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Influence of Rhizobium, Pseudomonas and Mycorrhiza on Some Physiological Traits of Red Beans (*Phaseolus vulgaris* L.) under different irrigation conditions

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ABSTRACT

This study was conducted to evaluate response red bean (CV. Goli) under drought stress conditions for three types of biological fertilizers in 2014 - 2015 years. A split plot experiment was conducted in Tabriz, Iran. Irrigation levels (irrigation after 70, 110 and 150 mm of evaporation from pan) were considered as main plots, and biological fertilizers Rhizobium (Rp), Mycorrhiza (My), Pseudomonas (Ps), Rp + My, Rp + Ps, Rp + My + Ps and non-inoculation (control) were considered as sub plots. Combination of Rhizobium, Mycorrhiza, and Pseudomonas at irrigation level of more than 70 mm of evaporation had the highest grain yield, RWC, CCI, stomata conductance, leaf water potential and lowest cell leakage. Triple inoculation increased grain yield in comparison to the control, or to the time they were used individually or simultaneously; it also reduced the negative effects of drought stress on the beans.

Key words: Arbuscular, Beans, Drought stress, Pseudomonas, *Rhizobium*.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) has always faced numerous limitations and problems. Only 7 % of the total land dedicated to beans plantation globally has sufficient water and 60 % of beans agriculture is carried out under intense drought stress conditions (Bourgault *et al.,* 2013). Due to absorption of more food and water from the soil, the plants with Mycorrhiza symbiosis have better growth and yield and have better resistance against environmental stresses (Haghighatnia *et al.,* 2013). Plant growth-promoting rhizobacteria (PGPR) increase the plant's productivity and safety as well as activate induced systemic tolerance (resistance) (ISR) against abiotic stresses including drought and salinity (Yang *et al.,* 2009).

Espidkar *et al.* (2016) claimed that Mycorrhiza increases the bacterial inoculation efficiency of pseudomonas in the young plant and increases grain yield by increasing the absorption of nutrients and resistance against drought. Suarez *et al.* (2008) reported that the inoculation of beans with Rhizobium under drought stress conditions increased performance by 30% in comparison to the control. Owing to the significance of using appropriate methods for reducing the negative effects of drought, the purpose of the present study is to investigate the effects of *Mycorrhiza arbuscular* and the growth promoting bacteria of Rhizobium and Pseudomonas on some physiological traits and grain yield of Goli red bean under drought conditions.

MATERIALS AND METHODS

In order to determine the effect of bio-fertilizers on Goli red bean under different irrigation conditions, an experiment was conducted in 2014 and 2015 in the Faculty of Agriculture,

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Islamic Azad University, Tabriz, Iran. The altitude of the site is 1360 m above sea level(asl), lying at longitude E46.17 and latitude N38.5.

The experiment was carried out as split plot in a completely randomized block design with three replications. The irrigation levels included irrigation after 70 (I_1), 110 (I_2), and 150 (I_3) mm evaporation from class A pan considered as the main plots and bio-fertilizers with seven levels that included (F_1) Rhizobium (*R. phaseoli*), (F_2) Mycorrhiza (*Glomus mosseae*), (F_3) Pseudomonas (*P. fluorescens*), (F_4) Rhizobium + Mycorrhiza, (F_5) Rhizobium + Pseudomonas, (F_6) Mycorrhiza+ Rhizobium+ Pseudomonas and (F_7) Control comprised the sub plots.

Table 1: The results of soil experiment of farm site in 2014-2015.

