

Improvement of Salt Tolerance and Growth in Common Bean (*Phaseolus vulgaris* L.) by Co-Inoculation with Native Rhizobial Strains

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ABSTRACT

Beneficial association of rhizobial strains with leguminous plants may result in the enhancement of nodulation in the root and overall plant performance. In this study, the efficacy of inoculation was tested with pre-isolated *Rhizobium phaseoli* R9 and *Mesorhizobium ciceri* R6 as a single and in combined treatments on common bean (*Phaseolus vulgaris* L.) in a field experiment at soil salinity level of 5.6 dS m⁻¹ over two growing seasons. The bacterial inoculations increased the plant height, root and shoot biomass, grain yield, number of nodules per plant, nodule dry weight, and root length of common bean, with a significant difference. Compared to single inoculation, co-inoculation of the rhizobial strains was more effective in all plant parameters and increased the seed yield by 35.1 and 37.9%, respectively, over the two consecutive seasons compared to the uninoculated control. Chemical analysis showed a significantly ($P < 0.05$) higher protein and oil content in the seeds of the co-inoculated plants than those of the control. The result of the experiment showed a strong correlation ($r^2 = 0.87$) between the increased nodule dry weight and seed yield of common bean. It might be concluded that co-inoculation with rhizobial strains could be the most effective biofertilization strategy for achieving greater nodulation and yield of common bean under saline conditions of Uzbekistan.

Keywords: Biofertilization, Co-inoculation, Nodulation, *Rhizobium*, Seed protein, Soil salinity.

INTRODUCTION

Increase in domestic crop production is required in order to meet the demand of an increasing population in Uzbekistan. In fact, by 2050, the population of the country is predicted to increase by over 40%, from approximately 32 million to 50 million people (Qushimov *et al.*, 2007), which requires the intensification of crop production. Although cotton production is important, the recent rise of legume production represents a completely new

contribution to regional food security in Uzbekistan (Kienzler *et al.*, 2011). Soil salinity is a major threat to agricultural productivity in arid and semi-arid regions of the world including Uzbekistan. The main challenges of crop production in saline condition are soil nutrient degradation, lack of water for irrigation, and improper crop rotation management, which in turn decrease soil microorganisms and enzyme activities in the soil. Salt stress inhibits plant growth by disturbing various biochemical and physiological processes, i.e. uptake of essential nutrients, respiration, translocation

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of carbohydrates, and photosynthesis (Pourbabaei *et al.*, 2016).

Common bean (*Phaseolus vulgaris* L.) is the most important edible legume crop with high nutritional value. Common bean provides 15% of the protein and 30% of the caloric requirement of the world's population and represents 50% of the grain legumes consumed worldwide (McConnell *et al.*, 2010). In Uzbekistan, common bean is widely used for a daily diet and cultivated all over the country, especially as a follow-up crop constituting approximately 6,500 ha in 2016, with the seed yield of 1.6 t ha⁻¹ (FAO, 2016). Like most of the other legume species, common bean is sensitive to soil salinity (Hungria *et al.*, 2003). The growth and symbiotic performance of legumes under saline condition depend on the salt tolerance conferred by associated rhizobial strains (Vakali *et al.*, 2017). Usually, abiotic factors such as soil salinity, high temperature, drought, and also nutrient deficiency in the soil suppress Nitrogen (N) fixation capacity of common bean in arid regions (Naseri *et al.*, 2009). Also, some other factors such as lack of irrigation and inefficiency of indigenous bacterial strains may cause losses in bean production (Remans *et al.*, 2008).

Legumes can carry out biological N fixation in root nodules through symbiosis with soil bacteria collectively called rhizobia. Nodule forming bacteria belonging to the genus *Rhizobium* have been frequently identified due to their ecological importance, soil fertility improvement and plant growth stimulation ability (De Meyer *et al.*, 2011; Rahmani *et al.*, 2011). However, salt stress can limit N₂-fixation capacity by reducing plant photosynthetic activity in legume crops, causing a shortage of photosynthate supply to the nodules and, subsequently, a low supply of energy to the bacteroids (Araújo *et al.*, 2015). Salt-tolerant rhizobial strains used as inoculants in legumes have been shown to alleviate the detrimental impacts of salt stress in plant growth and nodulation and reduce Na⁺ uptake (Tejera *et al.*, 2006). It is well known that rhizobia from harsh origin possess enormous potential for improving the

resilience of legumes in salt-stressed conditions (Keneni *et al.*, 2010). Simultaneous infection with specific rhizobial strains may increase nodulation and growth in a wide variety of legumes (Figueiredo *et al.*, 2008; Goettsch *et al.*, 2016). While studying the interrelated effect of rhizobial strains with legumes, Bertrand *et al.* (2015) reported greater symbiotic and plant growth parameters. Rhizobial inoculation had also a positive effect on non-legume crops in previous studies, increasing yield of paddy and wheat by 20 and 15%, respectively, but legumes with inoculation exhibited a substantial yield increase up to 50-60% (Oliveira-Franceschini *et al.*, 2017).

Inoculation of rice (Hussain *et al.*, 2009), wheat (Mehboob *et al.*, 2011) and chickpea (Tejera *et al.*, 2006) with *Mesorhizobium ciceri* showed an effective colonization of bacterial strains in the root rhizosphere and stimulation of plant growth, suggesting that *Mesorhizobium ciceri* has growth promoting mechanisms, i.e. uptake and mobilization of nutrients, solubilization of insoluble phosphates, enhancement of stress resistance, production of phytohormones, vitamins and siderophores. Large populations of indigenous rhizobial strains have high nitrogenase activity and can survive in the harsh soil environment for 2-3 weeks in order to effectively form nodules on the roots of legume seedlings (Vargas *et al.*, 2000). Previous experiments on common bean showed that inoculation with rhizobia enhanced plant physiological and morphological traits (Suárez *et al.*, 2008; McConnell *et al.*, 2010).

