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Role of potassium solublizing bacteria on nutrients uptake in red bean (*Phaseolus vulgaris* L. cv. Goli) under water deficit condition

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ABSTRACT

This experiment was carried out to evaluate the effect of halt irrigation and bio fertilizer on plant yield, seed nutrients content, relative water content (RWC) and chlorophyll index (SPAD) of Goli genotype of red bean (*Phaseolus vulgaris* L.), based on split plot design with three replications during two years of 2014-2015 at Arak, Iran. Halt irrigation was in main plots by four levels of control (full irrigation), halt irrigation in vegetative stage, at flowering stage and pod filling stage and four bio fertilizer were in sub plots, including: control (no bacteria), Phosphorus Solublizing bacteria (PSB), potassium solublizing bacteria (KSB) and concurrent use both of PSB and KSB. Analysis of variance showed that no significant effect of years but the effect of halt irrigation treatments was significant on plant yield, N, P, K and Zn percentage of seed, relative water content (RWC) and chlorophyll index (SPAD). Halt irrigation reduced plant yield by 67.81% in comparison to full irrigation during flowering stage. In the study, physiological characteristics such as RWC, N, P, K and Zn contents were also reduced significantly with halt irrigation at flowering stage than its effects on other stages of the crop. Also other characteristics was significantly reduced in this study with halt irrigation that this reduction in flowering stage was more than other stages.

Key words: Chlorophyll index, Grain yield, Halt irrigation, Potassium, Solublizing bacteria.

INTRODUCTION

The inappropriate environmental effects caused by use of chemical fertilizers is more obvious, and it is important to reduce their consumption or opt for replacement partially with biological fertilizers. A key pillar in the sustainable agriculture is use of bio fertilizers in agricultural ecosystems to remove or substantially reduce the use of chemical inputs (Rashid *et al.*, 2004). Phosphate solubilizing bacteria produce organic acids which increase solubility of poorly soluble inorganic phosphate such as rock phosphate (Goldstein *et al.*, 1999).

Beneficial microorganisms in soil such as mycorhizal fungi and phosphate solubilizing bacteria provide nitrates, exchangeable phosphorus, magnesium and potassium nutrients required for plant growth and yield of crops (Edwards *et al.*, 2004). Phosphate solubilizing bacteria stimulates plant growth through P nutrition, and increases the uptake of N, P, K and Fe. Phosphorous bio fertilizers help in increasing the availability of accumulated phosphates for plant growth by the increasing the efficiency of biological nitrogen fixation and the availability of Fe, Zn through production of plant growth promoting substances (Biswas *et al.*, 2000 ; Kaur *et al.*, 2015).

Some species of rhizobacteria are capable of mobilizing potassium in accessible form in soils. There are considerable populations of K solubilizing bacteria in soil and rhizosphere (Lingaraju *et al.*, 2016). It activates enzymes, maintains cell turgor, enhances photosynthesis, reduces respiration, helps in transport of sugars and starches, aids in nitrogen uptake, and is essential for protein synthesis. In addition to plant metabolism, potassium improves crop quality because it helps in grain filling and kernel weight. strengthens straw, increases disease resistance and helps the plant better to withstand stress. Microorganisms play a key role in the natural K cycle. The potassium is made available to plants when the minerals are slowly weathered or solubilized (Bertsch and Thomas, 1985; Kumar *et al.*, 2015). Mineral potassium when applied from a cheaper source of rock potassium may be agronomically more useful and environmentally feasible than soluble K (Rajan *et al.*, 1996). Potassium solubilizing bacteria are capable of solubilizing rock K, mineral powder such as mica, illite and orthoclases

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through production and excretion of organic acids (Friedrich *et al*., 1991). Relative water content (RWC) as an integrative indicator of internal plant water status under drought conditions has successfully been used to identify drought-resistant cultivars of common bean (Franca *et al*., 2000). Stoyanov (2005) reported that water stress may reduce RWC between 19% and 37% in the first trifoliate leaf. The purpose of this study was the study effect of deficit irrigation and bio fertilizers application on red bean yield and NPK accumulation in grains.