	Soil texture	% clay	% silt	% sandy	available K mg kg^{-1}	ava. P mg kg^{-1}	% T.N	% O.C	% CCE	pH	EC dS/m	depth cm
2014	Sandy lome	15	20	65	485	11.6	0.089	0.69	10.1	7.92	1.89	0-30
2015	Sandy lome	15	20	65	489	11.9	0.092	0.74	10.5	7.94	1.94	0-30

Each replication consisted of seven experimental units of (3×2) m^2 and each plot included four rows with a distance of 50 cm in between. In both years, planting was done in the second half of May. While preparing the land, 100 kg/ha of triple phosphate and 50 kg/ha of urea were added. The seeds were inoculated with the bacteria by seed dressing while planting in the early hours of the day. From each of bacterium 10 g was added to every 1 kg of seeds and mixed well. After inoculating the seeds and drying them under a shade, plantation was carried out immediately. Before plantation, 5 g of Mycorrhiza fungus (*Glomus mosseae*) was placed in the plantation hole at a depth of (3 to 4) cm. The bacteria population was 2×10^7, and Mycorrhiza had at least 80 propagules (living fungus unit) per gram. Planting was done manually and plant density was adjusted to 20 plant/m^2. Weeds were also controlled manually during the plant's growth period. Until 8 to 10 leaf phase, irrigation was done at every 70 mm of evaporation from the class A pan. After this phase, the control plots were irrigated 11 times, and after 110 mm and 150 mm of evaporation from the class A pan, they were irrigated seven and five times, respectively. At the late flowering stage, Relative Water Content (RWC), leaf water potential, cell leakage, chlorophyll content index, stomata conductance, and density of stomata on the abaxial and adaxial surface of leaves were measured. During harvesting, grain yield was calculated. For measuring the relative water content, the highest young leaf was selected, and it's wet, saturated and dry weights were determined, and RWC was calculated by using the following formula (Fazeli-Rostampour *et al.,* 2013):

RWC = (saturated weight - dry weight)/ (wet weight - dry weight) × 100

For measuring cellular leakage (in stress period), the method proposed by Hu *et al.,*(2009) was adopted. For counting the number of stomata during the pollination phase were used the Replica method by optic microscope with a 40X lens (Yano-Melo, 2003). The stomata resistance of the last leaf from the top in the early hours of the day was determined by using a porometer (AP_4 Model).

The chlorophyll content index was recorded with (KONICA MINOLTA) SPAD-502. For measuring the leaves' water potential (Ψw), three leaves from the top were separately placed inside a pressure chamber instrument of SANTA BARBARA. CA. USA model and Ψw were obtained (Hu *et al.,* 2009). Statistical data analysis was performed based on statistical model of split plot test using SAS 9.1 software and mean comparisons was compared by Duncan's multi-range test method at 5% level. Diagrams were obtained with Excel software.

RESULTS AND DISCUSSION

The results of combined analysis of variance of two years are showed in Table 2.

Physiological Characteristics

Chlorophyll content index

Highest level of chlorophyll content in first year with a mean

Table 2: Combined analysis of variance of measured traits in two years 2014-2015.

		MS							
S.O.V	df	Chlorophyll content index	Density of stomata on abbaxial	Density of stomata on addaxial	Stomata conductance	Cell Leakage	RWC	LWP	Grain Yield
Y	1	42.062ns	2032.032**	540.643**	0.199**	4965.722 **	65.563 ns	16.619**	66.868ns
R/Y	4	8.123	31.556	1.944	0.010	90.825	6.114	2.382	29.427
A	2	3866.956**	94886.09**	5906.198**	5.231**	17900.17**	1839.958**	383.321**	140251.7**
A×Y	2	22.389ns	1465.365**	298.5**	0.071*	401.484*	65.308ns	1.698ns	3981.414**
A/Y×R	8	15.535	15.278	7.218	0.013	66.504	17.014	0.659	18.565
B	6	487.935**	1280.368**	788.069**	0.060**	1315.624**	100.335**	9.802**	8877.31**
B×Y	6	43.916 **	840.31**	206.513**	0.053**	151.926**	29.599**	1.779**	352.98**
B×A	12	25.719**	438.169**	60.485**	0.024*	139.898**	17.927*	0.889ns	522.608**
B×A ×Y	12	19.531*	503.032**	68.62**	0.020*	67.493*	16.605*	0.479ns	235.628**
B/YA×R	72	10.198	13.755	12.108	0.010	32.722	7.467	0.548	60.334
CV(%)		11.64	4.22	11.49	21.61	16.47	4.89	8.11	8/17

ns, * and **: no significant and significant at 5% and 1% levels of probability, respectively.