Some researchers observed that specific co-inoculation generates synergism by improving the performance of other bacteria (Remans *et al.*, 2008). In order to create such co-inoculations, a combination of beneficial rhizospheric bacterial strains has to be selected after extensive and careful evaluations (Mehboob *et al.*, 2013). This combination can enhance nodule number and weight, root and shoot biomass, total plant N and flavonoid levels (Khaitov, 2016). Symbiosis with functionally distinct microorganisms such as free-living N-fixing bacteria, and/or plant

growth promoting rhizobacteria, such as *Pseudomonas*, *Rhizobium*, *Azotobacter* attracted much attention in recent studies (Pineda *et al.*, 2010). Many combinations of bacterial co-inoculation were studied in a laboratory; therefore, comprehensive researches in field conditions are important for further implementing this technique into practice. To date, only few number of experiments have been performed under field conditions in arid regions with co-inoculation of microorganisms in different crops.

In arid regions, due to a harsh environmental condition, limited populations of indigenous rhizobial strains exist in the soil that might naturally nodulate legume crops (Beiranvand *et al.*, 2018). Despite several attempts, poor nodulation and lack of response to inoculation in field experiments have been frequently reported worldwide, raising doubts about the usefulness of common bean inoculation under arid condition (Hardarson *et al.*, 1993). According to previous reports, co-inoculation of rhizobial strains compared to single inoculation has advantages to improve growth parameters and yield components of common bean under saline field conditions (Benidire *et al.*, 2017).

The aim of the present study was to investigate whether inoculation of common bean seeds with different rhizobial strains improves nodulation, plant growth, and yield in saline soil condition of Uzbekistan.

MATERIALS AND METHODS

Experimental Site Location and Soil Properties

The experiment was conducted in irrigated farmers' field located in the central part of the country, on the left bank of Syr-Darya

River under mildly saline soil conditions in Sirdarya region (40° 25' N 68° 40' E). The climate of the area is a typically arid continental climate with extreme differences between winter and summer temperatures. The coldest period is January with 0°C average monthly air temperature; the hottest is July with 37°C. A typical characteristic of the summer climate is drought and heat. Annual rainfall is about 200±40 mm. The soil has a low level of nutrient content due to continuous cotton monoculture accompanied by frequent tillage for many decades in this area.

Soil samples were collected at the beginning of the experiment in each year. In each of the sampling plots, at least four soil samples were taken at 0-30 and 30-50 cm depth across the two diagonals of the experimental field. All soil samples were air dried and passed through a sieve (2 mm and 4 mm) for chemical and physical analysis. Air-dried samples were analyzed for soil chemical properties i.e. soil pH, EC (electroconductivity), organic matter, available nutrients, and humus content. The EC value of the soil was around 560±61 dS per meter, pH 8.0. Soil characteristics with average nutrient content are listed in Table 1.

Soil particle distribution was determined using natrium phosphate. The total nitrogen, N_{tot}, content in the soil was determined by the Kjeldahl method while the molybdenum blue method was used to determine the total phosphorus content, P_{tot}. Potassium, K, was determined using the Flame Photometric Method (Riehm, 1985). The Atomic Absorption Spectrophotometer (AAS) was employed to measure Calcium Chlorite (CaCl₂). Soil pH and EC values were measured by appropriate electrometers.

On average, the soil contained 76±9 g kg⁻¹

Table 1. Soil chemical analysis of the experimental field.

Soil horizons (sm)	The total content (%)				Mobile forms (mg kg ⁻¹)		
	Humus	N	P	K	N-NO ₃	P ₂ O ₅	K ₂ O
0-30	0.925	0.085	0.153	2.30	4.8	30.0	180.5
30-50	0.715	0.066	0.139	1.80	3.2	15.0	141.0



sand, 668 ± 12 g kg⁻¹ silt, and 243 ± 13 g kg⁻¹ clay, bulk density changed in the range of 1.41–1.57 g cm⁻³. The main chemical soil properties were: Organic matter 0.93–0.72%; total organic C 0.12–0.34, N 0.09–0.07%, total P 0.15–0.14%, exchangeable potassium 181–141 mg kg⁻¹ at 0–30 and 30–50 cm depths, respectively. The high concentrations of Ca²⁺, K⁺, and Na⁺ are associated with CO₃²⁻ and Cl⁻, reflecting the dominance of carbonate and chloride in the saline soil.

Common Bean Inoculation with Bacterial Strains

Bacterial strains, namely, *Rhizobium phaseoli* R9 and *Mesorhizobium ciceri* R6, were previously isolated from the rhizosphere of soybean and chickpea, respectively, from cultivated saline soil of Uzbekistan (Khaitov et al., 2016). These strains were stored in 50% glycerol (v/v) inside a freezer at temperature -80°C until further use in experiments at Biotechnology Department of Tashkent State Agrarian University, Tashkent, Uzbekistan.

