

# The effects of rhizobium strains on drought resistance, yield and yield components of common bean in dry land conditions<sup>1</sup>

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## Abstract

**Introduction:** Drought resistance (DR) is defined as the mechanism causing minimum loss of yield in a water deficit environment relative to the maximum yield in a water constraint free management of the crop. Plants have evolved several mechanisms to cope with water deficit stress which includes drought escape and drought tolerance. Investigations have shown that the seed inoculation with rhizobium bacteria was caused higher nitrogen fixation and consequently higher grain yield of bean in dry lands. Rhizobium is a bacterium found in soil that helps in fixing nitrogen in leguminous plants. It attaches to the roots of the leguminous plant and produces nodules. These nodules fix atmospheric nitrogen and convert it into ammonia that can be used by the plant for its growth and development.

Beans are one of the longest-cultivated plants in history. Broad beans, also called fava beans, are in their wild state the size of a small fingernail, and were first gathered in Afghanistan and the Himalayan foothills. An early cultivated form were grown in Thailand from the early seventh millennium BCE, predating ceramics. Beans were deposited with the dead in ancient Egypt. Not until the second millennium BCE did cultivated, large-seeded broad beans appear in the Aegean region, Iberia, and transalpine Europe. In the Iliad (8th century BCE), there is a passing mention of beans and chickpeas cast on the threshing floor.

**Materials and Methods:** Both greenhouse (2018) and field (2019) Experiments were conducted at the Eqolid agricultural research station located at 2375 m above the sea level, latitude [30°55'] N and longitude [52°42'] E. The annual rain is 240 mm, with 12 °C mean

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temperature. Base of De Martonne climate classification, the dry index (I) is 10.6, then the climate of experimental site is semi -arid. The experimental design was a split plot arranged in three completely randomized blocks. The treatments were, irrigation methods including 30, 60 and 80% of soil field capacity as main plots and four types of biological fertilizers including Rh54, Rh58, Rh160 and Rh177, respectively. The experiment was conducted in spring 2018 in greenhouse of the Eqilid agricultural research station. The 72 pots were filled with a mixture of top soil and quartz sand to a total weight of 8 kg. The soil was not autoclaved or steamed. In each pot, five seeds were sown and cover with 1.5 cm quartz sand. The seeds were not surface- sterilized prior to planting. To equalize number of seedlings per pot they were to two on 20 days. During the growing period, each day a pot per plot were weight to determine the average water loss. This measurement was used to determine the water given to irrigation treatments. The field capacity (FC) of soil was determined by using the pressure plate apparatus. Permanent wilting point (PWP) was determined. The soil water deficit of each plot, was determined by weight plot at FC and present weight plot.

**Results and Discussion:** To investigate the effects of the seed inoculation with rhizobium bacteria's strains on yield and drought resistance of common bean, two years' study (greenhouse and field) was conducted on split plot experiment using Complete Block Design with 12 treatments in three replications 2018 and 2019 years. The experimental factors were irrigation methods 30, 60 and 80 % of soil field capacity (FC) as a main plots and four inoculants, including 54, 58, 160 and 177 rhizobia strains as a sub plots. In field the seed yield, biologic yield, irrigation water content, water use efficiency, 100seed weight, pods per plant and seed per pod were determined. In greenhouse the plant height, left area and number leaves per plant, fresh and dry tissue weight, plant nitrogen percent, nodule dry mater and nodule number per plant were determined.

**Conclusions:** The results in two years' pooled data showed that; the main effects of irrigation methods and rhizobia treatments have significant ( $P < 0.05$  %) effects on seed yield and water use efficiency. The highest yield ( $3066 \text{ kg ha}^{-1}$ ) and water use efficiency ( $0.81 \text{ kg m}^{-3}$ ), were obtained at 60 % FC irrigation level with application of 160 strains with no significant difference with 80 % FC. The results showed that; inoculation of seed bean with rhizobium strains mitigates some of the negative effects of drought stress.

**Keywords:** Bean, Drought Stress, Rhizobium, Yield.

## 1. Introduction

Dry bean (*Phaseolus vulgaris* L.) is one of the major leguminous crops grown in the different climatic conditions of Iran. Dry bean is an important legume for human health and provides major of protein content and calorie intake of Iranian people. Drought is important factor for reduction of bean yield in dry land of Iran. A numerous researches indicated that, dry bean is susceptible to some degree of drought stress or water deficiency (Serraj & et al., 2001; Stoyanov 2005). In contrast a lot of studies revealed that drought stress considerably decreased their N<sub>2</sub> fixation, and more broadly dry bean yield potential (Zlatev & Stoyanov, 2005; Cruz Caryalho & et al., 2004; Sinclair & et al., 2001). The quick and effective solutions among agricultural practices' management are smart irrigation and bio-fertilization that can contribute to enhance grain yield under drought stress conditions (Khan & et al., 2007). The most common bio-fertilization microbial inoculants are cost effective, ecofriendly, and renewable sources of plant nutrients (Mahdavi pour & et al., 2009). We hypothesize that inoculation of common bean with Rhizobium strains may be mitigates some of the negative effects of drought stress. It is one of the most beneficial bacteria in agricultural practices. Rhizobium could play an important role in adaptation strategies and increase of tolerance to water stress in bean plant (Emam & et al., 2010). Investigations has shown that certain rhizobium strains enhance plant tolerance to abiotic stresses such as drought (Sarma & Saikia, 2013). Furthermore, these bacteria increase nutrient uptake and plant growth through the effect on morphology and physiology of inoculated plants root (Bresson & et al., 2013; Cruz de Caryalho & et al., 2004). Zahir and et al., reported that, the rhizobacteria having ACC deaminas activity are effective in promoting plant growth and water use efficiency under drought condition (Zahir & et al., 2008). The inoculation of peanut, increased of total dry matter, and this result must be linked to better development and efficiency of root system, in which presents higher nitrogen absorption using the nodulation process (Delfini & et al., 2010).