Y, R, A and B mean year, replication, irrigation levels and biofertilizer, respectively.

Table 3: Mean comparisons of water deficit stress and biofertilizer and year interaction effects for two year experiments.

Treatment Combination	Chlorophyll content index		Density of stomata on abbaxial		Density of stomata on addaxial		Stomata content (Sec/cm)	
	2014	2015	2014	2015	2014	2015	2014	2015
I_1F_1	41.70 b-d	34.17 e-h	46.33 qr	44.67 qr	14.00 q-t	21.00 n-r	0.87 cd	0.84 c-e
I_1F_2	39.13 c-e	36.27 d-f	35.67 s-u	39.00 r-t	15.33 p-t	20.33 n-s	0.73 d-f	0.84 c-e
I_1F_3	33.47 e-i	33.11 e-i	47.33 qr	47.67 qr	12.00 st	21.67 m-q	0.78 de	0.79 de
I_1F_4	37.50 de	44.17 bc	28.00 u	31.33 tu	12.67 r-t	15.33 p-t	0.67 ef	1.02 bc
I_1F_5	35.60 e-g	38.69 c-e	47.00 qr	40.67 rs	18.67 o-t	18.67o-t	0.75 de	1.06 b
I_1F_6	46.70 ab	49.89 a	19.33 v	28.33 u	11.33 t	15.00 p-t	0.99 bc	1.30 a
I_1F_0	33.73 e-i	30.81 f-j	50.67 q	51.33 q	37.33 g-i	23.33 l-p	0.57 fg	0.71 def
I_2F_1	23.87 k-n	25.44 j-m	94.67 jk	80.67 l-n	27.00 k-o	28.00 j-n	0.39 g-k	0.34 h-m
I_2F_2	21.63 m-o	23.91 k-n	100.33 ij	77.33 m-o	38.67 f-i	27.00 k-o	0.36 h-l	0.36 h-l
I_2F_3	23.60 k-n	24.19 k-n	95.00 jk	85.33 lm	39.00 f-h	30.00 i-m	0.31 h-n	0.34 h-m
I_2F_4	24.60 k-n	29.75 g-k	104.33 i	71.00 op	31.67 h-l	22.00 m-q	0.42 g-i	0.43 gh
I_2F_5	21.93 m-o	27.81 i-m	98.67 ij	76.33 no	30.00 i-m	25.00 l-o	0.41 g-j	0.41 g-i
I_2F_6	29.03 h-l	35.17 e-g	83.67 l-n	67.00 p	26.33 k-o	20.00 n-t	0.38 g-k	0.47 gh
I_2F_0	16.00 o-q	13.69 pq	119.33 h	89.00 kl	47.33 c-e	35.00 g-k	0.28 h-n	0.31 h-n
I_3F_1	18.43 n-p	15.98 o-q	143.67 bc	136.33 c-e	35.00 g-k	39.00 f-h	0.17 l-n	0.15 mn
I_3F_2	14.07 pq	18.80 n-p	151.67 b	124.00 f-h	53.00 bc	36.00 g-j	0.13 n	0.18 l-n
I_3F_3	14.80 pq	16.46 o-q	129.0 e-g	139.00 cd	46.00 c-f	43.00 d-g	0.12 n	0.17 l-n
I_3F_4	26.07 j-m	27.02 j-m	102.00 ij	126.00 f-h	40.67 e-g	31.00 h-l	0.13 n	0.22 j-n
I_3F_5	21.87 m-o	23.00 l-n	138.00 cd	132.33 d-f	49.00 cd	34.33 g-k	0.15 mn	0.20 k-n
I_3F_6	28.40 h-l	29.02 h-l	105.67 i	123.00 gh	31.00 h-l	27.00 k-o	0.14 n	0.23 i-n
I_3F_0	12.07 q	11.11 q	188.00 a	149.33 b	63.67 a	60.00 ab	0.12 n	0.15 mn

Table 3: Continue...