In order to prepare inoculants, rhizobial strains were cultured in Yeast Extract Mannitol broth (YEM) medium. The growth rate of bacterial isolates was determined by spectrophotometer after 24, 48, and 72 hours. The suspension used for the inoculation was adjusted to a final concentration of approximately 10⁷ CFU mL⁻¹. For inoculation, common bean seeds were coated with rhizobial inoculants at a rate of 10 mL of inoculant 200 g⁻¹ seeds for 15 minutes and planted into the experimental plots. For control plots, common bean seeds were soaked in sterilized pure water for 15 minutes before planting. In the case of co-inoculation, inocula of desired rhizobial strains were mixed in equal proportion and shaken for 5 minutes to ensure homogenized cell density of different rhizobial strains before seed soaking.

Experimental Procedures

Common bean cultivar Orzu was used in the experiment. The seeds of the cultivar were provided by Seed Production Laboratory of Plant Science Department, Tashkent State Agrarian University, and planted manually in the middle of April. The experiment was carried out during two consecutive growing seasons in 2012 and 2013. This common bean cultivar has been used in previous studies, showing positive responses in yield and N₂ fixation with different inoculants. It has a capacity for high N₂ fixation and adaptation to saline and drought conditions, and is efficient in N and P uptake. The seeds of common bean were sorted to eliminate broken, small seeds. Then, they were surface-sterilized with a solution of 75 mL 0.1% mercuric chloride+25 mL water for 2–3 minutes, rinsed five times with sterile distilled water.

The following four treatments were used in the experiments such as no inoculation (control), inoculation with *Mesorhizobium ciceri* R6, inoculation with *Rhizobium phaseoli* R9, and dual inoculation with both inoculants. The inoculated and uninoculated (control) common bean seeds were planted in appropriate field plots in a Randomized Complete Block Design (RCBD) with three replicates. In all experimental plots, 100 kg ha⁻¹ of granular N as Ammonium Nitrate (34% N), 100 kg P ha⁻¹ as Triple Superphosphate (17.2% P) and 75 kg K ha⁻¹ as Muriate of Potash (21.5% K) were applied based on current agronomic practices. One-half of the N fertilizer was applied prior to planting and the other half was applied 40 DAP (Days After Planting). Main parts (70%) of P and K fertilizers were applied before sowing and the remaining portions (30%) were applied before the flowering stage. Irrigation was applied three times using 650–700 m³ ha⁻¹ to prevent drought stress.

Plant Analyses

For sampling, ten plants per plot of each replication were randomly harvested

resulting in 30 plants per treatment and fresh weight of shoots, roots and pods were measured immediately after removing the plants from the soil and cleaning the roots, respectively. During cleaning the roots, the number of nodules were counted in the plants inoculated with *Rhizobium*. Also, the plants grown in the control plots were checked for nodulation due to the possibility of infection with indigenous rhizobial strains. Subsequently, nodules were separated and dried for 48 hours at 65°C to determine the nodule dry weight.

Plants were harvested when the seeds and plants were dry, approximately 105 DAP. Before harvesting, the number of plants per plot was counted. Seed yield for each plot was calculated as follows: seed weight of common bean per plot divided by the number of plants harvested at that plot and multiplied by the number of plants calculated per hectare. Plant samples of each plot were further used to determine the number of pods and seeds per plant, dry weight of root and shoot of plants. The dry weight of shoot, root, and pods were measured after drying in an oven for 72 hours at 65°C. A near-infrared-reflectance spectroscopy instrument (Bruker Matrix-I) was used to determine seed protein and oil content.

Statistical analysis

Data were subjected to statistical analysis using the ANOVA by CropStat 7.2 program. Means were compared by the Least Significant Difference (LSD) test ($P \leq 0.05$).

RESULTS

Effect of Inoculation on Growth Parameters of Common Bean

Single and co-inoculation with *Rhizobium phaseoli* R9 and *Mesorhizobium ciceri* R6 significantly increased dry weight, height, nodulation, and seed yield of common bean (Table 2). Rhizobial inoculation enhanced morphological parameters and growth of common bean under saline soil condition; however, co-inoculation with selected rhizobia was superior in comparison to a single treatment. The tested rhizobial strains had the capability to promote plant growth of common bean significantly as compared with the control. The maximum increase in shoot dry weight of common bean with a value of 47.6% was observed in the co-inoculated treatment followed by single inoculations with *Rhizobium phaseoli* R9 and *Mesorhizobium ciceri* R6 exhibiting 16.7 and 14.6% increases, respectively, in comparison to the control. Likewise, root

Table 2. Effect of inoculation with rhizobial strains on shoot and root dry weight and nodulation of common bean in saline soil.^a

Inoculation treatments	Shoot dry weight (g plant ⁻¹)		Root dry weight (g plant ⁻¹)		Nodule number (plant ⁻¹)		Nodule dry weight (g plant ⁻¹)	
	2012	2013	2012	2013	2012	2013	2012	2013
Control	4.2d	4.8d	1.43c	1.67d	3.8d	6.2d	0.07d	0.12d
<i>Mesorhizobium ciceri</i> R6	4.8c	5.5bc	1.61bc	1.82c	31.2c	33.7c	0.67c	0.78c
<i>Rhizobium phaseoli</i> R9	5.0b	5.6b	1.89ab	1.98b	40.8b	43.2b	0.93b	1.06b
R6+R9	6.2a	6.7a	2.11a	2.28a	58.1a	64.7a	1.18a	1.35a
LSD 0.05	0.32	0.3	0.22	0.14	5.1	3.4	0.19	0.21
CV (%)	3.4	2.8	6.6	3.8	8.0	4.6	14.5	13.4

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



dry weight of common bean was increased by 32.2 and 15.6% after the single inoculation with *Rhizobium phaseoli* R9 and *Mesorhizobium ciceri* R6, respectively. The highest root dry weight of common bean was observed in the co-inoculated treatment with an increase of 47.5% compared to the control.

