizobium-6	0.10	0.95a	1.34a	0.57d	0.49c	0.33d	0.73d	1.43a	0.67bc
LSD 0.05	9.8	0.15	0.10	0.11	0.13	0.09	0.13	0.18	0.13
CV(%)	14.6	7.6	8.2	12.8	9.6	7.9	13.2	9.8	9.8

Data are means of 3 replicates/plot analysed. Values in the same column at the same index followed by different letters are significantly different according to the LSD test ( $P \leq 0.05$ )

**Table 5. Seed protein content of chickpea genotypes, (%), (averaged across 2014 and 2015 growing seasons)**

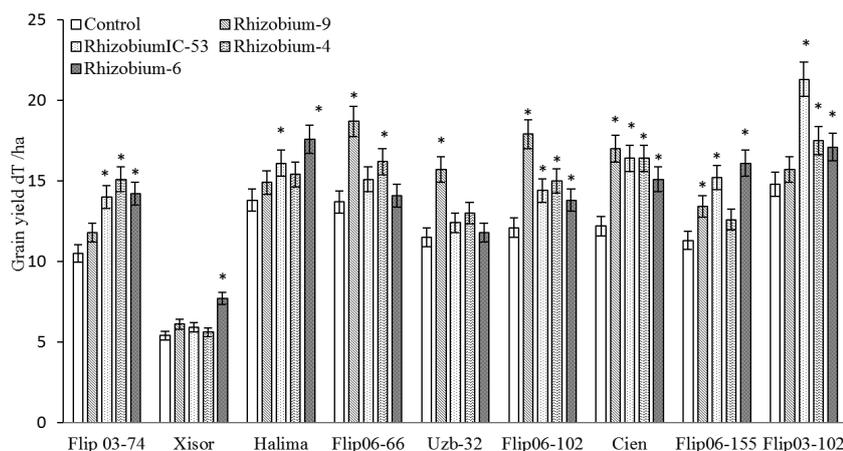
Rhizobium strains	Flip 03-74	Xisor	HALIMA	FLIP 06-66	Uzbekis-ton-32	FLIP 06-102	CIEN	FLIP 06-155	FLIP 03-102
Control	22.4b	20.8b	22.6c	21.9b	21.0b	22.2c	20.3c	22.4b	22.6b
Rhizobium-9	23.9a	20.8b	23.4ab	23.2a	21.6a	23.4a	21.2a	23.0ab	23.4ab
RhizobiumIC-53	23.5ab	21.8ab	22.9bc	23.1ab	21.5ab	22.4c	20.9ab	22.8b	23.5a
Rhizobium-4	23.4ab	21.2ab	23.3abc	23.1ab	21.5ab	23.3ab	20.6bc	23.0ab	23.4ab
Rhizobium-6	23.6ab	21.9a	23.7a	22.0d	21.3ab	23.4a	20.8abc	23.3a	23.3ab
LSD 0.05	1.04	1.03	0.79	0.59	0.52	0.97	0.59	0.81	0.87
CV(%)	2.4	2.8	1.8	1.4	1.3	2.2	1.5	1.9	2.0

Data are means of 3 replicates/plot analysed. Values in the same column at the same index followed by different letters are significantly different according to the LSD test ( $P \leq 0.05$ )

**Table 6. Seed oil content of chickpea genotypes, (%), (averaged across 2014 and 2015 growing seasons)**

Rhizobium strains	Flip 03-74	Xisor	HALIMA	FLIP 06-66	Uzbekis-ton-32	FLIP 06-102	CIEN	FLIP 06-155	FLIP 03-102
Control	6.48d	5.84b	6.75b	6.27d	6.13b	6.16c	5.19c	6.57d	6.76d
Rhizobium-9	6.69bc	5.93ab	6.91ab	6.56a	6.31a	6.41a	5.32a	6.80abc	6.84c
RhizobiumIC-53	6.75b	5.89b	6.94ab	6.47abc	6.26ab	6.35ab	5.28b	6.94ab	6.96a
Rhizobium-4	6.93a	5.91b	6.88ab	6.49ab	6.23ab	6.23bc	5.32a	6.63cd	6.81cd
Rhizobium-6	6.56cd	5.98a	6.97a	6.39bcd	6.26ab	6.21c	5.26bc	6.95a	6.93ab
LSD 0.05	0.14	0.09	0.18	0.14	0.14	0.12	0.08	0.20	0.05
CV(%)	1.1	0.8	1.4	1.2	1.2	1.0	0.8	1.6	0.4

Data are means of 3 replicates/plot analysed. Values in the same column at the same index followed by different letters are significantly different according to the LSD test ( $P \leq 0.05$ )



**Fig 2. Yield of chickpea inoculated with different rhizobial strains, dT/ha**

Data are means of 3 replicates/plot analysed. Asterisks indicate significantly different compared to the respective control according to the LSD test ( $P \leq 0.05$ ). (Averaged across 2014 and 2015 growing seasons)