Treatment Combination	Cell Leakage (m.mos/cm)		RWC (%)		Grain Yield (kg/ha)	
	2014	2015	2014	2015	2014	2015
I_1F_1	30.67 gh	15.00 k-p	62.33 b-f	61.38 c-h	3065 d	3020 d
I_1F_2	17.00 k-p	13.67 m-p	59.33 e-k	63.92 b-e	3052.6 d	3154.6 d
I_1F_3	29.33 g-i	16.33 k-p	59.67 e-k	61.93 b-g	2551 ef	2669 e
I_1F_4	10.33 op	10.67 op	61.67 c-h	66.98 ab	3550.4 c	4019 b
I_1F_5	14.33 l-p	12.00 n-p	60.67 d-i	65.94 a-c	3176.8 d	3371.8 cd
I_1F_6	9.33 p	8.000 p	65.33 a-d	69.53 a	3564 c	4621.4 a
I_1F_0	22.67 h-n	19.00 i-p	55.67 i-p	58.50 f-l	2378.6 e-g	2080.6 g-i
I_2F_1	43.00 d-f	25.33 h-l	53.00 m-r	54.82 j-q	1751.8 i-k	1251.l m-p
I_2F_2	25.67 h-k	23.67 h-m	56.33 h-p	57.89 f-m	1791.2 i-k	1460.1 k-n
I_2F_3	51.33 c-e	24.00 h-m	54.33 k-q	55.60 i-p	1576.4 k-m	1011.9 o-s
I_2F_4	28.33 g-j	17.67 j-p	58.67 e-k	60.11 e-j	2289.2 f-h	1667.3 j-l
I_2F_5	41.67 ef	20.67 h-o	54.33 k-q	59.38 e-k	2116.8 g-i	1538.7 k-m
I_2F_6	31.00 gh	14.67 k-p	57.00 f-n	62.18 b-f	2377.2 e-g	2003.4 h-j
I_2F_0	55.67 c	29.33 g-i	51.67 o-s	51.76 n-s	1311.8 l-p	941.6 p-s
I_3F_1	73.33 ab	54.67 c	53.33 l-r	47.32 s-v	555.8 t	721.5 r-t
I_3F_2	66.67 b	52.33 cd	50.00 q-u	47.35 s-v	719.8 r-t	819.6 q-t
I_3F_3	72.67 ab	53.33 cd	49.00 r-u	46.87 s-v	735.4 r-t	699.2 st
I_3F_4	53.33 cd	38.33 fg	50.00 q-u	51.05 p-t	1128.1 n-q	1360.4 l-o
I_3F_5	65.00 b	46.67 c-f	46.33 t-v	50.00 q-u	784.4 q-t	1085.9 n-r
I_3F_6	38.67 fg	30.33 gh	56.67 g-o	51.41 o-t	1277.6 m-p	1573.8 k-m
I_3F_0	81.33 a	72.00 ab	43.33 v	45.03 uv	482.3 t	553.3 t

I_1, I_2, I_3 are Irrigation after 70, 110, 170 mm evaporation, Respectively, F_0, F_1, F_2, F_3, F_4, F_5, F_6 are Control, Rhizobium, Mycorrhiza, Pseudomans, R+M, R+PS, R+M+PS, respectively.

of 46.70 was in treatment compound I_1F_6 and lowest chlorophyll content with a mean of 12.07 was in treatment compound I_3F_0. In second year, highest chlorophyll content belonged to the treatment compound I_1F_6 with a mean of 49.89 and lowest chlorophyll content in second year with a mean of 11.11 belonged to treatment compound I_3F_0 (Table 3). Combined inoculation of bean with Rhizobium, pseudomonas and mycorrhizal fungi under optimal irrigation conditions as well as in water shortage conditions compared to non-inoculated control increased significantly chlorophyll content of plant and under water shortage conditions highest resistance was seen against water shortage and finally highest seed yield has been obtained. Effect of biological fertilizers on leaf may depend on efficiency of fixed nitrogen supply biologically, soluble phosphorus and production of plant hormones, which can stimulate absorption of nutrients, enhance process of photosynthesis in plant and thereby enhance plant growth (Manbari *et al.,* 2017). Kaur *et al.* (2015) in a study on chickpea stated that *Rhizobium* inoculation significantly increased chlorophyll content as compared to control.