Inoculation significantly increased nodulation of common bean when both inoculants were employed. The inoculation with *Mesorhizobium ciceri* R6 and *Rhizobium phaseoli* R9 was found to have a positive influence on the root nodulation of common bean, presenting values of 43.7 and 43.2 nodule plant⁻¹, respectively. Maximum nodule number and nodule dry weight were recorded in the case of co-inoculation with values of 64.7 nodule plant⁻¹ and 1.35 g plant⁻¹, respectively. Single inoculations with *Mesorhizobium ciceri* R6 and *Rhizobium phaseoli* R9 presented the nodule dry weight values of 0.78 and 1.06 g plant⁻¹, respectively. A greater proportion of nodules were formed on the main root of common bean by the co-inoculation. The improved nodulation in the root may have resulted in increased N contents in the shoots and, in turn, increased plant growth and grain

yields. The high positive correlation between nodule dry weight and root dry matter ($r^2 = 0.84$) was recorded (Figure 1).

Effect of Inoculation on Yield Parameters of Common Bean

All the bacterial inoculation treatments significantly increased the seed yield of common bean in both seasons of the experiment (Table 3). The single inoculation of common seeds with *Rhizobium phaseoli* R9 and *Mesorhizobium ciceri* R6 increased the seed yield by 10.1 and 14.1% in 2012, whereas, in 2013, the common bean yield was higher by 17.7 and 22.3%, respectively, compared to the control. However, the seed yield of common bean increased by 35.1% (1731 kg ha⁻¹) in 2012 and 37.9% (1821 kg ha⁻¹) in 2013, in response to the co-inoculation with the selected bacterial strains as compared to the control. These results specified a positive influence of the co-inoculation with the selected rhizobial strains to the reproductive growth of common bean.

Protein content in the seeds was significantly influenced by the inoculation

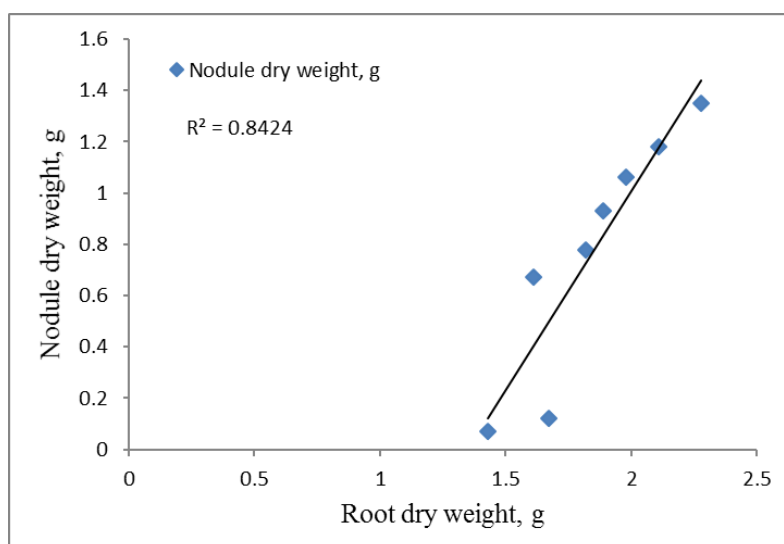


Figure 1. Effect of co-inoculation with rhizobial strains on correlation between root dry weight and nodule dry weight of common bean in saline soil under continental climate (averaged across 2012 and 2013 growth seasons).

Table 3. Effect of inoculation with rhizobial strains on yield, protein, and oil content of seeds of common bean in saline soil.^a

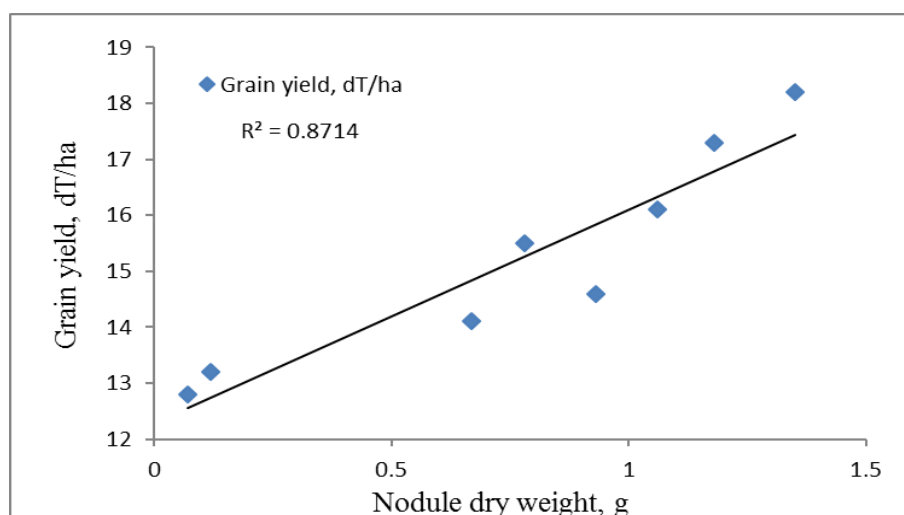
Inoculation treatments	Yield, (kg ha ⁻¹)		Protein content (%)		Oil content (%)	
	2012	2013	2012	2013	2012	2013
Control	1281d	1320d	22.6d	23.9d	2.2ab	2.2 c
<i>Mesorhizobium ciceri</i> R6	1410bc	1553bc	27.2b	26.6b	2.5ab	2.5b
<i>Rhizobium phaseoli</i> R9	1462b	1615b	25.7c	25.9c	2.3ab	2.3c
R6+R9	1731a	1821a	28.8a	29.2a	2.6a	2.6a
LSD 0.05	0.66	0.83	0.22	0.25	0.86	0.32
CV (%)	2.4	2.8	1.6	1.6	1.7	0.6