MATERIALS AND METHODS

This study was carried out in the years 2014 and 2015 at the Islamic Azad University Agricultural Research Station, Arak, Iran (49°48' E, 34°3' N with an altitude of 1698.4m above the). The soil chemical and physical properties are shown in Table 1. The experiment was conducted in split plot design with three replications. Halt irrigation consisted of four levels of control (full irrigation), and laid in main plots. Halt irrigation was imposed at vegetative stage, flowering stage and pod filling stage (Madani and Sharifi, 2014). The four bio fertilizers viz., phosphorus solubilizing bacteria[1] (PSB), potassium Solubilizing bacteria[2] (KSB) and Concurrent use both of PSB and KSB were applied in soil 30 days after sowing in sub plots, against a: control (no bacteria). The dimensions of each experimental plot were 4 m (length) in 3 m (width), and each plot contained six rows spaced by 50 cm. At maturity, the pods were harvested grains from the pods of each plant were separated manually. The grain weight was recorded for each treatment and expressed as $g.plant^{-1}$. The seeds from each plot were milled apart initially and then N content in it was estimated following the Kjeldahl method suggested by Mann (1963).

For determining seed nutrient contents, the seeds were oven dried, extracted with wet acid digestion method and analyzed for elemental content of P, K and Zn by Atomic Absorption Spectrophotometer, model-2380 (Jones *et al*., 1990). Chlorophyll index was measured using chlorophyll meter (Minolta SPAD-502, Tokyo, Japan). For this purpose, five leaves from the top and middle position of each plant were chosen. In each leaf, chlorophyll content was measured at five points using chlorophyll meter. Then, the mean values were determined as an indicator of chlorophyll in the leaves.

The relative water content (RWC) was determined from the youngest leaves developed. The selected leaves were cut and placed immediately in an ice flask and transported to the laboratory. Fresh weight of the leaf was determined and then dipped in distilled water for 24 hours at room temperature in darkness. The ensuing saturated weight was determined. Then, the leaves were dried to a constant weight in an hot air oven for 24 hours at 70°C. Leaf RWC was obtained by the following formula (Barrs and Kozlowski, 1968).

$$RWC= [(FW-DW)/(SW-DW)]*100$$

Where, FW: fresh weight, SW: saturated weight, DW: dry weight.

To determine the seed yield, the crop was harvested at physiological maturity. From the margins of each experimental unit, all plants in the two square meters of the central rows of each plot were considered for harvesting. Analysis of variance was computed using software MSTAT-C, and the mean comparisons were made using LSD test at P(0.05).

RESULTS AND DISCUSSION

Yield per plant: Data presented in Table 2, showed that weather conditions (year) were not effective on bush yield. The effects of halt irrigation and bio fertilizers on bush yield were significant at the P (0.01) level. The interactive effect of halt irrigation × bio fertilizers on bush yield was significant at P(0.05). Mean comparison of the interactive effect of halt irrigation and bio fertilizers showed that the highest bush yield belonged to concurrent use of both of PSB and KSB with full irrigation quantifying the bush yield as 9.94 $g.plant^{-1}$ (Fig. 1).

The greatest reduction in seed yield occurs due to at the drought stress in vegetative flowering, and end of the pod filling stages (Araújo and Teixeira, 2008; Ghanbari *et al*., 2013; Muuhouche *et al*., 1998; Rauthan and Schnitzer, 1981). In the present study, the highest bush yield loss was found with biofertilizer application at flowering stage, which is a critical one. Despite, the pod filling stage associated with biofertilizers had the lowest reduction as compared to the control. The potential of PSB and KSB were evaluated using pepper and cucumber as test crops. Rock phosphorus and potassium applied either singly or in combination did not enhance availability of soil phosphorus and potassium significantly, which indicates their unsuitability for direct

Table 1: Soil physical and chemical characteristics

EC	pH	%				ppm							%			year	type
		SP	T.N.V	O.C	N	P	k	Fe	Zn	Mn	Cu	B	Sand	Silt	Clay		
1.2	7.7	31.5	11.5	1.5	0.2	24.9	408	2.9	4.1	6.8	1.1	1.3	41	35	22.3	2014	loam
1.4	7.6	33.2	10.3	1.7	0.2	19.3	414	3.0	4.3	6.9	1.2	1.1	42	36.5	20.7	2015	loam

[1]*Panteoa agglomerans* (strain P5) and *Psuedomonas putida* (strain P13)

[2] *Bacillus circulans* (strain KSB1) and *Bacillus megatherium(*strain KSB2)

