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Research Article

Effect of Rhizobacteria and Microalgae Treatments on Development and Physically Parameters of Fenugreek (*Trigonella foenum-graecum* L.) Grown Under Deficit Water Conditions

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Abstract

In this study, the effects of deficit water (DW) (normal=control, ½ reduced and $^3/_4$ reduced) and some beneficial rhizobacteria (*Azospirillum lipoferum, Bacillus megaterium*) and microalgae (*Chlorella saccharophilia*) on some growth and physiological parameters of fenugreek (*Trigonella foenum-graecum* L.) were investigated. The experiment was carried out in a fully controlled climate cabinet with 3 replications in factorial order according to the Random Plots Trial Design. Fenugreek (*Trigonella foenum-graecum* L.) plant was used as material. The study aims to investigate the effects of different deficit water treatments (normal=control, ½ reducedand $^3/_4$ reduced) and some rhizobacteria (Control=R0, *Azospirillum lipoferum*=R1, *Bacillus megaterium*=R2) and microalgae (*Chlorella saccharophilia*=R3) on the growth and development of fenugreek. The effects of deficit irrigation practices on stem length, root fresh and dry weight, stem fresh and dry weight, and leaf area were found to be statistically significant. As the deficit irrigation doses increased, all growth parameters decreased. Root length, root age and stem weight were statistically significantly affected in rhizobacteria and microalgae applications. The highest stem length (19.32 cm) and root wet (1.34 g) and dry (0.18 g) weight were obtained from *Bacillus megaterium* applications.

Keywords: Deficit water, rhizobacteria, fenugreek, physiological, growth parameters

INTRODUCTION

Drought is determined as an environmental factor that affects the fertility and productivity of plants (Bartels and Sunkar, 2005). Plant growth, crop productivity, and development of plants are deficit as a result of drought and insufficient irrigation. Climatic change is worsening the severity of drought (Wu et al., 2017). On the earth, around 30% of the whole land is arid land and inadequate for crop production (Bray, 2004). Water deficiency is considered one of the main environmental factors that limit the improvement and growth of plants (Dudley, 1996). Crop production and plants are strongly influenced by insufficient water which causes water deficit stress (Passioura, 1996). Water deficiency causes the reduction of tissue concentration of chlorophylls at plants (Jaleel et al., 2009). Bartels and Sunkar (2005) reported that water stress leads to changes in turgor pressure, cell volume, and membrane shape and causes disruption of water potential gradients. Currently, many researchers spend their efforts on understanding plants' tolerance to drought conditions and the adaptation of plants to inadequate irrigation that influences plant development (Bray, 2004). Plant growth promoting rhizobacteria (PGPR) is a significant and environmentally friendly solution for sustainable agricultural production (Rashid et al., 2021). It has been reported that PGPR has the potential in increasing plant tolerance to water deficiency which is an abiotic stress (Jaleel et al., 2007; Sandhya et al., 2010). Many researches demonstrate that plants exposed to water stress increased secondary metabolites. Thus compared to plant genetics, environmental factors have effects on secondary metabolites as well (Aliabadi-Farahani et al., 2009). The mechanism mitigating water deficiency stress by PGPR includes the process of increasing essential nutrients uptake and production of phytohormones such as gibberellic acid (GA), indole acetic acid (IAA), and ethylene (Oleńska et al., 2020). Phyto hormones produced by PGPR influence the development and growth stages of plants such as the expansion of cell and tissue specialization (Vessey, 2003). As auxin treatment is applied for the long-term, plant root development highly increases. Preventing diseases contaminated by pathogens (virus, fungi, bacteria, etc.), strengthening plants to environmentally unfavorable conditions, and a wide range of actions that can help plant growth are counted among the positive effects of PGPR (Kloepper et al., 2004; Yang et al., 2009; Sharghi et al., 2018). Fenugreek (*Trigonella foenum-graecum* L.), which is one of the significant medicinal plants, is native to India and Mediterranean region. It is in the Fabaceae family and cultivated as a spice, supplement for foods, and vegetables. Leaves and seeds of fenugreek are preferred for medicinal purposes, especially in the treatment of diabetes (Sharma and Raghuram, 1990; Basch et al., 2003; Fernández-Aparicio et al., 2008). Although fenugreek's significance, benefits, and usage in the medicinal and other field have been searched by many, understanding its adaptation to abiotic stress and drought condition is among the major research objects. The aim of this study was to investigate the effects of different PGPR (*Azospirillum lipoferum, Bacillus megaterium*, *Chlorella saccharophilia*) inoculations on Fenugreek's (*Trigonella foenum-graecum* L.) tolerance to drought conditions and physiological and biochemical properties under different water deficit levels.