Inoculation with *Rhizobium* strains obviously enhanced the yield of nine tested chickpea genotypes and was found statistically ( $P < 0.05$ ) different over the control (Figure 2). Inoculation with R9 showed the highest efficiency in increasing the seed yield. The grain yield of FLIP 06-66, Uzbekistan-32, Flip 06-102, and CIEN genotypes were increased by 36.5%; 31.1%; 47.9% and 39.3%, respectively over the uninoculated control. Rhizobium IC-53 showed the best result with FLIP 03-102 genotype by increasing the seed yield up to 43.9%. The highest increase in yield was 35.2% by the interaction of R4 with Flip 03-74 genotype. The R6 bacterial strain increased the yield of Xisor, Halima and Flip 06-155 genotypes 42.6%, 27.5%, and 42.5%, respectively. The lowest yield was observed in the control plot of Xisor genotype with 5.4 dT/ha, which also performed the highest increase of 42.6% by inoculation R6 strain.

Inoculation with rhizobial strains significantly affected seed protein and oil content, which was mainly reflected in the treatments with the increased seed weight following inoculation. Seed protein content is considered as a consistent index to assess inoculation efficiency, which could directly represent the advantages of N fixation in the symbiotic system of *Rhizobium* and chickpea. Seed protein content was increased in Flip 06-66, Halima and Flip 06-155 up to 5.9%, 4.6%, and 4%, respectively, in which Flip 06-66 showed the highest increase (Table 5).

Halima, Flip 06-155 and Flip 06-102 generated significantly higher seed oil content 6.97%, 6.95%, and 6.96%, respectively with *Rhizobium* inoculation than other genotypes. Whereas, the lowest seed oil content was recorded in CIEN genotype (5.19%) without inoculation (Table 6). There was

also significant variation among chickpeas genotypes by the inoculation with different *Rhizobium* strains regarding seed oil content. The highest seed oil content was recorded when R6 and R9 inoculants were applied, while the lowest seed oil content was recorded in the control of all tested genotypes.

The results indicated that inoculation with rhizobial strains could significantly increase seed protein and oil contents of all chickpea genotypes. There were significant genotypic differences in terms of chickpea response to inoculation with rhizobial strains. The results of this experiment are in agreement with previous studies that inoculation of legume seed with rhizobial inoculants increased settings of pods, the number of seeds per pod and per plant, and finally the quality of seeds (Rokhzadi et al., 2008). Many researchers declared that inoculation with appropriate *Rhizobium* strains is an effective measure to increase  $N_2$  fixation, promote N nutrition and enhance yield in legumes (Mirza et al., 2007; Thomas & Bakker, 2015). However, rhizobial strains cannot express its full capacity for  $N_2$  fixation if limiting factors (soil salinity, nutrient deficiency, mineral toxicity, extreme temperatures, unfavourable soil pH, insufficient or excessive soil moisture, plant diseases, and etc) impose threats on the growth of the host legume (Soussi et al., 1998; Wani et al., 2007). Meanwhile, in this experiment, inoculation with rhizobial inoculants showed its advantages in saline soil with significantly increasing chickpea shoot and root biomass and improve seed yield. It was revealed that different chickpea genotypes and rhizobial strains differed in their tolerance to environmental stresses. Availability of soil moisture and mineral nutrients are the most important factors in the nitrogen fixation process. The salinity problems observed in

this study can be surmounted by wisely selection of chickpea genotypes and inoculation with *Rhizobial* strains.

## Conclusion

Chickpea genotypes Flip 06-155 and Flip 03-102 showed superiority by the highest yield and seed quality over local Uzbekistan-32 chickpea variety. Based on these results, these two chickpea genotypes have been recommended for further evaluation through the State Varietal Commission of Agricultural Crops for cultivation in Uzbekistan. Our experimental results showed that rhizobial inoculants not only produce a higher number of nodules formed in chickpea roots but also increased chickpea plant vegetative and generative growth as well as its tolerance. We noted a strong correlation between the rhizobia inoculations and the studied plant variables such as plant dry weight, nodulation, yield, seed oil, and protein content. It can be concluded that if favorably interacting *Rhizobium* strains R6 and R9 used as microbial inoculants, nodulation will be improved as well as growth and yield of chickpeas with enhanced seed quality. Our results provided a practical basis of benefits to select the highest yielding chickpea genotypes and generated the efficient rhizobial inoculants adapted to saline soils. This study showed the advantages of bacterial treatment in the chickpea cultivation and identified its success in terms of yield and quality; especially these bio-inoculants are environment-friendly and licensed for the organic cultivation. Unlike, the excessive use of chemical N fertilizers, which may result the extended vegetative period, the N supplied by the nodule from biological N fixation could effectively improve chickpea growth and promote its transition reproductive stage, and then significantly increase chickpea production.

Therefore, inoculation with the effective rhizobial inoculants might be an important approach to improve chickpea production in saline soils of Uzbekistan.

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