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ORIGINAL ARTICLE

The effects of Plant Growth Promoting Rhizobacteria (PGPR) on growth characteristics of fenugreek under water deficit stress

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ABSTRACT
Plant growth-promoting rhizobacteria (PGPR) are root-colonizing bacteria which can improve the plant growth by increasing the availability of nutrients and protecting them from pathogens. For a long-serving period, the PGPRs have been applied as biofertilizers in crops culture. Recent studies indicated the importance of PGPR for controlling the water deficit. The present study investigates the effects of two different PGPRs on some physiological and morphological characteristics in fenugreek (Trigonella foenum –graecum L.) under water deficit stress. A factorial design based on randomized complete block design with four water deficit levels (100%, 80%, 60% and 40% FC) and four PGPR condition (control, Rhizobium meliloti, Pseudomonas fluorescens and combination of R. meliloti and P. fluorescens with three replications were carried out. The results showed that leaf area, shoot and root fresh and dry weight, phosphorus and potassium content, and water use efficacy (WUE) were significantly improved by PGPR inoculation and individual use of PGPR was more effective. Whereas seed yield was decreased in PGPR treated plants.
Keywords: PGPR, water stress, WUE, medicinal plant, shoot weight, seed yield

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INTRODUCTION

Insufficient water would induce a stress in plants called water deficit stress [8]. Water deficit stress has major effects on plant growth and development, limiting crop production in the worldwide. Water deficits negatively affects the plant growth and reproduction and disrupts the whole-plant functions [4, 1]. Water deficit causes cellular changes such as solutes concentration, cell volume alteration, disruption of water potential gradients, changes in membrane shape and disrupting its integrity, loss in turgor pressure, and protein denaturation [5]. Water deficit is a major threat to agricultural production and tolerance to drought conditions is the main target for crop improvement [25].

Plant growth-promoting rhizobacteria (PGPR) are rhizosphere bacteria which constitute symbiotic relationships in large varieties of plants and are used as a biofertilizer [27]. PGPR have been reported to confer positive effects and induce plant resistances to environmental stresses and diseases caused by pathogens [12, 17, 19, 20, 21]. A wide variety of mechanisms that can improve the plant growth, have been suggested to be impressed by PGPR. These involved mechanisms are as follows: nitrogen fixation [29, 30], production of 1-Aminocyclopropane-1- carboxylate deaminase (ACC) [10], production of volatile organic compounds [24], induction of systemic resistance [6,7], phytohormone production [30], siderophore production [9] and phosphate solubilization [24].

Fenugreek (*Trigonella foenum- graecum* L.) is a member of the Fabaceae family, cultivated worldwide as a semiarid crop, and traditionally used as a medicinal plant. Fenugreek is grown as a spice and a vegetable crop and also has been used as a traditional therapy for the remedy of diabetes [21]. And its effects as an antidiabetic and antiatherosclerotic have been documented [2]. Fenugreek's leaves are a rich source of

iron, calcium, β-carotene and other vitamins and its seeds contain tannic acid, diosgenin, trigocoumarin, alkaloids trigonelline, trigomethyl coumarin, gitogenin and vitamin A [31]. Whereas water deficit has limited many crop production worldwide and negatively affects the plant growth and reproduction; recently published literatures indicated that plant growth-promoting rhizobacteria ameliorate the plants tolerance to abiotic stresses through a variety of mechanisms [26, 28]. Also beneficial effects of PGPRs on medicinal plants have been reported [13, 14]. Because of the high importance of fenugreek as a medicinal plant in the present investigation the impacts of PGPR on some physiological and morphological

MATERIALS AND METHODS

The seeds of fenugreek with good germination quality was provided from "Jahad daneshgahi Iranian institute of medicinal plants," Karaj, Iran. And the present investigation, carried out in research greenhouse of Faculty of Agriculture at the University of Tabriz during 2015-2016. The experiments were conducted in a factorial design based on completely randomized block design with three replications. The first factor was application of PGPR in 4 levels (1.*Rhizobium meliloti* as nitrogen fixing bacteria 2. *Pseudomonas fluorescens* as phosphorous solubilizing bacteria 3. Combination of *R. meliloti* and *P. fluorescens* 4. negative control without any bacteria and fertilizer. The second factor was soil water content treatment based on field capacity (FC) in 4 levels (100, 80, 60 and 40% of FC). Seed of fenugreek was sown in a plastic pot which had 5 kg soil after establishment 5 plants remained in each pot. Soil water content was maintained aforementioned values by daily weighting of pots. Plants kept in a greenhouse under a 16 h photoperiod, 24±4 / 18±3ºC day/ night temperatures, and 40-60% relative humidity. At end of experiment leaf area measured, by the leaf area meter (LI 3100C area meter, LI-COR, USA). Dry weight of each part was determined after drying at 72˚C until constant weight. The fresh and dry weight plants were determined using a digital weighing scale.