Therefore, in this study we highlighted a new concept by which rhizobacteria can enhance plant performance under both normal irrigation water supply and drought soil conditions. This study was conducted to evaluate the effects of rhizobium strains on drought resistance, growth and symbiotic related parameters, yield and yield components of common bean in dry land conditions. At harvest the seed yield, biologic yield, irrigation water content, water use efficiency, 100seed weight, pods per plant and seed per pod were determined.



## 2. Materials and Methods

### 2-1. experimental site

Both greenhouse (2018) and field (2019) Experiments were conducted at the Eqlid agricultural research station located at 2375 m above the sea level, latitude [30°55'] N and longitude [52°42'] E. The annual rain is 240 mm, with 12° mean temperature. Base of De Martonne climate classification, the dry index (I) is 10.6, then the climate of experimental site is semi -arid.

### 2-2. Experimental design and treatments

The experimental design was a split plot arranged in three completely randomized blocks. The treatments were, irrigation methods including 30, 60 and 80% of soil field capacity as main plots and four types of biological fertilizers including Rh54, Rh58, Rh160 and Rh177 inoculants as subplots (Figure1).

FC=30%				FC=60%				FC=80%			
RH177	RH54	RH58	RH160	RH177	RH54	RH58	RH160	RH177	RH54	RH58	RH160
RH160	RH177	RH54	RH58	RH54	RH58	RH160	RH177	RH54	RH160	RH177	RH58
RH54	RH58	RH160	RH177	RH160	RH177	RH54	RH58	RH160	RH58	RH54	RH177

**Fig- 1.** Pilot design of treatments and replications.

### 2-3. General Greenhouse Techniques

The experiment was conducted in spring 2018 in greenhouse of the Eqlid agricultural research station. The 72 pots were filled with a mixture of top soil and quartz sand to a total weight of 8 kg. The soil was not autoclaved or steamed. In each pot, five seeds were sown and cover with 1.5 cm quartz sand. The seeds were not surface- sterilized prior to planting. To equalize number of seedlings per pot they were to two on 20 days. During the growing period, each day a pot per plot were weight to determine the average water loss. This measurement was used to determine the water given to irrigation treatments. The field capacity (FC) of soil was determined by using the pressure plate apparatus. Permanent wilting point (PWP) was determined. The soil water deficit of each plot, was determined by weight plot at FC and present weight plot.

Growth data were collected after 60 days from sowing were plant height, left area and number leaves per plant, fresh and dry tissue weight, plant

nitrogen percent, nodule dry mater and nodule number per plant. All of the growth data were collected in one pot and outer pot were left for yield-related parameters.

## 2-4. Field trial

This study was conducted on a farm that was located at a distance of 270 km from north of Shiraz city. The gross area of this project, was approximately 600 m<sup>2</sup> with longitude and latitude of 52o42 and 30o55 respectively and its height was 2375 meters above sea level. Each subplot had four rows with 2.0 m width and 5.0 m length (10 m<sup>2</sup>). The rows were 0.50 m apart from each other. Bean variety used in this project, entitled as the Talash. The bean variety of this plan was planted manually in May.

For applying different water regimes, the following equation (1) was used. This method was according to the usage of soil moisture index or soil matric potential. In this method, the soil moisture percentage was measured through sampling of plant root at before irrigation. When the weighted of soil moisture reached the allowed depletion to deficit irrigation treatment, the irrigation process happened. The deficit irrigation treatments took place in the 5 to 6 leaf stage, after full settlement of seedlings.

$$\text{SMD} = (\text{FC}-\text{WP}) \text{ Bd. Dp. F} \quad (1)$$

**Where SMD:** soil moisture deficit (cm), FC: field capacity moisture, WP: weight percent of available moisture in the soil of the farm, F: each treatment coefficient (0.5, 0.75 and 1), Bd: bulk density (gr/cm<sup>3</sup>) and Dp: plant root development depth (cm).

All vegetative growth-related parameters were collected from middle row of each plot while the other four rows out of five were left for yield-related parameters. The boarder rows were left untouched. Growth data were collected after 60 days from sowing were plant height, left area and number leaves per plant, fresh and dry tissue weight, plant nitrogen percent, nodule dry mater and nodule number per plant.

At harvest the seed yield, biologic yield, irrigation water content, water use efficiency, 100seed weight, pods per plant and seed per pod were determined.