The density of stomata on abbaxial and addaxial

According to Table 3, in first year, highest density of stomata on abbaxial (d = 188) was obtained from compound I_3F_0 and lowest density of stomata (d = 19) was obtained from the compound I_1F_6. In second year, highest density of stomata on abbaxial (d = 149) was obtained from the compound I_3F_0 and lowest density of stomata (d = 28) was obtained from compound I_1F_6. In all compounds, increasing irrigation range from 70 mm to 150 mm evaporation increased density of stomata on abbaxial. This may be due to reduced leaf area and cell size due to water shortage stress and as a result of increased stomatal density. The large number of stomata on addaxial is also directly related to the loss of water from the leaf (Doheny-Adams *et al.,* 2012).

In first year, highest density of stomata on addaxial (d = 63) was in compound I_3F_0 and lowest density of stomata (d = 11) was in compound I_1F_6. In second year, the maximum density of stomata on addaxial (d = 60) was in compound I_3F_0 and minimum density of stomata (d = 15) was in compound I_1F_6. Results showed that triple application of biological fertilizers under no stress and water shortage conditions had less density of stomata.

Stomata conductance

According to Table 3, highest stomata conductance (0.99 sec/cm) in first year was seen in compound I_1F_6 and lowest (12.2 Sec/cm) was seen in the treatment compound I_3F_0. In second year, highest stomata conductance (1.30 Sec/cm) belonged to compound I_1F_6 and lowest (15 sec/cm) belonged to compound I_3F_0. Bean stomata conduction response to biological fertilizers under maximum water shortage conditions has not been significantly influenced by biological fertilizers but under optimal irrigation conditions it has been more affected by biological fertilizers, so that in first year, the compounds I_1F_1, I_1F_3, I_1F_5 and I_1F_6 showed an increase in stomata conductance by 52.63 %, 36.84 %, 31.57 % and 73.68 % compared to compound I_1F_0, respectively. In second year, compounds I_1F_4, I_1F_5 and I_1F_6 compared to compound I_1F_0 showed an increase in stomata conductance by 43.66 %, 49.29 % and 83.06 % (Table 3). Manafi *et al.* (2010) reported that both species of mycorrhizal fungus increased stomata conductance significantly compared to the control, and with increasing humidity stress, stomata conduction reduced. In this case, we can say that mycorrhizal plants can better drain soil water, thus keep stomata more open and less exposed to water shortage stress.

Cell leakage

According to Table 3, highest leakage rate in first year was observed in compound I_3F_0 (81.33 mmho/cm), and lowest was observed in the compound I_1F_6 (9.33 mmho/cm). In second year, highest leakage rate was observed in compound I_3F_0 (72 mmho/cm) and lowest was observed in treatment compound I_1F_6 (8 mmho/cm). In compounds with increasing levels of water shortage, cellular leakage increased due to the fact that the cytoplasmic membrane of plants' cells under stress had low stability, as well as plants under water shortage conditions compared to plants under common conditions of irrigation had higher EC. Higher EC indicates low cytoplasmic membranes. As a result, under water shortage conditions, membrane has low stability, resulting in an increase in intracellular leakage. Water shortage with affecting the structure of membrane of cell increases the permeability of membrane relative to ions and macromolecules. Triple application of biological fertilizers under both normal irrigation and maximum water shortage conditions had least leakage among other biological fertilizers, it is likely that triple application of biological fertilizers provided plant with more suitable conditions and causes increasing diameter of plant cell wall and reducing cell leakage in plants.