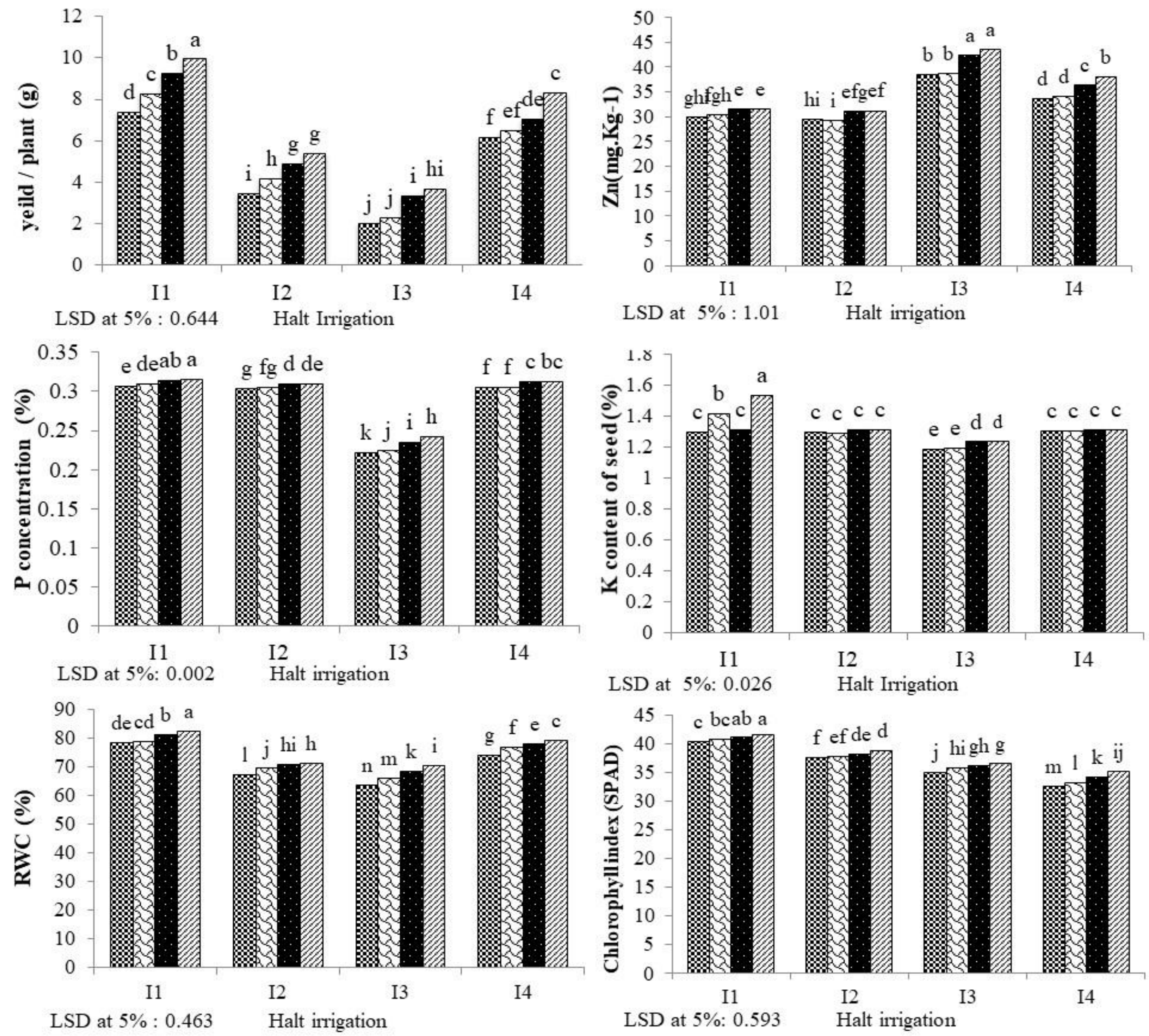

Fig 1: Plant yield, seed nutrients content, relative water content (RWC) and chlorophyll index (SPAD) under different halt irrigation and bio fertilizer treatments.

Table 2: Means of squares for halt Irrigation (I) and Bio fertilizer (B) on plant yield, seed nutrients content, relative water content (RWC) and leaf chlorophyll index (SPAD Value)