## 2-5. Statistical analysis

Data were analyzed using SAS statistical software program. The treatment means were compared using Duncan's test significance difference.



### **3. Result and Discussion**

#### **3-1. Seed Yield and Biological Yield (in field experiment)**

There was a significant difference ( $P < 0.05$ ) in yield grain and biological yield between the irrigation methods as well as between different bacterial strains. The most of yield grain (3066 kg/ha) and biological yield (6882 kg/ha) were obtained in irrigation at 60% field capacity with 160 rhizobium strain seed inoculated treatment (Table1).

The results showed that, rhizobacteria, had substantial effects on seed yield and biological yield particularly under moderate drought stress (Figure2). The seed yield and biological yield of bean inoculated in severe stress (S30=irrigation at 30% FC) was strongly decreased, but the yield and biological yield in the moderate stress (S60) was same in normal irrigation treatment (S80). Alleviation of drought stress by rhizobium and increasing the yield in drought condition was reported by some researchers (Ghorbanpour & et al., 2013; Zahir & et al., 2008 & Zahran 199). Inoculation of peas with rhizobacteria significantly decreased the “drought stress imposed effects” on growth of peas, although with variable efficacy at different moisture levels. It is highly likely that rhizobacteria might have decreased the drought-stress induced ethylene in inoculated plants, which resulted in better growth of plants even at low moisture levels. Therefore, inoculation with rhizobacteria could be helpful in eliminating the inhibitory effects of drought stress on the growth of peas (Zahir & et al., 2008). Prolonged vegetative growth and delayed flowering induced by PGPR are new in the context of plant–microorganism interactions and may be promising for agronomy. Delaying flowering time by rhizobacteria inoculation could represent a valuable strategy for increasing biomass yield (Bresson & et al., 2013). Inoculation of *Hyoscyamus niger* roots with PGPR strain not only alleviated the deleterious stress effects on plant growth to some extent, it also improved the growth parameters under WDS and increased elicitation of tropane alkaloid production.

#### **3-2. Water use efficiency in field**

As water irrigation purpose becomes increasingly scarce because of climate change and population growth, there is growing interest in regulated deficit irrigation as a way to improve efficiency of water usage and farm productivity in arid and semi-arid areas (Burgault & et al., 2010; Urmi Jahan & et al., 2022). The results (table1) indicated that, the water irrigation content was decreased by increasing of drought stress. Mean of water irrigation were 3288,

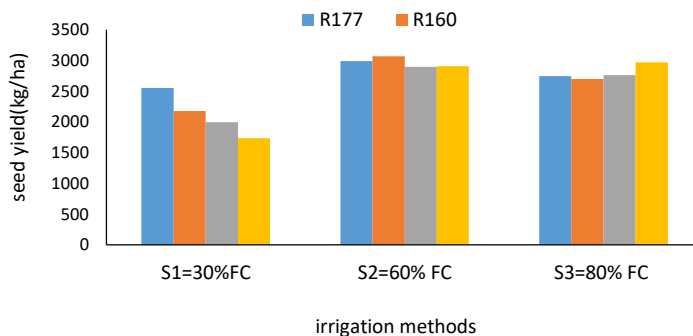


3794 and 4523  $\text{m}^3\text{ha}^{-1}$  in 30, 60 and 80 % irrigation respectively. The results show that, there was a significant difference ( $P < 0.05$ ) in water use efficiency between treatments, and the highest water use efficiency ( $0.81 \text{ kgm}^{-3}$ ) was obtained in moderate stress ( $S_{60}$ ) with 160 rhizobium strain seed inoculated (Table1). The highest of water use efficiency was due to the improvement the yield and decreasing the water irrigation content in moderate water stress. By reaching soil water potential to  $-0.9 \text{ MPa}$  and exerting drought stress for 14 days, osmotic potential and turgor pressure in first leaf of bean were strongly decreased (Tagor & et al., 2013). The WUE of inoculated plants was significantly improved under water deficit and there is not any significant change in WUE in response to drought in noninoculated plants (Brsson & et al., 2013). The higher WUE of inoculated plants was mainly a result of a significantly lower water loss through daytime and night-time transpiration, which may reflect a better drought avoidance strategy. Zahir & et al. (2008) reported that, the rhizobacteria having ACC deaminase activity are effective in promoting plant growth and water use efficiency under drought conditions, by lowering the ethylene or ACC accumulation whose higher levels have inhibitory effects on root and shoot growth.