Relative water content (RWC)

Highest RWC in first year with a mean of 61.67 % was in compound I_1F_6 and lowest RWC with a mean of 43.33 % belonged to compound I_3F_0. In second year, highest RWC (69.53 %) belonged to the compound I_1F_6 and lowest RWC (45.03 %) was observed in compound I_3F_0 (Table 3). Mean comparison of treatments showed that F_6 biological fertilizer had the most significant effect on RWC at all three levels of irrigation compared to other biological fertilizers, and could be more effective on reducing the side effects of water shortage. Higher RWC of leaf in triple-inoculation treatment may possibly be attributed to fact that mycorrhiza fungus with producing a network of hyphae provided plant with much soil volume, thus providing plant roots with much water. Auge *et al.* (2015) stated that mycorrhiza absorbs more water and improves the aqueous interactions of plants through changes in the root morphology, prolongation of the host plant root system and increase in absorption through the fungi hyphae. Also, accumulation of ions or organic matter

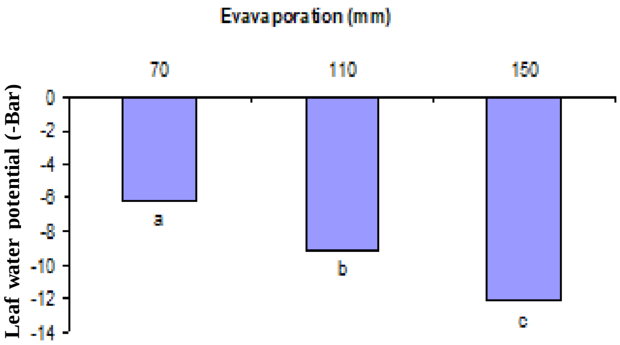
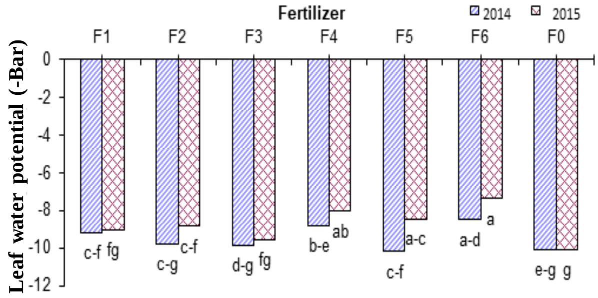


Fig 1: The mean effect of irrigation levels on leaf water potential.



F0, F1, F2, F3, F4, F5, F6 Respectively Control, Rhizobium, Mycorrhiza, Pseudomans, R + M, R +PS, R+M+PS

Fig 2: Mean comparisons interaction effects year and biofertilizer for two year experiment.

in leaf cells' vacuola under water shortage stress occurs more in mycorrhizal plants and reduces osmotic potential of leaf cells.

Leaf water potential (LWP)

LWP under optimal irrigation conditions (irrigation after 70 mm evaporation) with a range of -6.134 bar was significantly higher than irrigation withdrawal treatments and under water shortage conditions (irrigation after 110 mm and 150 mm evaporation) with a significant difference was in two different statistical group. Loss of LWP in irrigation after 110 mm and 150 mm evaporation are 45% and 98%, respectively, compared to irrigation after 70 mm evaporation (Fig 1). With reducing water potential in soil, transpiration rate of plant reduced and growth limited (Mullet and Whitsitt, 1996).

Highest LWP was obtained in first year in F_6, which was -8.49 bar. Lowest LWP was observed in F_0 treatment. In second year, highest LWP was obtained in F_6 treatment equivalent to -7.36 bar, and lowest LWP was obtained in F_0 treatment (Table 4). In first year, application of F_4 and F_6 biological fertilizers increased LWP by 12.69% and 15.77%, respectively, in comparison with control (Table 4). In second year, application of F_1, F_2, F_4, F_5 and F_6 fertilizers increased LWP by 10.31%, 12.30%, 20.33%, 16.33% and 26.98%, respectively, compared to F_0 treatment (Fig 2). *Mycorrhiza* fungus increased leaf water potential in comparison with non-fungal plants by opening plant stomata, increasing evaporation and water absorption (Zarei *et al.,* 2013).