The composition of potassium and phosphorus was determined by nitric perchloric and nitric digestion methods [11, 33]. Phosphorous was analyzed by a vanadate-molybdate method using a spectrophotometer (Motic, CL-45240-00, China) and K was analyzed using a flame photometer (Model 405G, Iran). Also the seed yield was recorded at maturity. Toward the end of the experiment, plants were cut at the soil level and their roots were cleaned and washed from soil and separately were oven-dried at 70° C and their water use efficiency (WUE) was calculated by following formula:

WUE = DW / UW

In this formula, DW and UW represent dry mass production and the amount of consumed water, respectively [15].

All collected data were subjected to two-way analysis of variance (ANOVA) through PROC GLM procedure, using a SAS statistical package (SAS Institute, software Version 9.4, Cary, NC, USA). If interactions were significant, means were compared by Duncan's multiple rage tests to determine whether means of the dependent variable were significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

Analysis of data variances indicated that the effect of PGPR and soil water content and their interaction on leaf area, shoot fresh and dry weight was significant ($P \leq 0.01$). Means comparison showed that PGPR inoculation increased fenugreek leaf area; shoot dry and fresh weight especially *R. meliloti* (Table 1). By increasing of water deficit stress leaf area, shoot fresh and dry weight was decreased (Table 2). In aspect of interaction between PGPR inoculation and water stress, the highest and lowest leaf area, shoot fresh and dry weight was observed in well watered (100% FC) and *R. meliloti* treated plants and sever water stressed control ones respectively (Table 3). It seems that in normal condition *R. meliloti* bacteria improved aerial growth of fenugreek better than *P. fluorescens*, whereas under water stress condition *P. fluorescens* was more or less effective than *R. meliloti* application. Combination use of two PGPR bacteria was not successful in enhancement of shoot growth unexpectedly (Table 3). Whereas water deficit has limited many crop production worldwide and negatively affects the plant growth and reproduction; recently published literatures indicated that plant growth-promoting rhizobacteria ameliorate the plants tolerance to abiotic stresses through a variety of mechanisms [26, 28]. In keeping with our results Mishra *et al.* [22] indicated that PGPRs could ameliorate the negative effects of salinity stress conditions by positive effects on parameters such as increasing the germination in plants, and also increasing the yield, drought tolerance, and growth. It also have reported that even in the presence of optimum levels of nitrogenous fertilizers, inoculating with PGPR containing ACC-deaminase activity can improve the yield and growth of inoculated plants. According to the results of this investigation inoculation with PGPR containing ACC-deaminase considerably decreased the damages caused by drought stress on the growth

and yield. They reported that un-inoculated plants exposed to drought stress at vegetative growth stage had significantly decreased shoot growth by 41%, while in the inoculated plants the decreased shoot growth was only 18%. Similar to our finding in this study the grain yield was improved in inoculated plants in comparison to their un-inoculated counterparts [3].

As was shown in the table of mean comparison (Table 1) individual and dual PGPR treatments improved root fresh and dry weight. Root expansion was limited under moderate (60% FC) and severs (40% FC) water deficit stress significantly (Table 2). The highest and lowest root fresh weight was observed in *P. fluorescens* inoculated in 80% FC soil water content treatment and all pants which were under sever (40% FC) water deficit stress (Table 3). Plant growth-promoting rhizobacteria ameliorate the plants tolerance to abiotic stresses and increases the root growth parameters [26]. Also it have been indicated that PGPRs could ameliorate the negative effects of salinity stress conditions by increasing the roots dray and fresh weight and overall growth [22]. In a study, the effects of *Pseudomonas fluorescens* as a PGPR on growth parameters and the production of ajmalicine were investigated under drought stress. Similar to our findings in this study in the cases treated with *Pseudomonas fluorescens,* the growth parameters were increased under drought stress and improved the drought induced growth inhibition through increasing the fresh and dry weights. This findings indicates that PGPRs could be used for increasing the biomass and yield in plants and can be used as a tool in water deficit stress amelioration [14].

