The effects of biofertilizers on physiological traits and biomass accumulation of red beans (*Phaseolus vulgaris* cv.Goli) under water stress

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Abstract

This field experiment was executed as the split plot in a randomized complete block design with three replications in 2014. In a red bean irrigated farm, halt irrigation was implemented in main plots and the treatments included the control (full irrigation) and halt irrigation at vegetation, flowering, and pod development stages. In subplots utilization of biofertilizers were applied at four cases of control, phosphorus solubilizing bacteria, potassium solubilizing bacteria, and combined application of these bacteria in soil. Results showed that halt irrigation and biofertilizers had significant interaction on red bean biomass. Normally, to achieve the highest biomass full irrigation and application of phosphorus and potassium solubilizing bacteria (8023 and 7978 kg ha-1) are suggested. But the use of biofertilizers instead of chemical fertilizers led to more water efficiency under low water condition. Finally, our results showed that maximum biomass in red bean (about 7985 kg. ha^{-1}) was recorded in ecological nutrition management under limited water resources. In other words, halt irrigation at flowering stage was compensated by Bio-P and Bio-K fertilizer application as a logical method to mitigate and moderate biomass losses about 37%.

Keywords: bio fertilizer; water stress; red beans; biomass accumulation; chlorophyll

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Introduction

Beans are one of the most important grains for food security and healthy human consumption (Beebe et al., 2008). Being rich in protein, a great deal of protein from plant sources are beans. Therefore, they play a very significant role in human diet (FAO, 2001).

A large part of the farm lands in arid and semi-arid regions are affected by water stress and water deficit (Rezaei et al., 2009). In fact, drought stress is a common issue throughout the world, posing challenges for crops including beans (Munoz-Perea et al., 2006). It is believed that water stress is the most important factor in arid and semi-arid areas limiting bean production as a

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summer crop (Teran and Singh, 2002). Effects of drought on beans depend on the severity, type, and duration of stress in plant growth stages (Munoz-Perea et al., 2006). Thus, it is crucial to investigate the response of these crops to drought stress conditions and determine the sensitivity of crops to water deficit at different growth stages of the plants. The use of modern methods to improve irrigation may increase water use efficiency and crop production in arid and semi-arid regions (Mintesinot et al. 2002).

In addition to improved irrigation systems and implementation of new irrigation methods introduced to conserve water resources, there are other water management strategies that can increase water use efficiency in agriculture (Horst et al., 2005). One of these methods is deficit irrigation. This approach to the management and exploitation of water is has been reported to increase yields in a number of crops (Haouari and Azaiez 2001). Hindrance in the evolution of flowers, young pods, and seeds occur due to drought stress during and before flowering stage (10 to 12 days before pollination) and reproductive stage. Severe drought stress reduces biomass and grain yield (from 20 to 90 %), harvest index, the number of pods and seeds, grain weight, and remaining days to maturity (Nunez-Barrios et al. 2005). According to some researchers, the appropriate time for full irrigation is an important factor to achieve full production and without taking into account the most appropriate irrigation time for each crops, full irrigation is just a waste of water (Mousavi, 2005). Studies have also shown that using biofertilizers may increase the plants' tolerance to drought conditions (Zahir et al., 2008, Aulakh et al. 2003). Understanding the way biological interactions can improve tolerance to drought stress is a challenge for cropping systems (Mohammadi and Sohrabi 2012). The use of beneficial bacteria is increasing in the agricultural systems and there are much evidence that show their ability to increase plant tolerance to abiotic stress Including salinity (Egamberdieva and Islam 2008), drought stress (Zahir et al., 2008), weed infestation (Babalola, 2010), and nutrient deficiency.

The delay in reproductive growth of bacteria leads to increased biomass that is independent of the irrigation regimes. The coordinated changes in transpiration, content of ABA, photosynthesis, and growth leads to higher water use efficiency and better tolerance to drought-induced plants. This avenue of research can provide new insights in the field of ecophysiology by which PGPR can improve stress tolerance in plants.

Drought stress is one of the major constraints to food production around the world. Therefore, the development of drought-resistant varieties of plants and the efficient use of water is a global concern. Plants are not the only organisms in their habitat; there are also a large population of diverse microorganisms that may interact with them. Some of these microorganism-plant interactions are useful for plant growth helping them to better cope with biotic and abiotic stresses (Yang et al. 2009). In their article, Adesemoye et al. (2009) reviewed benefits of PGPR-Plant interaction to improve the speed of seed germination, root and shoot weight, yield, leaf area, chlorophyll, hydraulic activity, protein content, and absorbed nutrients such as phosphorus and nitrogen. The present study was carried out to investigate the effect of halt irrigation at different growth stages and use of phosphorus and potassium solubilizing bacteria on biomass and physiological traits in common bean *(Phaseolus vulgaris* cv. Goli).