Grain yield

In first year, highest grain yield (3564 kg/ha) was seen in the treatment compound I_1F_6 and lowest yield (482.3 kg/ha) was seen in the treatment compound. In second year, highest grain yield (4621.4 kg/ha) belonged to the treatment compound I_1F_6 and lowest yield (553.3 kg/ha) belonged to the treatment compound I_3F_0 (Table 3). All treatments of biological fertilizers at all irrigation levels increased grain yield of beans compared to the control treatment (Table 3). With increasing the intensity of water shortage stress, yield reduced, and biological fertilizers, especially in combination with F_6, had a positive effect on grain yield and increased yield compared to the treatments with no biological fertilizer. The highest yield was in the treatments with biological fertilizers when water needed for the plant is provided and the lowest grain yield was observed in the treatments with no biological fertilizers and maximum water shortage conditions. These results indicate a positive correlation between *Arbuscular mycorrhiza* fungus, Rhizobium bacteria and Pseudomonas bacteria, the application of which increased yield compared to the separate application of these three microorganisms. It seems that rhizobium biological fertilizer increases the yield of beans under optimal irrigation conditions with increasing the efficiency of biological fixation of nitrogen. Rhizobacteria stimulating the growth of plants with increasing the plant access to important nutrients such as nitrogen and phosphorus and increasing root growth increased bean grain yield under both optimal irrigation and water shortage conditions. Also combined application of (F1+F2+F3) might have helped to enhance the biological activity in the soil, soil characters' improvements, better root development, improved transport of nutritional elements, enhanced chlorophyll content synthesis and photosynthesis, solubilization of nutrients resulting in higher nutrients uptake by red bean compared to other treatments. Khandelwal *et al.,*(2012) reported that the inoculation of Rhizobium + PSB increased cowpea grain yield by 13.80 % compared to the control Lingaraju *et al.* (2016) reported higher seed yield of soybean was obtained with the treatment combination of dual inoculation of PSB+VAM compared to control.

CONCLUSION

The results showed that although water shortage stress reduced studied traits more or less, use of biological fertilizers could reduce negative impacts of water shortage stress. Meanwhile, application of F6 fertilizer in both years under optimal irrigation and water shortage conditions is very important. Results indicated a positive correlation between Mycorrhiza, Rhizobium and Pseudomonas. Their application, through increasing absorption of nutrients and tolerance of water shortage conditions, increased grain yield relative to the three microorganisms' separate and combined application. Therefore, it is suggested to use these microorganisms as a promising technology in combined and simultaneous application (Rhizobium, mycorrhiza and pseudomonas) in arid and semi-arid regions, both under optimal irrigation conditions and under conditions of irrigation shortage or uncertain rainfall in order to cope with water shortage stress for sustainable agriculture goals.

REFERENCES

Auge, R.M., Toler, H.D. and Saxton, A.M. (2015). Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. Mycorrhiza. 25(1): 13-24.

Bourgault, M., Madramootoo, C.A., Webber, H.A., Dutilleul, P., Stulina, G., Horst, M.G. and Smith, D.L. (2013). Legume production and irrigation strategies in the Aral sea basin: yield, yield components,water relations and crop development of Common Bean (*Phaseolus vulgaris* L.) and Mungbean (*Vigna radiate* L.) Wilczek. Journal of Agronomy and Crop Science. 199(4): 241-252.