The phosphorus and potassium concentration was significantly affected by PGPRs and water deficit. As it was the table of mean comparison (Table 1) for the effects of PGPR treatments, the maximum P belonged to plants treated with *R. meliloti* (0.719 mg g^{-1}) follows by *P. fluorescens* (0.674 mg g^{-1}) and treatments containing both *R. meliloti* and *P. fluorescens* (0.639 mg g^{-1}). By decreasing of soil water content, P concentration was increased significantly with an exception at 80% of FC treatment able 2). The highest and the lowest P concentration was belonged to dual application of PGPR bacteria at mild (80% FC)water stress treatment and well watered *R. meliloti* inoculated fenugreek respectively (Table 3). Similar to our findings in a study the bacterial root inoculations significantly affected the plant nutrient element contents in apple compared to controls and significantly increased the Phosphorus content of treated plants [16]. Also potassium content was significantly affected by PGPR treatment and control plants (33.91mg g^{-1}) showed higher K than bacteria treated ones and *R. meliloti* inoculated fenugreek had lower K (30.02mg g^{-1}) (Table1). According to the interaction effects between PGPRs and drought stress the highest K concentration was observed in control plant under sever water stress (40% FC) and *P. fluorescens* treated plants under moderate water deficit stress (60%FC) (Table3). Żuk-Gołaszewska *et al.* [34] reported that *Rhizobium* inoculation of fenugreek did not improved K uptake. Whereas similar to our findings, *P. fluorescens* improved potassium uptake especially under water stress in tomato plant [23]..

Water use efficiency (WUE) significantly was affect by PGPR and soil water content. Mean comparison indicated that PGPR inoculate plants produced more areal biomass per water unit than control ones (Table 1). By increasing water deficit stress WUE was increased significantly (Table 2). In aspect of interaction between PGPR and water stress it was shown that dual application of *P. fluorescens* and *R. meliloti* under severe water deficit stress (40%FC) led to highest WUE and well watered control plants presented lowest WUE (Table 3). Similar to the present findings Maqshoof *et al* (2013) demonstrated that the minimum WUE was belong to un-inoculated cases which was improved by inoculation with *Rhizobium* and PGPR. Also beneficial effects of PGPRs on medicinal plants have been reported [14]. It have been reported that PGPR could delay the flowering time and increase the biomass yield also can confer stress tolerance to plants. In the present investigation shoot and root weight, phosphorus and potassium content, and WUE increased significantly by treating both PGPRs. PGPR colonizes the plant's root system and modulates its growth through increasing the availability of nutrients it also protects the plants from plant pathogens [18].

Un-inoculated control plants produced significantly higher seed yield per pot than PGPR treated fenugreek (Table 1). It should be noted that the experiment duration was 5 month and fenugreek has indeterminate flowering habit, so when they continued flowering plants were harvested. It have been reported that PGPR could delay the flowering time [14]. Water limitation except mild water deficit stress led to decrease in seed yield (Table 2). The maximum seed weight was observed in control plants the reason may be due to this fact that in the absence of stress conditions, more photosynthates material have been stored in the organs such as stems and leaves which by transferring to the seeds have been increased the grain weight. In contrary, stress conditions the water and food absorption by the plant is disrupted which decreases the plant growth and reduces the transmission of photosynthates material in leaf and other organs to the grain [14]. Integrative use of PGPRs and water deficit stress could be an enhance the eco-friendly strategy of PGPRs and plants and could increasing the alkaloid yields in

medicinal plants [1]. From there that the Fenugreek is used as a medicinal plant this strategy could be applied for increasing its useful secondary metabolites.

In conclusion, the results of the present investigation indicate that both *R. meliloti* and *P. fluorescens* could effectively increase vegetative growth, phosphorus and potassium content and WUE in fenugreek regardless water deficit stress. Also under water stress condition PGPR increased plant growth. However in present study seed yield because of delaying bolting time was decrease by application of PGPR.

Table 1. Effect of PGPR on growth characteristics, P and K concentration and WUE of fenugreek

Bacteria	Leaf area (cm^2)	Shoot fresh weight(g)	Shoot dry weight(g)	Root fresh weight(g)	Root dry weight (g)	Seed yield ($g\ pot^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	WUE ($g\ kg^{-1}$)
Control	709c	17.41c	3.99d	5.21b	0.882c	106.14a	0.5456d	33.91a	0.131c
R. meliloti (R)	1303a	28.51a	5.87a	8.14a	1.422ab	56.93b	0.719a	30.02c	0.220a
P. fluorescens(P)	1277ab	28.13a	5.56b	8.12a	1.455a	53.03b	0.674b	31.27b	0.241a
*R * P*	1000b	21.09b	5.22c	8.10a	1.330b	56.78b	0.6393c	31.19b	0.196b

*R * P*= treatment containing both *R. meliloti* and *P. fluorescens* Dissimilar letter indicating significant differences (Duncan's multiple range test P≤0.01).