S.O.V	df	Mean of square						
		Yield/ Plant	RWC	Zn content	K content	P content	N content	SPAD Value
Year	1	$2.097^{n.s}$	$36.977^{n.s}$	$0.03375^{n.s}$	$0.0001193^{n.s}$	$0.00030^{n.s}$	$0.510^{n.s}$	$0.621^{n.s}$
Year * Rep	4	4.643	33.597	0.46608	0.0035643	0.00693	58.365	0.608
Halt Irrigation(I)	3	163.765^{**}	904.182^{**}	2.70059^{**}	0.0367659^{**}	0.12564^{**}	583.010^{**}	223.621^{**}
Y * I	3	$0.027^{n.s}$	$1.171^{n.s}$	$0.00219^{n.s}$	$0.0000002^{n.s}$	$0.00001^{n.s}$	6.288^{**}	2.596^{*}
Erorr1	6	0.063	9.159	0.11364	0.0000460	0.00144	0.389	1.349
Biofertilizer(B)	3	20.268^{**}	107.620^{**}	0.07170^{**}	0.0006352^{**}	0.02568^{**}	59.899^{**}	12.252^{**}
B * Y	3	$0.159^{n.s}$	$0.306^{n.s}$	$0.00146^{n.s}$	$0.0000065^{n.s}$	$0.00023^{n.s}$	$0.899^{n.s}$	$0.041^{n.s}$
B * I	9	0.351^{*}	$3.670^{n.s}$	$0.00601^{n.s}$	0.0000524^{**}	0.01683^{**}	4.603^{**}	0.629^{*}
B * I * Y	9	$0.243^{n.s}$	$0.126^{n.s}$	$0.00107^{n.s}$	$0.0000022^{n.s}$	$0.00038^{n.s}$	$0.603^{n.s}$	$0.206^{n.s}$
Erorr2	54	0.145	3.608	0.01106	0.0000172	0.00064	1.596	0.289
C.V (%)		6.63	2.59	3.39	1.44	1.93	3.68	1.49

n.s, non-significant, *and ** significant at 0.05 and 0.01 probability respectively application. The co-incubation of PSB and KSB resulted in higher P and K available consistently in comparison to the control (Vassilev *et al*., 2006). In a field experiment, Balasubramaniam and Subramanian (2006) reported 15.31% increase in rice grain yield due to effect of silicate solubilizing bacteria.

Relative water content (RWC): There were significant effects of different halt irrigation and bio fertilizer on RWC (Table 2). Full irrigation (Control) had the highest RWC, and halt irrigations at flowering stage showed the lowest RWC (Table 3). Severe drought stress reduces RWC and seed yield (Aydi *et al*., 2008; Bajji *et al*., 2000; Ghanbari *et al*., 2013; Muñoz-Perea *et al*., 2006; Vazan *et al*., 2002). Anjum *et al*., 2011; Rauf and Sadaqat, 2008 also reported that drought affected leaves exhibit large reduction in RWC. Therefore, the pod yield and RWC may be considered as important indexes for sorting different cultivars under water deficit condition (Link *et al*., 2007).

Nitrogen content of seeds: Analysis of variance showed that the effects of halt irrigation and bio fertilizers on N contents of seeds were significant at the P(0.05) level (Table 2). The lowest N content (2.641 %,) was found due to halt irrigation at flowering stage (Table 3). Drought changes a number of physiological and biochemical processes in plants including inhibition of protein synthesis (Fresneau et al., 2007). Water deficit stress causes decrease in N and protein contents in legumes (Allahmoradi *et al*., 2011; Nielsen and Nelson, 1998; Rosales-Serna *et al*., 2004). Photosynthetic activity is related to leaf N. The photosynthetic rate increases with higher levels of leaf N and increases yield (Nakayama *et al*., 2007).

Phosphorus content of seeds: The halt irrigation and bio fertilizers had significant effects on P content of seeds at P(0.05) level (Table 2). Full irrigation had higher P content than the other treatments *viz.*, halt irrigation at pod, vegetative and flowering stages. Mean comparison of bio fertilizer showed that the highest P content belonged to PSB and PSB+KSB, whereas the lowest P content was found in the control plants (Table 3). Han *et al.* (2006) also evaluated the potential of PSB and KSB inoculated in nutrient limited soil planted with pepper and cucumber. Their results showed that co-inoculation of plants with PSB and KSB showed higher P and K contents as well as more plant growth as compared to the control. They concluded that transfer of elements between vegetative and reproductive organs (leaves and seeds), and the phenomenon of dilution could be the reasons for the change of concentration (Kamble *et al*., 2016).