**Table1-** Mean values for water use efficiency (WUE), water irrigation, biological yield and seed yield of bean inoculated with Rhizobium strains at different irrigation levels. (In field trial)

Treatments	WUE (kgm <sup>-3</sup> )	Irrigation content (m <sup>3</sup> ha <sup>-1</sup> )	Biol. Yield (kg ha <sup>-1</sup> )	Seed yield (kg ha <sup>-1</sup> )	
S <sub>30</sub>	Rh <sub>54</sub>	0.52 <sup>c</sup>	3288 <sup>c</sup>	4495 <sup>c</sup>	1733 <sup>c</sup>
	Rh <sub>160</sub>	0.66 <sup>b</sup>	3288 <sup>c</sup>	4991 <sup>c</sup>	2177 <sup>bc</sup>
	Rh <sub>177</sub>	0.77 <sup>ab</sup>	3288 <sup>c</sup>	5890 <sup>b</sup>	2551 <sup>ab</sup>
	Rh <sub>58</sub>	0.61 <sup>b</sup>	3288 <sup>c</sup>	4961 <sup>c</sup>	1996 <sup>c</sup>
S <sub>60</sub>	Rh <sub>54</sub>	0.77 <sup>ab</sup>	3794 <sup>b</sup>	6603 <sup>ab</sup>	2908 <sup>a</sup>
	Rh <sub>160</sub>	0.81 <sup>a</sup>	3794 <sup>b</sup>	6882 <sup>a</sup>	3066 <sup>a</sup>
	Rh <sub>177</sub>	0.79 <sup>ab</sup>	3794 <sup>b</sup>	6659 <sup>a</sup>	2991 <sup>a</sup>
	Rh <sub>58</sub>	0.76 <sup>ab</sup>	3794 <sup>b</sup>	6728 <sup>ab</sup>	2895 <sup>a</sup>
S <sub>80</sub>	Rh <sub>54</sub>	0.61 <sup>b</sup>	4523 <sup>a</sup>	5983 <sup>b</sup>	2743 <sup>a</sup>
	Rh <sub>160</sub>	0.65 <sup>b</sup>	4523 <sup>a</sup>	6495 <sup>ab</sup>	2968 <sup>a</sup>
	Rh <sub>177</sub>	0.59 <sup>c</sup>	4523 <sup>a</sup>	5921 <sup>b</sup>	2700 <sup>a</sup>
	Rh <sub>58</sub>	0.61 <sup>c</sup>	4523 <sup>a</sup>	6138 <sup>ab</sup>	2759 <sup>a</sup>

The number with same character are no significant at %5.



**Fig- 2.** Intraction Rhizobium strain and drought stress on seed yield of bean

### 3-3. Nodule Dry Mater and Nodule Number per

There was a significant difference ( $P < 0.05$ ) in nodule dry mater between drought stress and rhizobium strains (Table 2). The drought stress was a significant difference on nodule dry mater but haven't a significant difference on nodule number, reverse, rhizobium strains have a significant difference on nodule number and haven't a significant difference on nodule dry matters. The most of nodule number (63 per plant) and nodule dry matters (0.458 gr/plant) were obtained in irrigation at 80% filed capacity (S80=normal irrigation) and 177 rhizobium strain seed inoculated treatment. Nodule dry mater was reduced by increasing drought stress levels. Nodule number and nodule dry matter of legumes were all reduced by low drought stress levels. A favorable rhizosphere environment is highly important for the interaction between root hairs and Rhizobium as it does not only encourage the growth and multiplication of Rhizobia, but also ensures the healthy development of root hairs (Zahran, 1999). Any environmental stress that affecting these processes is also likely to effect on infection and nodulation (Cordovilla & et al., 1999). In a study was showed that, the native strains had significant effect on the number of nodule and nodule weight (Karaca & Uyanoz, 2012). Other studies with native inoculation or Rhizobium sp. and dry bean have shown that isolated strains used significantly ( $P < 0.05$ ) increased nodulation and other morphological parameters (Ozdemir 2002; Slattery & et al., 2004). Beside co-inoculation studies with PGPR and Rhizobium have been shown to increase nodule dry matter,  $N_2$ -fixation, and grain yield in common bean (Burdman & et al., 1997).



### 3-4. Plant Nitrogen Percent

There was a significant difference ( $P < 0.05$ ) in plant nitrogen between drought stress and rhizobium strains (Table 2). Highest the plant nitrogen (3.36 %) was obtained in moderate stress ( $S_{60}$ ) with 160 rhizobium strain seed inoculated of bean. Increasing of tissue nitrogen was due to improve of plant height and leaf area in this treatment. The nitrogen fixation was increased with improvement of number and matter nodules (Table 2) and growth of plant (Table 3), therefore the plant nitrogen content was increased in moderate stress. We know that, nodule size affects rate of oxygen uptake and it thus seem clear that transport of oxygen across a nodule may be a limiting factor in nitrogen fixation under normal condition. Effects of this type offer a possible explanation for the reversible effects of moderate moisture stress on nitrogen fixation (Sprent, 1971). Pate (1969) suggested that water could be a limiting factor in nitrogen fixation by restricting export from nodules.