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

treatments and interactions between common bean and rhizobial strains. Common bean had markedly higher protein content in the seeds by 20.4 and 13.7% upon inoculation with *Mesorhizobium ciceri* R6 and *Rhizobium phaseoli* R9, respectively, but the highest increase was 27.4% upon the co-inoculation with the selected bacterial strains compared to the control. This result shows that even though seed protein content decreases with the severity of salt stress, it was superior in the seed following the co-inoculation treatment with *Mesorhizobium ciceri* R6 and *Rhizobium phaseoli* R9. Likewise, a significant increase was

observed in response to the co-inoculation when oil content in the seeds was monitored.

Furthermore, the experiment revealed that rhizobial strains and their interaction influence the symbiotic outcome by the application of single or simultaneous inoculations with the two different rhizobial strains. A strong positive correlation ($r^2 = 0.87$) was determined between the increased nodule dry weight and seed yield of common bean and hypothesized that the nodulation has a positive effect on performance of common bean under salt stress (Figure 2). Together, the two microorganisms had additive effects on both

**Figure 2.** Effect of co-inoculation with rhizobial strains on correlation between nodule dry weight and seed yield of common bean in saline soil under continental climate (averaged across 2012 and 2013 growth seasons).



root nodulation and plant yield, while proving N_2 fixation by rhizobia increases the N content of host plant tissue. The co-inoculation additionally increased the weight of seed yield, that is, enhanced growth of reproductive tissue, reflecting the strong dependency of plant performance on N availability. Thus, the reproductive development of common bean was positively affected by single inoculation and exceeded in co-inoculation of the strains.

DISCUSSION

The experimental results showed that common bean responded positively to the inoculations with rhizobial strains under salt stress. This outcome confirms some previous reports that inoculation of common bean with rhizobial strains increased nodule numbers, plant weight and seed yields in field conditions (Goettsch *et al.*, 2017). Makoi *et al.* (2013) reported that *Rhizobium* inoculation significantly increases the uptake of N, P, K, Ca and Mg in common bean plant tissues and improves soil pH, which in turn increases the availability of most mineral elements. Some researchers also observed that soil microorganisms such as rhizobacteria influenced the quality of soils in many ways and enhance nutrients uptake by plants (Saharan and Nehra, 2011). Beneficial soil bacteria can contribute to plant growth by increasing nutrient uptake, synthesis of phytohormones (auxin, cytokinin), and solubilizing minerals in the soil (Bowen and Rovira, 1999; Gopalakrishnan *et al.*, 2015). Moreover, *Rhizobium* has the ability to tolerate abiotic stresses such as extremes of salinity and drought, temperature, pH, heavy metal and pesticide pollution (Saharan and Nehra, 2011). However, some previous studies with microbial symbioses have failed not to perform the measurable improvements in crop yield and quality benefits in extreme field conditions (Coyne *et al.*, 2015).

The effect of bacterial stimulation on common bean growth and yield was higher,

especially in the second year of the experiment, as trial plots were the same in both seasons. In the first season, the plants were probably affected by soil composition and fertility. Shoot and root dry matter increased by 12.5-19.1 and 12.6-32.2%, respectively in the inoculated common beans compared to the control (Table 2). However, the combined effect of bacterial strains was more pronounced in increasing root and shoot biomass, seed yield, and seed quality of common bean. Similar to shoot and root growth in response to bacterial inoculations, the same trend was observed in all other vegetative and generative parameters of the common bean. These results are in congruent with previous studies that a bacterial mixture containing *Rhizobium* sp., *Azospirillum* sp. and *Bacillus* increased plant growth, nodulation and yield of common bean as compared with sole inoculation and the control plants (Massoud *et al.*, 2009). Hungria *et al.* (2003) found that *Rhizobium tropici* inoculation of common bean increased nodule dry weight by 33%, root weight by 32%, and shoot weight by 26%. These results could be explained by symbiosis efficiency between common bean and rhizobial strains that increased uptake of nutrients under low levels of available soil N (Goettsch *et al.*, 2017). Similar observations are reported in which a rhizobial strain stimulated root and shoot growth as well as improved salt tolerance of crops through various mechanisms (Bertrand *et al.*, 2015).

Inoculation with rhizobial strains improved morphological parameters and growth of common bean under salt stress in this study. Also, the protein and oil content in the seed were conceivably enhanced with a likely increase in N and other elements uptake due to the inoculation, all of which are highly important for seed quality. Moreover, the co-inoculation of rhizobial strains was more beneficial than their sole application. These results are in agreement with some previous studies where co-inoculation with *Rhizobium* and *Pseudomonas fluorescens* increased growth and yield components such as number of

Pods per plant, number of seeds per pod, weight of 100 seed, mass of seeds per plant, plant biomass, as well as seed yield and protein content in common beans (Yadegari *et al.*, 2010). Stajković *et al.* (2011) conducted an experiment using a mixture of *Rhizobium* and *Pseudomonas* sp. or *Bacillus* sp. that promoted plant growth, N and P contents in common bean. They also reported solubilization of phosphate, production of indole acetic acid, ammonia, and siderophore production by bacterial strains *in vitro*, which altogether could explain common bean growth promotion.