Doheny-Adams, T., Hunt, L., Franks. P.J., Beerling, D.J. and Gray, J.E. (2012). Genetic manipulation of stomatal density influences stomatal size, plant growth and tolerance to restricted water supply across a growth carbon dioxide gradient. Philosophical transactions of the Royal Society of London Series Biological. 367: 547–555

Espidkar, Z., Yarnia, M., Ansari, M.H., Mirshekari, B. and Asadi-Rahmani, H. (2016). Differences in nitrogen and phosphorus uptake and yield components between barley cultivars grown under arbuscular mycorrhizal fungus and pseudomonas strains coinoculation in rainfed condition. Applied Ecology and Environmental Research. 15(4): 195-216.

Fazeli-Rostampour, M., Yarnia, M. Rahimzadeh Khoee, F. Seghatoleslami, M.J, Moosavi, G.R. (2013): Physiological response of frage sorghum to polymer under water deficit conditions. Agronomy Journal. 105(4): 951-959

Haghighatnia, H., Nadian, H., Rejali, F. and Tavakoli, A.R. (2013). Effect of two species of *Arbuscular-Mycorrhizal* fungi on vegetative growth and phosphorous uptake of Mexican Lime Rootstock (*Citrus aurantifolia* L.) under drought stress conditions. Seed and Plant Production Journal. 28(4): 403-417.

Hu, L., Wang, Z., Du, H. and Huang, B. (2009). Differential accumulation of dehydrins in response to water stress for hybrid and common Bermuda grass genotypes differing in drought tolerance. Journal of Plant Physiology, 167: 103-109.

Kaur, N., Sharma, P. and Sharma, S. (2015). Co-inoculation of Mesorhizobium sp. and plant growth promoting rhizobacteria Pseudomonas sp. as bio-enhancer and biofertilizer in chickpea (*Cicer arietinum* L.). Legume Research-An International Journal. 38(3): 367-374.

Khandelwal, R., Choudhary, S.K., Khangarot, S.S., Jat, M.K. and Singh, P. (2012). Effect of inorganic and bio-fertilizers on productivity and nutrients uptake in cowpea [*Vigna unguiculata* (L.) Walp]. Legume Research-An International Journal. 35(3): 235- 238.

Lingaraju, N.N., Hunshal, C.S. and Salakinkop, S.R. (2016). Effect of biofertilizers and foliar application of organic acids on yield, nutrient uptake and soil microbial activity in soybean. Legume Research An International Journal. 39(2): 256-261.

Manafi, H., Aliasgharzad, N., Neyshabouri, M.R. and Rejali, F. (2010). Tolerance to water deficit stress in tomato inoculated with Arbuscular Mycorrhizal fungi. Journal Water and Soil Science. 22(2): 97-117.

Menbari, S., Alizadeh-Salteh, S., Bolandnazar, S. and Sarikhani, M.R. (2017). Evaluation of the effects of biological and chemical fertilizers on some physiological and growth characteristics of fenugreek (*Trigonella foenum-graecum*). Journal of Agricultural Science and Sustainable Production, 27(4): 154-169.

Mullet, J.E. and Whitsitt, M.S. (1996). Plant cellular responses. Plant Growth Regulation. 20: 119-124.

Suarez, R., Wong, A., Ramirez, M., Barraza, A., Orozco, M., Cevallos, M., Lara, M., Hernandez, G. and Iturriaga, G. (2008). Improvement of drought tolerance and grain yield in common bean by overexpressing trehalose-6-phosphate synthase in rhizobia. Molecular Plant–Microb Interactions, 21: 958-966.

Yang, J., Kloepper, J.W. and Ryu, C.M. (2009). Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Scienc. 14: 1–4.

Yano-Melo, A.M., Saggin, O.J. and Maia, L.C. (2003). Tolerance of mycorrhized banana (Musa sp. cv. Pacovan) plantlets to saline stress. Agriculture Ecosystem Environment. 95: 343-348.

Zarei, M., Paymaneh, Z., Ronaghi, A., Kamgar-Haghighi, A.A. and Shahsavar, A. (2013). Effects of Arbuscular Mycorrhizal fungus on growth and physiological parameters of Rough Lemon rootstock under water deficit conditions. Journal of Water and Soil. 27(3): 485-494.