### 3-5. Plant Dry Weight and Fresh Plant Weight

The shoot dry weight was significantly different among the treatments (Table 2). The maximum dry shoot weight was recorded in moderate stress ( $S_{60}$ ) with 177 rhizobium strain seed inoculated of bean. Of course the shoot dry weight was no significantly different between moderate ( $S_{60}$ ) and non-stress ( $S_{80}$ ) treatments. The shoot dry weight was decreased with increasing drought stress. Rosales & et al. (2004) have also reported significant differences in shoot biomass accumulation among dry bean cultivars grown under moderate to severe drought stress conditions. Increase the shoot dry weight in moderate stress may be due to the effect of 177 rhizobium strain on growth and symbiotic related parameters of bean. Bacterial inoculations improved common bean growth. Tagore (2013) was reported, symbiotic traits of inoculated chickpea indicated that shoot dry weight increased progressively and nodule number, nodule fresh weight, nodule dry weight also followed the similar trend at 35 and 55 date after sowing (DAS), but the decline was noted in nodule number, fresh weight, and dry weight of nodules at 75 DAS. This was mainly due to decay of nodular tissues at pod formation, which start from 60 to 65 DAS. In the present study, the shoot fresh weight was no significantly different among the treatments. The highest fresh weight same as dry weight was in moderate stress ( $S_{60}$ ) with 177 rhizobium strain seed inoculated.



**Table-2.** Mean values for Plant dry weight, fresh plant weight, plant nitrogen, nodule number and nodule weight of bean inoculated with Rhizobium strains at different drought stress levels (greenhouse).

Treatments	Shoot Dry Weight (gplant <sup>-1</sup> )	Shoot Fresh Weight (gplant <sup>-1</sup> )	Plant Nitrogen (%)	Nodule No. per Plant	Nodule Dry Mater (gplant <sup>-1</sup> )	
S <sub>30</sub>	Rh <sub>54</sub>	8 <sup>bc</sup>	49 <sup>a</sup>	3.15 <sup>ab</sup>	2 <sup>ef</sup>	0.1223 <sup>b</sup>
	Rh <sub>160</sub>	9 <sup>b</sup> <sup>c</sup>	47 <sup>a</sup>	3.35 <sup>ab</sup>	1 <sup>ef</sup>	0.1000 <sup>b</sup>
	Rh <sub>177</sub>	10 <sup>b</sup>	51 <sup>a</sup>	2.85 <sup>b</sup>	9 <sup>d</sup>	0.1693 <sup>b</sup>
	Rh <sub>58</sub>	8 <sup>b</sup> <sup>c</sup>	49 <sup>a</sup>	2.85 <sup>b</sup>	3 <sup>ef</sup>	0.1178 <sup>b</sup>
S <sub>60</sub>	Rh <sub>54</sub>	15 <sup>ab</sup>	62 <sup>a</sup>	2.98 <sup>b</sup>	25 <sup>bc</sup>	0.1777 <sup>b</sup>
	Rh <sub>160</sub>	16 <sup>ab</sup>	63 <sup>a</sup>	3.69 <sup>a</sup>	35 <sup>b</sup>	0.1893 <sup>b</sup>
	Rh <sub>177</sub>	19 <sup>ab</sup>	75 <sup>a</sup>	3.36 <sup>ab</sup>	16 <sup>cd</sup>	0.2397 <sup>ab</sup>
	Rh <sub>58</sub>	17 <sup>ab</sup>	72 <sup>a</sup>	3.11 <sup>ab</sup>	13 <sup>cd</sup>	0.2083 <sup>ab</sup>
S <sub>80</sub>	Rh <sub>54</sub>	15 <sup>ab</sup>	61 <sup>a</sup>	3.25 <sup>ab</sup>	63 <sup>a</sup>	0.4583 <sup>a</sup>
	Rh <sub>160</sub>	16 <sup>ab</sup>	63 <sup>a</sup>	3.25 <sup>ab</sup>	33 <sup>b</sup>	0.3357 <sup>ab</sup>
	Rh <sub>177</sub>	17 <sup>ab</sup>	70 <sup>a</sup>	3.45 <sup>ab</sup>	19 <sup>bcd</sup>	0.2090 <sup>ab</sup>
	Rh <sub>58</sub>	16 <sup>ab</sup>	67 <sup>a</sup>	2.99 <sup>b</sup>	34 <sup>b</sup>	0.2640 <sup>ab</sup>

The number with same character are no significant at %5

### 3-6. 100 Seed Weight

There was a significant difference ( $P < 0.05$ ) in 100 seed weight between in rhizobium strains and irrigation treatments (Table 3). The 100 seed weight was decreased by increasing of water deficit stress (Figure3). The highest 100 seed weight was obtained in moderate water stress (60 % FC) with 160 rhizobium strain seed inoculated treatment (Figure4). The increasing of seed weight by rhizobium in drought stress was reported by Bresson & et al. (2013).

### 3-7. Pods per Plant and Seeds per Pod

Highest of pod per plant (17.3) and seed per pod (10.3) were obtained in moderate stress (S60) with 177 and 160 rhizobium strains respectively (Table 3). These results showed that, the increasing of yield and yield components in seed bean inoculated with rhizobium strains was due to enhanced of pods per plant and seed per pod in drought condition. The results are in agreement with Sarma and Saikia, (2013) who found there was an increase in pod numbers and dry weight by 43.5 to 48.6 % and 37.9 to 39.2 %, respectively, in bacteria-inoculated stress-experienced plants as compared with the untreated stressed plants. Means comparison for bacteria applications indicated that the



maximum number of pods per plant (71.83) was obtained from 'Khomein' variety also for this treatment. The minimum number of pods per plant (29) belonged to control treatment of bacteria (without grain inoculation) in local variety (Naseri & et al., 2014)

### **3-8. Plant height**

There was a significant difference in bean growth between the levels of drought stress as well as between different bacterial strains. The plant height was significantly affected by irrigation treatments (Table3). We found that, the highest plant height (134cm) was obtained in moderate stress (S60) that have no significant difference with normal irrigation treatment (S80). Although the plant height was decreased in severe stress (S30). Water stress depressed plant height and the shortest plants were produced at higher water stress levels (Emam & et al., 2010). Increasing of plant height in moderate stress was due to rhizobium strains, especially 160 rhizobium strain. Naseri rad (2014) reported that the tallest variety of common bean ('Talash') was positively affected by bacteria compared to the plot with no bacteria applied.