This study showed advantages of bio-inoculants in the common bean production and identified its success in terms of yield, quality, and nutrient use efficiency; particularly bio-inoculants are environment-friendly and licensed for the organic cultivation. Regarding the synergistic effects of the microorganisms, this experiment showed that the rhizobial strains in saline land have the ability to increase the level of root nodulation and plant growth stimulation. The current results highlighted the use of seed inoculation of common bean with *Rhizobium phaseoli* R9 strain, and with additional gains when using *Mesorhizobium ciceri* R6 in co-inoculation. If *Mesorhizobium ciceri* could enhance nodulation of *Rhizobium phaseoli*, then it is also possible that *Mesorhizobium ciceri* established synergistic relations with indigenous rhizobial strains and improved the nodulation of common bean. Furthermore, our study also upholds that co-inoculation of different rhizobia (isolated from different legumes) is a better option than single strain inoculation for enhancing plant growth and yield under soil salinity condition.

Assessment of different beneficial microorganisms and common bean cultivars under indigenous soil-climatic conditions is a potential alternative to enhance bean yield in Uzbekistan by means of sustainable crop production practices. However, in Uzbekistan, this study is the first one, in which native rhizobial strains exhibited a

stimulation effect and improved bean productivity under saline soil condition. These results are important because the use of rhizobial strains as a bacterial fertilizer provides a new technological approach that may reduce chemical fertilizers and help to produce healthy foods. Moreover, rhizobial strains application as an inoculant may improve soil properties by increasing microbial biomass and enzymatic activities. The development of farm management systems that encourage the use of bacterial inoculants with salinity tolerance is necessary to improve common bean yield in saline soil.

CONCLUSIONS

This study confirms that the use of indigenous rhizobial strains improves the productivity of common bean under saline soil condition. The superior performance of this symbiosis contributed to the growth and yield of common bean. A strong correlation between increased nodule dry weight and yield of common bean led to the point that nodule formation is the main factor influencing plant performance under salt stress.

Natural manipulation of plant growth and nutrient uptake by bacterial inoculation would be a potentially useful technology for sustainable crop production without harmful effect to the natural resources. Further efforts are needed to widely use these soil beneficial bacterial strains as a bio-inoculant for common bean production in salt-affected soils of Uzbekistan.

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REFERENCES

1. Araújo, S. S., Beebe, S., Crespi, M., Delbreil, B., Gonzalez, E. M., Gruber, V., Lejeune-Henaut, I., Link, W., Monteros, M. J., Prats, E., Rao, I., Vadez, V. and Patto, M. C. 2015. Abiotic Stress Responses in Legumes: Strategies Used to Cope with Environmental Challenges, *Crit. Rev. Plant Sci.*, 34: 237-280.
2. Benidire, L., Lahrouni, M., El-Khalloufi, F., Göttfert, M. and Oufdou, K. 2017. Effects of *Rhizobium leguminosarum* Inoculation on Growth, Nitrogen Uptake and Mineral Assimilation in *Vicia faba* Plants under Salinity Stress. *J. Agr. Sci. Tech. (JAST)*, 19(4): 889-901.
3. Beiranvand, J. P., Pourbabae, A. A., Shirmardi, S. P., Alikhani, H. A., Abbasi, A. R. and Motesharezadeh, B. 2018. Symbiotic Nitrogen Fixation, Phosphorus and Potassium Uptake Capacity of a Number of Soybean Mutant Lines in a Calcareous Soil. *J. Agr. Sci. Tech. (JAST)*, 20: 1555-1564.
4. Bertrand, A., Dhont, C., Bipfubusa, M., Chalifour, F. P., Drouin, P. and Beauchamp, C. J. 2015. Improving Salt-Stress Responses of the Symbiosis in Alfalfa Using Salt Tolerant Cultivar and Rhizobial Strain. *Appl. Soil Ecol.*, 87: 108-117.
5. Bowen G. D. and Rovira, A. D. 1999. The Rhizosphere and Its Management to Improve Plant Growth. *Adv. Agron.*, 1: 102.
6. Coyne, M. S., Mikkelsen, R. and Mineralization, S. 2015. Soil Microorganisms Contribute to Plant Nutrition and Root Health. *Better Crops*, 99: 18-20.
7. De Meyer, S. E., Van Hoorde, K., Vekemana, B., Braeckmana, T. and Willems, A. 2011. Genetic Diversity of Rhizobia Associated with Indigenous Legumes in Different Regions of Flanders (Belgium). *Soil Biol. Biochem.*, 43: 2384-2396.
8. Figueiredo, M. V., Burity, H. A., Martínez, C. R. and Chanway, C. P. 2008. Alleviation of Drought Stress in the Common Bean (*Phaseolus vulgaris* L.) by Co-Inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici*. *Appl. Soil Ecol.*, 40(1): 182-188.
9. Goettsch, L., Lenssen, A., Yost, R., Luvaga, E., Semalulu, O., Tenywa, M. and Mazur, R. 2016. Improved Production Systems for Common Bean on Phaeozem Soil in South-Central Uganda. *Afr. J. Agric. Res.*, 11(46): 4796-4809.
10. Goettsch, L. H., Lenssen, A.W., Yost, R. S., Luvaga, E. S., Semalulu, O., Tenywa, M., Miiro, R. and Mazur, R. E. 2017. Improved Production Systems for Common Bean on Ferralsol Soil in South-Central Uganda. *Afr. J. Agric. Res.*, 12(23): 1959-1969.
11. Gopalakrishnan, S., Sathya, A., Vijayabharathi, R., Varshney, R. K., Gowda, C. L. and Krishnamurthy, L. 2015. Plant Growth Promoting Rhizobia: Challenges and Opportunities. *3 Biotech.*, 5(4): 355-377.
12. Hardarson, G., Bliss, F. A., Cigalesrivero, M. R., Henson, R. A., Kipenolt, J. A., Longeri, L., Manrique, A., Penacabriales, J. J., Pereira, P. A., Sanabria, C. A. and Tsai, S. M. 1993. Genotypic Variation in Biological Nitrogen-Fixation by Common Bean. *Plant Soil*, 152: 59-70.
13. Hungria, M., Campo, R. J. and Mendes, I. C. 2003. Benefits of Inoculation of the Common Bean (*Phaseolus vulgaris*) Crop with Efficient and Competitive *Rhizobium tropici* Strains. *Biol. Fertil. Soil*, 39(2): 88-93.
14. Hussain, M. B., Mehboob, I., Zahir, Z. A., Naveed, M. and Asghar, H. N. 2009. Potential of Rhizobium spp. for Improving Growth and Yield of Rice (*Oryza sativa* L.). *Soil Environ.*, 28(1): 49-55.
15. Khaitov, B. 2016. Effect of Synergetic Interactions of *Arbuscula micorrhiza* Fungi and Rhizobia on Soybean Yield under Saline Condition. *Genet. Plant Physiol.*, 6(3-4): 176-185.
16. Khaitov, B., Kurbonov, A., Abdiev, A. and Adilov, M. 2016. Effect of Chickpea in Association with Rhizobium to Crop Productivity and Soil Fertility. *Eurasian J. Soil Sci.*, 5(2): 105-112.
17. Kienzler, K. M., Rudenko, I., Ruzimov, J., Ibragimov, N. and Lamers, J. P. A. 2011. Winter Wheat Quantity or Quality? Assessing Food Security in Uzbekistan. *Food Secur.*, 3: 53-64.
18. Keneni, A., Prabu, P. C. and Assefa, F. 2010. Characterization of Acid and Salt Tolerant Rhizobial Strains Isolated from Faba Bean Fields of Wollo, Northern