Materials and Methods

This experiment was carried out in Agricultural Research Station of Islamic Azad University, Arak, Iran (49°48' E, 34°3' N, 1698.4 m above the sea level) in 2014. Table 1 summarizes the regional climate status of the experiment location. Also, the chemical and physical properties of the soil are shown in Table 2. The soil was initially prepared using a moldboard plow during the fall prior to the study. The field was prepared by plowing twice perpendicularly in the spring and according to the results of the soil test no chemical fertilizer was before sowing. To control weeds Treflan 1-liter ha^{-1} was sprayed twenty days before the planting date.

Table 1
Characteristics of the regional climate

Month	Precipitation (mm)	Temperature (°C)	Sunshine (h)	Precipitation (mm)	Temperature (°C)	Sunshine (h)
	Average 50 year					
January	41.6	-0.8	152.0	41.0	3.3	179.8
February	38.6	1.9	170.0	22.5	0.9	154.0
March	49.1	7.8	206.2	85.6	8.9	165.9
April	50.6	13.4	225.7	61.6	14.1	230.1
May	25.6	18.2	288.3	30.5	19.0	249.0
June	2.8	24.0	345.0	0.0	25.9	323.1
July	1.2	27.3	334.8	1.0	29.0	369.2
August	1.6	26.4	330.9	0.0	27.7	306.1
September	0.9	21.9	305.1	0.0	23.6	310.0
October	14.5	15.6	259.3	47.4	14.6	290.7
November	30.7	8.4	185.9	23.5	5.9	246.4
December	40.1	2.8	154.3	15.8	3.9	212.3

Table 2
Soil physical and chemical characteristics

Type	Percent %			Parts Per Million (ppm)							Percent %				pH	EC
	Clay	Silt	Sand	B	Cu	Mn	Zn	Fe	K	P	N	O.C	T.N.V	SP		
L	22.3	35.0	41.0	1.3	1.1	6.8	4.1	2.9	408	24.9	0.2	1.5	11.5	31.5	7.7	1.2

Common bean seeds were obtained from the Khomein National Research Centre of pulse crops. The experiment was run a split plot in a randomized complete block design with three replications in the farm where corn was grown in the previous year. Sowing date and first irrigation were carried out in the first year on June 11th. Drip irrigation was carried out on the basis of halt irrigation treatments. Halt irrigation was applied at three different phases, i.e. at the end of vegetative stage, 50% flowering stage, and 50% pod filling stage, respectively on July 12th, August and 19th August 5th, and control (full irrigation by FC levels) in main plots. Biofertilizer treatment was applied at four levels of control (no bacteria), phosphorus solubilizing bacteria[1] (PSB), potassium solubilizing bacteria[2] (KSB), and combined application of PSB and KSB, arranged in subplots and applied 30 days after sowing time in the soil near roots.

In order to ensure was performed treatment of bio fertilizer, applied in the bushes twice with an interval of 3 days. The dimensions of each plots were considered 4 meters long and 3 meters wide. So that each plot contained six rows spaced 0.5 m. The plant densities were set of 40 plants per square meter for all the plots to identical. During the period of growth and development of beans was carried out by hand weeding and weed control in several steps. As well as to control pests and plant diseases was conducted in accordance with the technical advice necessary monitoring during the growing season. Harvesting was performed at the end of the growing season respectively on 15th September, at 96 days after planting. To measure the leaf cell membrane stability leaves samples were taken from each plot and measured the EC of cell electrolyte according to Krizek et al. (1998) method.

To determine the biomass at physiological maturity and considering the margins of each experimental unit, all plants in the two square meters of the central rows of each plot were harvested. The following formula was used to measure the harvest index:

[1] *Panteoa agglomerans* (strain P5) and *Psuedomonas putida* (strain P13) mixture included $1*10^9$ CFU bacteria per 100 grams bio-fertilizer registered as Barvar II®.