### **3-9. Leaf Area and Number of leaves per plant**

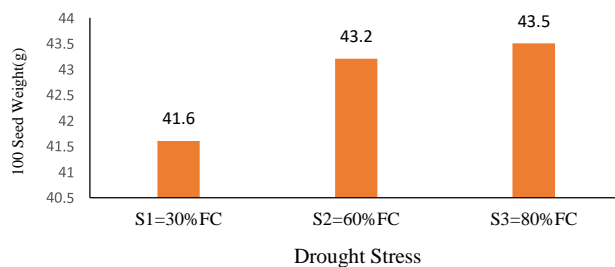
There was a significant difference ( $P < 0.05$ ) in leaf area between irrigation treatments. But there isn't significant difference in leaf area between rhizobium strains (Table3). Highest leaf area ( $632 \text{ cm}^2 \text{ plant}^{-1}$ ) was obtained in moderate water stress (S<sub>60</sub>) with 177 rhizobium strain seed inoculated of bean (Table3). Highest leaf number per plant (50) was obtained in irrigation at 60 % FC with 160 rhizobium seed inoculated treatment. Our results showed that, leaf area and leaves number of bean were decreased by increasing drought stress. But seed rhizobium inoculated in moderate water stress (S60) have significant effect on leaf area and leaf number per plant. Highest effect of rhizobium on improve of leaf is in flowering and pod filling stages. Inoculation of bean plants with rhizobium partially or completely eliminated the "drought stress imposed effects" on root and shoot growth, leaf area, and number of leaves per plant of bean. Leaf area is an important physiological component of crop yield being itself a complex character. Leaf morphology within a canopy usually reflects a tradeoff between photosynthesis per unit leaf area and light interception per leaf; thicker leaves allow greater photosynthetic apparatus per unit leaf area, while larger and thinner leaves can intercept more light (White & Montes 2005). The leaf area of common bean genotypes was highly correlated at pod setting and also at early pod filling (Trindade & et al., 2010).



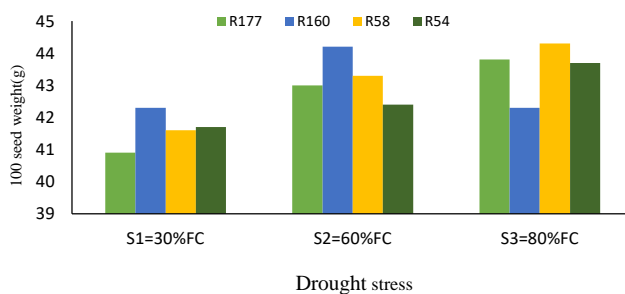
**Table- 3.** Mean values for leaf area, seed per pod, pods per plant, and 100 seed weight of bean inoculated with Rhizobium strains at different drought stress levels in field trial.

Treatments	100-Seed Weight(g)	Pods/plant	Seed/pod	Leaf area (cm <sup>2</sup> /plant)	Plant height (cm)	
S <sub>30</sub>	Rh <sub>54</sub>	40 <sup>abcd</sup>	15 <sup>b</sup>	2.3 <sup>c</sup>	380 <sup>b</sup>	86 <sup>b</sup>
	Rh <sub>160</sub>	42 <sup>abc</sup>	16 <sup>ab</sup>	5.3 <sup>b</sup>	361 <sup>b</sup>	84 <sup>b</sup>
	Rh <sub>177</sub>	37 <sup>cde</sup>	15.6 <sup>b</sup>	3 <sup>c</sup>	354 <sup>b</sup>	88 <sup>b</sup>
	Rh <sub>58</sub>	39b <sup>cd</sup>	15.6 <sup>b</sup>	4.3 <sup>bc</sup>	356 <sup>b</sup>	90 <sup>b</sup>
S <sub>60</sub>	Rh <sub>54</sub>	35 <sup>c</sup>	16 <sup>ab</sup>	10.3 <sup>a</sup>	551 <sup>ab</sup>	86 <sup>b</sup>
	Rh <sub>160</sub>	44 <sup>a</sup>	17.3 <sup>a</sup>	5.3 <sup>b</sup>	531 <sup>ab</sup>	134 <sup>a</sup>
	Rh <sub>177</sub>	43 <sup>ab</sup>	16.6 <sup>ab</sup>	3 <sup>c</sup>	632 <sup>a</sup>	105 <sup>ab</sup>
	Rh <sub>58</sub>	41 <sup>abc</sup>	17 <sup>a</sup>	5.3 <sup>b</sup>	616 <sup>a</sup>	127 <sup>a</sup>
S <sub>80</sub>	Rh <sub>54</sub>	39 <sup>bcd</sup>	15.6 <sup>b</sup>	4.3 <sup>bc</sup>	334 <sup>b</sup>	100 <sup>ab</sup>
	Rh <sub>160</sub>	38 <sup>dc</sup>	14.6 <sup>b</sup>	4b <sup>c</sup>	360 <sup>b</sup>	105 <sup>ab</sup>
	Rh <sub>177</sub>	43 <sup>ab</sup>	16 <sup>ab</sup>	2 <sup>c</sup>	365 <sup>b</sup>	102 <sup>ab</sup>
	Rh <sub>58</sub>	39 <sup>bvd</sup>	13.3 <sup>c</sup>	8 <sup>ab</sup>	388 <sup>b</sup>	101 <sup>ab</sup>