- Ethiopia. *J. Agr. Sci. Tech. (JAST)*, 12: 365-376.
19. Makoi, J. H., Bambara, S. and Ndakidemi, P. A. 2013. Rhizobium Inoculation and the Supply of Molybdenum and Lime Affect the Uptake of Macroelements in Common Bean (*P. vulgaris* L.) Plants. *Aust. J. Crop Sci.*, 7: 784-794.
 20. Massoud, O. N., Morsy, E. M. and El-Batanony, N. H. 2009. Field Response of Snap Bean (*Phaseolus vulgaris* L.) to N₂-Fixers *Bacillus circulans* and Arbuscular Mycorrhizal Fungi Inoculation through Accelerating Rock Phosphate and Feldspar Weathering. *Aust. J. Basic. Appl. Sci.*, 3: 844-852.
 21. McConnell, M., Mamidi, S., Lee, R., Chikara, S., Rossi, M., Papa, R. and McClean, P. 2010. Syntenic Relationships among Legumes Revealed Using a Gene-Based Genetic Linkage Map of Common Bean (*Phaseolus vulgaris* L.). *Theoretic. Appl. Genet.*, 121(6): 1103-1116.
 22. Mehboob, I., Naveed, M., Zahir, Z.A. and Sessitch, A. 2013. Potential of rhizosphere bacteria for improving Rhizobium-Legume symbiosis. In: "Plant Microbe Symbiosis: Fundamentals and Advances", (Ed.): Arora, N. K. Springer, India, PP. 305-349.
 23. Mehboob, I., Zahir, Z. A., Arshad, M., Tanveer, A. and Azam, F. 2011. Growth Promoting Activities of Different Rhizobium spp. in Wheat. *Pak. J. Bot.*, 43(3): 1643-1650.
 24. Naseri, B., Fathipour, Y. and Talebi, A. A. 2009. Population Density and Spatial Distribution Pattern of *Empoasca decipiens* (Hemiptera: Cicadellidae) on Different Bean Species. *J. Agr. Sci. Tech. (JAST)*, 11: 239-248.
 25. Oliveira-Francesquini, J. P. D., Hungria, M., Savi, D. C., Glienke, C., Aluizio, R., Kava, V. and Galli-Terasawa, L. V. 2017. Differential Colonization by Bioprospected Rhizobial Bacteria Associated to Common Bean in Different Cropping Systems. *Can. J. Microbiol.*, 63(8): 682-689.
 26. Pineda, A., Zheng S. J., van Loon, J. J. A., Pieterse, C. M. J. and Dicke, M. 2010. Helping Plants to Deal with Insects: The Role of Beneficial Soilborne Microbes. *Trends Plant Sci.*, 15: 507-514.
 27. Pourbabaei, A. A., Bahmani, E., Alikhani, H. A. and Emami, S. 2016. Promotion of Wheat Growth under Salt Stress by Halotolerant Bacteria Containing ACC Deaminase. *J. Agr. Sci. Tech. (JAST)*, 18(3): 855-864.
 28. Qushimov, B., Ganiev, I. M., Rustamova, I., Haitov, B. and Islam, K.R. 2007. *Land Degradation by Agricultural Activities in Central Asia*. Climate Change and Terrestrial Carbon Sequestration in Central Asia, Taylor & Francis, London, PP. 137-147.
 29. Rahmani, H. A., Räsänen, L. A., Afshari, M. and Lindström, K. 2011. Genetic Diversity and Symbiotic Effectiveness of Rhizobia Isolated from Root Nodules of *Phaseolus vulgaris* L. Grown in Soils of Iran. *Appl. Soil Ecol.*, 48(3): 287-293.
 30. Remans, R., Ramaekers, L., Schelkens, S., Hernandez, G., Garcia, A., Reyes, J. L., Mendez, N., Toscano, V., Mulling, M., Galvez, L. and Vanderleyden, J. 2008. Effect of Rhizobium-Azospirillum Coinoculation on Nitrogen Fixation and Yield of Two Contrasting *Phaseolus vulgaris* L. Cultivars Cultivated across Different Environments in Cuba. *Plant Soil*, 312: 25-37.
 31. Saharan, B. and Nehra, V. 2011. Plant Growth Promoting Rhizobacteria: A Critical Review. *Life Sci. Med. Res.*, 21: 1-30.
 32. Riehm, H. 1985. Arbeitsvorschrift zur Bestimmung der Phosphorsäure und des Kaliums nach der Laktatmethode. *Zeitschrift für Pflanzen Düngung und Bodenkunde*, 40: 152-156.
 33. Stajković, O., Delić, D., Jošić, D., Kuzmanović, Đ., Rasulić, N. and Knežević-Vukčević, J. 2011. Improvement of Common Bean Growth by Co-Inoculation with Rhizobium and Plant Growth-Promoting Bacteria. *Romanian Biotechnol. Lett.*, 16: 5919-5926.
 34. Suárez, R., Wong, A., Ramírez, M., Barraza, A., Orozco, M. D. C., Cevallos, M. A., Lara, M., Hernández, G. and Iturriaga, G. 2008. Improvement of Drought Tolerance and Grain Yield in Common Bean by Overexpressing Trehalose-6-Phosphate Synthase in Rhizobia. *Mol. Plant Microbe Interact.*, 21(7): 958-966.
 35. Tejera, N.A., Soussi, M. and Lluch, C. 2006. Physiological and Nutritional Indicators of Tolerance to Salinity in Chickpea Plants Growing under Symbiotic Conditions. *Environ. Exp. Bot.*, 58: 17-24.