[2] *Bacillus circulans* (strain KSB1) and *Bacillus megatherium (*strain KSB2) mixture included $1*10^9$ CFU bacteria per 100 grams' bio-fertilizer registered as PotaBarvar ®.

halt irrigation and use of biofertilizers on biomass

Table 3
Analysis of variance for the effects of halt irrigation and biofertilizer on biomass and important physiological traits

S.O.V	D.F	Mean of squares						
		Biomass yield	Harvest index	Chlorophyll a/b ratio	Total chlorophyll	Protein content	Seeds K/P ratio	EC
R	2	1427562.9	17.98	0.068	0.97	14.52	0.35	16410.1
Halt Irrigation (I)	3	34853274.0**	782.07**	0.848**	4.03**	52.99**	2.92**	155325.0**
Error I	6	81339.7	4.89	0.035	0.17	4.44	0.02	3103.1
Bio fertilizer (B)	3	4013312.8**	79.72**	0.206**	2.12**	1.68**	0.06**	35341.7**
I *B	9	315495.6**	2.59 ns	0.068ns	0.43ns	0.20*	0.09**	3523.8**
Error B	24	14428.3	1.94	0.036	0.20	0.08	0.01	865.5
C.V(%)		2.01	3.89	14.28	8.57	1.45	2.06	6.60

ns: non-significant; *and ** significant at 0.05 and 0.01 probability level, respectively

HI = GY/BY*100

where GY and BY are the grain yield (Kg ha^{-1}**)** and biomass yield (Kg ha^{-1}), respectively (Beebe et al., 2010).

Photosynthetic pigments were measured in 80% acetone-extracted samples according to (Lichtenthaler and Wellburn, 1983). The absorbance of chlorophyll and carotenoid content was measured at 470, 645, and 663 nm using spectrophotometry. The chlorophyll and carotenoid concentrations were calculated as follows:

Chlorophyll a = (11.75 A663 – 2.350 A645)

Chlorophyll b = (18.61 A645 – 3.960 A663)

Carotenoids = (1000 A470 – 2.270 Chl a – 81.4 Chl b)/ 227

Analysis of variance was conducted using Mstat-C and mean comparisons were done using LSD test at P≤0.05.

Results

Results showed that the effects of halt irrigation were significant on biomass yield, harvesting index, chlorophyll a/b ratio, total chlorophyll, seed protein content, seeds K/P ratio, and leaf ion leakage. Also, biofertilizer treatments had a significant effect on all above traits. Interaction effects were also observed between yield, protein content, seeds K/P ratio, and ion leakage (Table 3).

Biomass yield

Effects of halt irrigation and biofertilizer treatments as well as the interaction between halt irrigation and biofertilizers had a significant effect on biomass yield (P≤0.01) (Tables 4). Mean comparisons of interaction effects between halt irrigation and biofertilizers indicated that the maximum biomass yield (8022.8 kg ha^{-1}) was recorded for concurrent use of both PSB and KSB while the minimum biomass yield (3100.5 kg ha^{-1}) was recorded in halt irrigation group at flowering stage and without the use of biological fertilizers (Table 4).

Harvest index

Effects of halt irrigation and use of biological fertilizers on harvest index were significant at P≤0.01, but their interaction was not significant (Table 3). Mean comparison suggested the highest harvest index (46.09%) in the full irrigation to a low of 26.56% under halt irrigation at the flowering stage (Table 4). The maximum effect on the harvest index was obtained in the treatment involving the combined application of biological fertilizers (38.69%).

Results of analysis of variance indicated that the effects of irrigation treatment and application of biological fertilizers were significant at 1% probability level on the chlorophyll a to b ratio in red beans but their interaction effects were not significant. The maximum value of this trait was recorded in full irrigation and halt irrigation in vegetative stage, and the minimum chlorophyll a to b ratio (50%) was recorded under halt irrigation at pod filling stage, which was 36.2% less than the control.

Also the results of mean comparison of the effects of biofertilizers showed that the highest value of chlorophyll a to b ratio was obtained under combined treatment of PSB and KSB (1.48) showing 21% increase compared to control.

Total chlorophyll

Analysis of variance showed that the effect of halt irrigation and biofertilizers on the total chlorophyll content was significant at 1% probability level in red beans but their interaction was not significant.

Grain protein content

Analysis of variance showed that the effects of halt irrigation and applying biological fertilizers ($P \leq 0.01$) and their interaction ($P \leq 0.05$) were significant on protein contents of seeds (Table 3). The highest protein content (21.73%) was recorded in full irrigation and concurrent use of phosphorus and potassium solubilizing bacteria and the lowest (16.13%) was observed in halt irrigation at flowering stage and use of potassium biofertilizer.

Results showed that halt irrigation had low impact on the pod filling and at vegetative growth stage and the maximum reduction in protein content was observed at flowering stage. The simultaneous application of phosphorus and potassium soluble bacteria was effective in increasing the protein content of the seeds which can partly compensate for the damage caused by water stress on this trait (Table 4).