The number with same character are no significant at %5.



**Fig- 3.** 100 seed weight of bean in water stress treatments



**Fig- 4.** Intraction between rhizobium inoculation and drought stress on 100 seed weight of bean



All the strains that used in this experiment were effective on agronomic properties, yield and yield components of common bean in drought condition. Base of the results in this experiment we can say that, there are some organisms same as rhizobium bacteria that can withstand long periods in a dried state and have the capacity to rehydrate and restart their metabolic functions after being in contact with water. It has been reported that the some rhizobacteria help plants to maintain a favorable water status under water deficit by enhancing the development of shoot and root system (Clegg, 2001; Cruz de Carylho & et al., 2004).

#### **4. Conclusions**

It is evident from the results that exposure of bean plants to decreasing soil moisture levels caused reduction in all growth parameters of bean. This effect could be due to the sensitivity of beans to drought stress that might have affected different physiological and metabolic processes contributing to the growth and development processes of bean plants. Rhizobacteria have substantial effects on plant growth, particularly under stress conditions, and play an important role in plant physiology by a combination of direct and indirect mechanisms. In our study, the rhizobium inoculation had positive effects on plant growth parameters, yield, yield components and water use efficiency in moderate water stress (60% FC). The highest yield (3066 kg/ha) and water use efficiency (0.81 kg m<sup>-3</sup>), were obtained at 60 % FC irrigation level with application of 160 strains that, haven't significant difference with 80 % FC. We consider that the isolated strains of rhizobium have an ability to alleviate of drought stress in the common bean and thus have a commercial potential. However, Rhizobium strains are to be genetically identified before they are being recommended for commercial inoculations.

#### **5. Acknowledgments**

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#### **6. Conflict of interest**

None declared.



## References

- Bresson, J., Varoquaux, F., Bontpart, T., Touraine, B. & Vile, D. (2013). The PGPR strain *Phyllobacterium brassicacearum* STM196 induces a reproductive delay and physiological changes that result in improved drought tolerance in *Arabidopsis*. *New Phytologist*, 200: 558–569.
- Burdman, S., Kigel, J. & Okon, Y. (1997). Effect of *Azospirillum brasilense* on nodulation and growth of common bean (*Phaseolus vulgaris* L.). *Soil Biology and Biochemistry*, 29: 923–929.
- Burgault, M., Madramootoo, C.A. & Webber, H.A. (2010). Effects of deficit irrigation and salinity stress on common bean (*Phaseolus Vulgalis* L.) and mung bean (*Vigna radiate* (L) Wilczek) grown in a controlled environment. *Journal Agronomy and Crop Science*, 196(4): 262-272.
- Clegg, J.S. (2001). Cryptobiosis-a peculiar state of biological organization Comp. *Biochemistry Physiology*, 128: 613-624.
- Cordovilla, M.P., Ligerio, F. & Lluch C. (1999). Effect of salinity on growth nodulation and nitrogen assimilation in nodules of faba bean (*Vicia faba* L.). *Applied Soil Ecology*, 11: 1-7.
- Cruz de Carvalho, M.H., Laffray, D. & Louguet, P. (2004). Comparison of the physiological responses of *Phaseolus vulgaris* and *Vigna unguiculata* cultivars when submitted to drought conditions. *Environmental and Experimental Botany*, 40: 197-207.
- Delfini, R., Belgoff, C., Fernández, E., Fabra, A. & Castro, S. (2010). Symbiotic nitrogen fixation and nitrate reduction in two peanut cultivars with different growth habit and branching pattern structures. *Plant Growth Regulation*, 6: 153-159.
- Emam, Y., Shekoofa, A., Salehi, F. & Jalali, A.H. (2010). Water Stress Effects on Two Common Bean Cultivars with Contrasting Growth Habits. *American-Eurasian Journal of Agricultural and Environmental Sciences*, 9(5): 495-499.
- Ghorbanpour, M., Hatami M. & Khavazi K. (2013). Role of plant growth promoting rhizobacteria on antioxidant enzyme activities and tropane alkaloid production of *Hyoscyamus niger* under water deficit stress. *Turkish Journal of Biology*, 37: 350-360.
- Karaca, U. & Uyanöz, R. (2012). Effectiveness of native *Rhizobium* on nodulation and growth properties of dry bean (*Phaseolus vulgaris* L.). *African Journal of Biotechnology*, 11(37): 8986-8991.
- Khan, M.S., Zaidi, A. & Wani, P.A. (2007). Role of phosphate-solubilizing microorganisms in sustainable agriculture—a review. *Agronomy for Sustainable Development*, 27(1): 29–43.