Table 2. Effect of soil water content on growth characteristics, P and K concentration and WUE of fenugreek

Soil water content (FC)	**Leaf area** (cm^2)	**Shoot fresh weight (g)**	**Shoot dry weight** (g)	**Root fresh weight** (g)	**Root dry weight** (g)	**Seed yield** ($g\ pot^{-1}$)	**P** ($mg\ g^{-1}$)	**K** ($mg\ g^{-1}$)	**WUE** ($g\ kg^{-1}$)
100%	1448a	33.59a	7.07a	8.91a	1.457a	86.74a	0.6337c	29.35b	0.152c
80%	1264b	29.92b	6.33b	9.34a	1.42a	94.11a	0.5938d	33.89a	0.181b
60%	881c	17.97c	3.94c	7.51b	1.289a	48.31b	0.656b	29.98b	0.168bc
40%	694d	13.65d	3.32d	3.82c	0.933c	43.73b	0.694a	33.17a	0.287a

Dissimilar letter indicating significant differences (Duncan's multiple range test P≤0.01).

Table 3. The interaction effects between PGPR and soil water content on some characteristics of fenugreek

		Leaf area (cm^2)	**Shoot fresh weight**(g)	**Shoot dry weight(g)**	**Root fresh weight**(g)	**Root dry Weight**(g)	**Seed yield** ($g\ pot^{-1}$)	**P** ($mg\ g^{-1}$)	**K** ($mg\ g^{-1}$)	**WUE** ($g\ kg^{-1}$)
I_1	**B_1**	979.9de	20.36d	4.4de	6.05d	0.87f	148.89a	0.55g	32.85cd	0.098e
I_2	**B_2**	2057.3a	49.01a	9.76a	11.15a	1.74a	66.37cd	0.35h	30.15e	0.208c
I_3	**B_3**	1571b	37.33b	7.33b	8.89cd	1.66ab	42.86fe	0.53g	21.78f	0.163cd
I_4	**B_4**	1185c	27.33c	6.78bc	9.53bc	1.56abc	88.83bcd	0.74b	32.6cd	0.138de
I_1	**B_1**	717.8f	20.03d	4.89d	6.75d	1.12e	105.68b	0.67dce	35.22b	0.136de
I_2	**B_2**	1533.3b	35.73b	7.19b	11.67a	1.47bc	92.00bcd	0.68dc	34.62bc	0.200c
I_3	**B_3**	1570.3b	36.63b	6.4c	9.21bc	1.50bc	93.77bcd	0.67dce	34.29bc	0.185cd
I_4	**B_4**	1236.7c	27.30c	6.8bc	9.77bc	1.59ab	84.98bcd	0.83a	31.42de	0.201c
I_1	**B_1**	603.3gh	15.73f	3.80fg	4.37e	0.87f	99.99bc	0.6f	29.64e	0.140de
I_2	**B_2**	951.2e	20.51d	3.44gh	6.10d	1.35cd	27.01f	0.68dce	23.14f	0.160cd
I_3	**B_3**	1063.7d	17.87e	4.46de	9.96b	1.49bc	43.5fe	0.74b	37.7a	0.204c
I_4	**B_4**	904.8e	17.76e	4.07ef	9.60bc	1.45bc	22.75f	0.66de	29.64e	0.168cd
I_1	**B_1**	536.4h	13.50g	2.88h	3.69e	0.67f	70.cde	0.7c	37.95a	0.149d
I_2	**B_2**	671.3fg	8.80h	3.1h	3.66e	1.13e	42.33fe	0.64e	32.51cd	0.276b
I_3	**B_3**	903e	20.33d	4.07ef	4.42e	1.17de	32.00f	0.66dce	31.16de	0.314b
I_4	**B_4**	675fg	11.97g	3.23h	3.48e	0.76f	30.57f	0.53g	31.08de	0.411a

B1= Control (no bacterial treatment), B2= *R. meliloti*, B3= *P. fluorescens,* B4= combination of *R. meliloti* and *P. fluorescens*, I1= 100% irrigation, I2=80% irrigation, I3= 60% irrigation, I4= 40% irrigation. Dissimilar letter indicating significant differences (Duncan's multiple range test P≤0.01).

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