Seeds K/P ratio

According to the results of variance analysis, it was observed that effects of halt irrigation, applying biological fertilizers, and their interaction were significant at the 0.01 level on P content of seeds (Table 3). The highest ratio of this attribute (5.42) was observed in halt irrigation at the flowering stage and no application of biofertilizers while the other halt irrigation treatments did not have much effect on this ratio even with the application of biological fertilizers. This shows that this trait is mostly affected by drought stress and bio-fertilizer does not help to improve it. Results showed that halt irrigation had maximal impact at the flowering stage (Table 4).

Ion leakage

Significant differences were found in the study ($P \leq 0.01$) in the use of biological fertilizers, halt irrigation, and their interaction on ion leakage of the plants (Table 3). Since in all treatments of irrigation, biofertilizers with phosphorus plus potassium had the lowest EC and the best condition, it can be concluded that bacteria in the biological fertilizers could increase plant tolerance to dehydration.

Discussion

It seems that combined use of biological phosphorus and potassium fertilizers can maintain biomass yields in the event of water deficit in plants. According to these results, water deficit seems to be extremely influential on biomass yield at the flowering stage of red bean and declining this trait by 61.4%. Studies have shown that lack of water reduces the plants' ability in biosynthesis and transportation of assimilates thereby reducing the accumulation of dry matter and the biomass in plants. Water deficit also reduces dry matter production in plants whereas the reduction in biological yield depends on stress intensity during the stages of plant growth (Spaeth et al. 1984). Shekari (2001) reported of loss biomass yield due to increased level of drought stress during different growth stages.

Severe drought stress reduces biomass and seed yield (by 20 - 90%), harvest index, number of pods and seeds, grain weight and remaining days to maturity (Munoz-Perea et al., 2005; Khaghani et al., 2017). German and Teran (2006), observed that drought reduced the biomass, grain yield, harvest index, and the grain weight. Their study also showed a positive correlation between seed yields under normal and drought conditions.

Reduced harvest index under drought stress in vegetative and reproductive stages can be due to the loss of photosynthetic area reducing the remobilization of assimilates at grain filling stage (Gebeyehu, 2006 and Khoshvaghti, 2006). The results showed that the drought reduced harvest index in beans (German and Teran 2006). Padilla-Ramirez et al., (2005) reported that drought stress reduced grain yield, biomass and the harvest index. Besides the dry matter accumulation is also important to assimilate partitioning between different plant organs in drought stress treatment in vegetative stage to spend more of assimilates produced more for the plant roots to provide more water. So reduced harvest index in such conditions (Taleie et al. 2000). In relation to the impact of moisture turned out on economic yield and biomass relationship balance between vegetative and reproductive stages the effect of drought stress on the vegetative and reproductive parts and finally reduced the stability of harvest index (Spaeth et al., 1984).

Reductions in chlorophyll content are most likely due to increased catalytic activity of chlorophylls and degradation of photosynthetic pigments and this process is also the result of not providing the necessary factors for the synthesis of chlorophyll and the destruction of its structure under stress conditions (Ahmadi et al., 2005; Mohtashami et al., 2016). Therefore, chlorophyll can be reduced considered as a Non-stomatal limiting factor under water stress conditions (Jiang and Hung., 2001; Sairam et al., 1997; Nematollahi et al., 2017).

Biological fertilizers have been effective in preventing severe protein depletion and can be partially offset for damage caused by water stress. At this time of grain filling increases nitrogen absorption that the water stress it will disrupt in this process biological fertilizers has improved protein recovery to reduce the negative effects of this situation (Chauhan and Bagyaraj, 2015).

It seems that the transfer of elements between vegetative and reproductive organs (leaf and seed) and the occurrence of dilution phenomenon can be considered as the reasons for the changes in the concentration of the elements in seeds K/P ratio. Drought stress has long-term effect on the uptake and accumulation phosphorus in beans (Dos Santos et al., 2004), In particular, be reduced absorb and store by beans (Fageria et al. 1997).

Electrical conductivity has reverse proportion with cytoplasm membrane stability and it’s been the best each of the treatments can reduce the amount of electrical conductivity (Farshadfar et al. 2008).

Conclusion

The phosphorus and potassium solubilizing bacteria mixture were able to improve the red bean biomass and important physiological traits under limited irrigation condition significantly. In fact, in red bean farms, halt irrigation at the flowering stage had more harmful effects compared to halt irrigation at vegetative stage. Obviously, the study recommends full irrigation and combination of PSB and KSB to achieve the maximum biomass in red beans. This is followed by halt irrigation at 50% pod filling stages and then vegetative stage. Finally, the application of both types of biological fertilizers resulted in the highest index of all traits under study including biomass in all different irrigation conditions.

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