- Mahdavi pour, A., Rezaei, M., Asgharzadeh, A. & Cheraty, A. (2009). Effect of different strains of bacteria brady rhizobium japonicum on micronutrient uptake in shoot and seed yield of soybean. *Journal of Vegetation Science*, 4(16).
- Mundree, S.G., Baker, B., Mowla, S., Peters, S., Marais, S., Willigen, C.V., Govender, K., Maredza, A., Muyanga, S., Farrant, J.M. & Thomson, J.A., (2002). Physiological and molecular insights into drought tolerance. *African Journal of Biotechnology*, 1: 28–38.
- Naseri rad, H., Sayadi, V. & Naseri rad, A. (2014). Effect of Rhizobium Bacteria (*Rhizobium leguminosarum*) and Nano-Iron Application on Yield and Yield Components of Different Pinto Beans Genotypes. *Agricultural Communications*, 2(2): 22-27.
- Özdemir, S. (2002). Yemeklik tane baklagiller. *Hasad yayıncılık*: 28-46.
- Pate, J.S., Gunning, B.E.S. & Briarty, L.G. (1969). Ultrastructure and functioning of the transport system of the leguminous root nodules. *Planta*, 85, II.
- Rosales-Serna, R., Kohashi-Shibata, J., Acosta- Gallegos, J.A., Lopez, C.T., Ortiz-Cereceres, J. & Kelly, J.D. (2004). Biomass distribution, maturity acceleration and yield in drought-stressed common bean cultivars. *Field Crops Research*, 85: 203-211.
- Sarma, R.K. & Saikia, R. (2013). Alleviation of drought stress in mung bean by strain *Pseudomonas aeruginosa* GGRJ21. *Plant Soil*. DOI:10.1007/s11104-013-1981-9
- Serraj, R., Vadez, V. & Sinclair, T.R. (2001). Feedback regulation of symbiotic N<sub>2</sub> fixation under drought stress. *Agronomy Journal*, 21: 621–626.
- Sinclair, T.R., Purcell, L.C., Vadez, V. & Serraj, R. (2001). Selection of soybean (*Glycine max*) lines for increased tolerance of N<sub>2</sub> fixation to drying soil. *Agronomy Journal*, 21: 653–657.
- Slaterry, J.F., Pearce, D.J. & Slaterry, W.J. (2004). Effects of resident rhizobial communities and soil type on the effective nodulation of pulse legumes. *Soil Biology, Biochemistry*, 36: 1339-1346.
- Sprent, J.I. (1971). The effects of water stress on nitrogen fixing root nodules. *New Phytology*, 70: 9-17.
- Stoyanov, Z.Z. (2005). Effects of water stress on leaf water relations of young bean (*Phaseolus vulgaris* L.). *Journal Central European Agriculture*, 6: 5-14.
- Tagore, G.S., Namdeo, S.L., Sharma, S.K. & Kumar, N. (2013). Effect of Rhizobium and Phosphate Solubilizing Bacterial Inoculants on Symbiotic Traits, Nodule Leghemoglobin, and Yield of Chickpea Genotypes. *International Journal of Agronomy*. DOI: <http://dx.doi.org/10.1155/2013/581627>.
- Trindade, R.S., Araújo, A.P. & Teixeira, M.G. (2010). Leaf area of common bean genotypes during early pod filling as related to plant adaptation to limited phosphorus supply. *Revista Brasileira Ciencia do Solo*, 34: 115-124.



- Urmi Jahan, T., Nahrin Jannat, H., Md Mahfuzul, H. & Md Redwanul, I. (2022). Socio-hydrological Resilience to Climate-induced Drought: A case of Naogaon, Bangladesh. *Water Productivity Journal*, Articles in Press,  
**DOI:** <http://dx.doi.org/10.22034/wpj.2021.270875.1031>
- White, J.W. & Montes, C. (2005). Variation in parameters related to leaf thickness in common bean (*Phaseolus vulgaris* L.). *Field Crops Research*, 91: 7-21.
- Zahir, Z.A., Munir, A., Asghar, H.N., Shaharoona, B. & Arshad, M. (2008). Effectiveness of Rhizobacteria Containing ACC Deaminase for Growth Promotion of Peas (*Pisum sativum*) Under Drought Conditions. *Journal Microbiology Biotechnol*, 18(5): 958–963.
- Zahran, H.H. (1999). Rhizobium-Legume Symbiosis and Nitrogen Fixation under Severe Conditions and in an Arid Climate. *Microbiology and Molecular Biology Reviews*, 63(4): 968-989.
- Zlatev, Z. & Stoyanov, Z. (2005). Effects of water stress on leaf water relations of young bean plants. *Journal Central European Agriculture*, 6: 5-14.