Potassium content of seeds: Results displayed in Table 2 portray that there were significant effects of different levels of halt irrigation and bio fertilizers on K content of seeds at P(0.01). The full irrigation increased K content by 1.392 % than halt irrigation imposed at other growth stages. The reduction in K content in flowering stage was 12.72 % as compared to control. The biofertilizer treatment viz., KSB+PSB could maintain maximum (1.349 %) K contents in seeds (Table 3). Moreover, seed K contents in case of full irrigation and KSB+PSB treatments were higher than the amount found in halt irrigation at flowering stage and with non-use bio fertilizer (Table 3). It means that flowering is a sensitive phase to scarcity of water. Ramarethinam and Chandra (2005) in a field experiment on drought recorded significantly higher fruit yield, plant height and K uptake in Brinjal as compared to control due to inoculation of potash solubilizing bacteria. Sheng (2005) worked on potassium releasing bacterial strain *B. edaphicus* to study plant growth promoting effects and nutrient uptake by cotton and rape seed in K-deficient soil. There were increases in root-shoot growth and potassium contents by 30% and 26% respectively.

Page(1982) and Krieg and Holt (1984) isolated potassium solubilizing bacteria from rock and mineral samples, and demonstrated higher activity of these bacteria in respect of potassium release from acid leached soil and improving green gram seedling growth. Phosphorus solubilizing bacteria and silicate bacteria play a important role in plant nutrition through the increase in P and K uptake by plant (Datta *et al*., 1982; Nyanikova *et al*., 2002; Neelamet *et al.* (2016). Han and Lee (2006) found that the co-inoculation of PSB and KSB in combination with direct application of rock P and K materials into the soil increased

Table 3: Means comparison of plant yield, seed nutrients content, relative water content (RWC) and chlorophyll index under different halt irrigation and bio fertilizer treatments.

Treatment	Yield /plant(g)	RWC(%)	Ncontent (%)	Pcontent (%)	Kcontent (%)	ZnContent ($mg.Kg^{-1}$)	SPAD Value
Control (full irrigation) (I_1)	8.707	80.14	3.439	0.311	1.392	30.833	40.91
Halt irrigation at vegetative stage (I_2)	4.475	69.64	3.120	0.306	1.302	30.208	38.04
Halt irrigation at flowering stage (I_3)	2.827	67.07	2.641	0.231	1.215	40.833	35.87
Halt irrigation at pod stage (I_4)	6.998	77.06	3.210	0.308	1.308	35.500	33.77
LSDi	0.151	0.99	0.043	0.000	0.003	2.304	1.48
Control (Non-use) (B_1)	4.744	70.84	3.056	0.284	1.272	32.917	36.33
KSB (B_2)	5.296	72.85	3.055	0.286	1.303	33.083	36.87
PSB (B_3)	6.138	74.51	3.143	0.292	1.293	35.333	37.40
KSB+PSB (B_4)	6.829	75.72	3.156	0.295	1.349	36.042	38.00
LSDb	0.367	0.51	0.035	0.002	0.014	0.871	0.19

in N, P and K uptake, photosynthesis and the yield of eggplant grown on P and K limited soil.

Zinc content of seed: In the experiment, he effects of halt irrigation and bio fertilizers on Zn content of seeds were significant at P(0.05) level. The interactive effect of halt irrigation × bio fertilizers on Zn content was also significant (Table 2). The lowest Zn content was due to non-use and KSB bio fertilizer treatment under the halt irrigation at vegetative stage were 29.17 (mg.Kg^{-1}) and 29.50 (mg.Kg^{-1}) respectively. There were higher Zn contents at KSB+PSB (43.50) and PSB (42.50) bio fertilizer treatments under the halt irrigation at flowering stage (Fig. 1) as compared to the control.

Leaf chlorophyll index (SPAD Value): The effects of halt irrigation and bio fertilizers on leaf chlorophyll index (SPAD values) were significant (Table 2). Full irrigation showed higher SPAD than the other treatments. The highest leaf chlorophyll index belonged to full irrigation as well as concurrent use of both of PSB and KSB. The lowest SPAD belonged to halt irrigation at pod filling stage×non-use bio fertilizer (Fig. 1). Water deficit causes decrease in SPAD value (Dalil *et al.*, 2010; Jing *et al.*, 2005; Karipçin, 2009). Drought stress affects plant growth and causes in physiological disorder in plants. i.e. reduction in chlorophyll content (Colom and Vazzana, 2001).

CONCLUSION

The results showed that the halt irrigation, bio fertilizer and its effect on grain yield was significant at the 1% level. Mean comparison of the treatments interactive effects showed that the highest bush yield belonged to concurrent use of both of PSB and KSB with full irrigation quantifying the bush yield as 12.47%. Thus, the greatest risk in seed yield production will occurs due to at the irrigation halt in vegetative flowering. Although, replacing inorganic fertilizer with biofertilizers in red bean under water deficit condition could be reduce the risk.

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