36. Vakali, C., Baxevanos, D., Vlachostergios, D., Tamoutsidis, E., Papathanasiou, F. and Papadopoulos, I. 2017. Genetic Characterization of Agronomic, Physiochemical, and Quality Parameters of Dry Bean Landraces under Low-Input Farming. *J. Agr. Sci. Tech. (JAST)*, 19(4): 757-767.
37. Vargas, M. A. T., Mendes, I. C. and Hungria, M. 2000. The Response of Field Grown *Phaseolus vulgaris* to Rhizobium Inoculation and Nitrogen Fertilization in Two Cerrado Soils. *Biol. Fertil. Soil*, 32: 228-233.
38. FAO 2016. www.fao.org/faostat/en/
39. Yadegari, M. and Asadi Rahmani, H. 2010. Evaluation of Bean (*Phaseolus vulgaris*) Seeds' Inoculation with Rhizobium Phaseoli and Plant Growth Promoting Rhizobacteria (PGPR) on Yield and Yield Components. *Afr. J. Agric. Res.*, 5: 792-799.

بهبود رشد و مقاومت به شوری در لوبیای معمولی (*Phaseolus vulgaris* L.) با تلقیح همزمان با ریشه های ریزوبیوم بومی

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چکیده

همراهی (همزیستی) سودمند ریشه های ریزوبیومی با گیاهان لگومینه احتمالا منجر به ارتقای گره بندی در ریشه و بهبود عملکرد کلی گیاه می شود. در این پژوهش، موثر بودن تلقیح با ریشه های قبل جدا شده *Rhizobium phaseoli* R9 و *Mesorhizobium ciceri* R6 به صورت تلقیح تکی یا همراه با هم روی لوبیای معمولی (*Phaseolus vulgaris* L.) در یک مزرعه که دارای خاک با شوری ۵/۶ دسی زیمنس بر متر بود در طی دو فصل رشد بررسی شد. تلقیح باکتریایی به طور معناداری باعث افزایش طول گیاه، زیست توده ریشه و شاخسار، عملکرد دانه، تعداد گره ها در هر بوته، وزن خشک گره ها، و طول ریشه لوبیای معمولی شد. در مقایسه با تلقیح تکی، تلقیح همزمان ریشه های ریزوبیومی بر همه پارامترهای گیاهی موثر تر بود و در مقایسه با تیمار تلقیح نشده در طی دو فصل آزمایش به ترتیب باعث افزایشی در حد ۳۵/۱٪ و ۳۷/۹٪ در عملکرد دانه شد. تجزیه شیمیایی دانه، مقدار پروتئین و روغن را در تیمارهای تلقیح همزمان در مقایسه با تیمار شاهد به طور معناداری ($P < 0.05$) بیشتر نشان داد. نتایج آزمایش حاکی از همبستگی قوی ($r^2 = 0.87$) بین افزایش وزن خشک گره ها و عملکرد دانه لوبیای معمولی بود. بنا بر این، می توان نتیجه گرفت که در شرایط شور در ازبکستان، تلقیح همزمان ریشه های ریزوبیوم میتواند موثرترین استراتژی کاربرد زیست کود (biofertilization) برای به دست آوردن گره بندی و عملکرد بیشتر لوبیای معمولی باشد.