MATERIAL and METHODS

The study was carried out in 2020 in the controlled climate room of the Field Crops Department of Van Yuzuncu Yil University, Faculty of Agriculture. In the research, Gürarslan cultivar were used as seed material. The experiment was carried out in a factorial order with 3 replications according to the complete randomized design. In the research, three different water levels (normal (control), ½ reduced and $^3/_4$ reduced)) and some bacterial species (*Azospirillum lipoferum* ($1x10^6$ cfu/ml) and *Bacillus megaterium* ($1x10^5$ cfu/ml)) that survive in the root zone of plants and are known to contribute to the growth and development of the plant and protect the plant against environmental stress and an algae species (*Chlorella saccharophilia* ($2x10^4$ cfu/ml)) that lives in water and has functions in macro-micro nutrient uptake in plant development were used. Before sowing seed, surfaces were sterilized with 3% sodium hypochlorite. The seeds, except the sterilized control group, were treated separately for two hours in 10 ml/L doses of *Azospirillum lipoferum* and Bacillus megaterium rhizobacteria solutions and 5% *Chlorella saccharophila* algae solution prepared. Treated and untreated seeds were sown 6 each in 1-liter pots filled with 1/3 perlite, 1/3 peat and 1/3 garden soil by volume. After planting, the pots were placed in a climate room with a temperature of 25 ^oC and a humidity of 65% in a light/dark photoperiod of 16/8 hours. The seeds started to emerge homogeneously on the soil 4 days after sowing. Bacteria prepared at the rate of 10 ml/L and algae solutions prepared at the rate of 5% were given in the pots containing the seeds treated with bacteria and algae instead of irrigation water, 2 times with 5 days intervals, 3 days after emergence. It was given NPK as basic fertilization was given to all pots after two weeks from emergence. The thinning process was done before the deficited irrigation application to remain one plant in each pot. Different irrigation regimes were started 30 days after planting and the experiment was ended 45th day after planting.

Growth Parameters

The shoot lengths of the plants were determined by measuring the distance between the soil and the extreme point, and the root lengths were measured with the help of a ruler after the soil extracted from the pot was softened with tap water and the roots were separated. The wet weights of the above-ground and underground parts were determined by weighing these parted root sand shoot separately on precision scales. Dry weights of root sand shoots; It was determined by measuring the roots and shoots on a sensitive scale after putting them in separate paper bags and keeping them in an oven at 70 ^oC for 48 hours.

Leaf Area and Temperature

Before the end of the experiment, the leaf temperatures were determined as ^oC with the help of an infrared the riometer, and leaf are an index; It was determined as cm^2 using the Easy Leaf Area program.

Statistics Data

Statistical analyzes of the obtained data were made using the COSTAT (version 6.03) package program and multiple comparison tests were performed according to the Duncan test (Düzgüneş ve ark. 1987).

RESULTS and DISCUSSION

As a result of the study; The effect of deficit irrigation, rhizobacteria, and R x KS interaction on root length was not found statistically significant. Root length was found to be between 17.92-19.03 cm in terms of deficit irrigation, and between 17.85-19.04 cm in terms of rhizobacteria applications (Table 1).Contrary to what we found, it was reported that inoculation of *Bacillus megaterium* isolated from semi-arid conditions increased the root length of wheat significantly under deficit water stress (Rashid et al., 2021). Oral et al., (2021) found that the application of rhizo bacterium increased soybean root length significantly in the fully controlled climate while root length decreased as water stress increased. Decrease in root length is due to insufficient moisture in the soil whereas the increase is because inoculation of bacteria and microalgae produce IAA that helps to increase water efficiency (Blum, 2009; Gururani et al., 2013). In the study, the effect of deficit irrigation and R x KS interaction on shoot length was found to be statistically significant at the level of 5%, while the effect of rhizobacteria applications was determined at the level of 1%. In terms of deficit irrigation applications, the highest value was obtained from DW1 applications with 19.98 cm. However, it was determined that there was no statistically signify can't difference with DW2 applications in the same Duncan group. The lowest value was measured at 13.85 cm from DW3 applications. After the application of rhizobacteria, the highest value was obtained from R2 with19.32 cm, while the lowest value was determined as 15.75 cm from R3 treatments.

Table 1. Effects of deficit water and some rhizobacteria, microalgae on some growth parameters in fenugreek

APPLICATION		Root Length (cm)	Shoot Length (cm)	Root Fresh Weight (g)	Shoot Fresh Weight (g)
Rhizobacteria (R)	**Deficit Water (DW)**				
	DW1 (control)	21,1	21,20 ab	1,50 ab	3,18
Control (R0)	**DW2 (½ reduced)**	17,87	17,40abc	0,73ef	2,25
	DW ($^3/_4$ reduced)	18,17	11,70 d	0,54 f	1,72
Average		**19,04**	**16,76 B**	**0,92 C**	**2,38**
	DW1 (control)	17,73	21,40 ab	1,39abc	3,35
***Azospirillum lipoferum* (R1)**	**DW2 (½ reduced)**	19	19,57 ab	0,80 def	2,97
	DW ($^3/_4$ reduced)	16,83	12,07 d	1,26abc	1,58
Average		**17,85**	**17,67 AB**	**1,15 B**	**2,63**
	DW1 (control)	17,73	22,43a	1,24abcd	3,68
***Bacillus megaterium* (R2)**	**DW2 (½ reduced)**	20,2	17,93abc	1,70a	2,67
	DW ($^3/_4$ reduced)	19,17	17,60abc	1,10bcd	1,9
Average		**19,03**	**19,32 A**	**1,34A**	**2,75**
	DW1 (control)	18,47	14,90 bcd	1,38 abc	3,36
***Chlorella saccharophilia* (R3)**	**DW2 (½ reduced)**	19,07	18,33 abc	1,43 ab	2,74
	DW ($^3/_4$ reduced)	17,53	14,03 cd	1,01 cde	1,77
Average		**18,35**	**15,75 B**	**1,27AB**	**2,62**
	DW1 (control)	18,75	19,98 *A*	1,37 *A*	3,39 *A*
Deficit Water Average	**DW2 (½ reduced)**	19,03	18,30 *A*	1,16 *B*	2,65 *B*
	DW ($^3/_4$ reduced)	17,92	13,85 *B*	0,97 *C*	1,74 *C*
LSD (%5) (R)		ns	*	**	ns
LSD (%5) (DW)		ns	**	**	**
R×DW		ns	*	**	ns
CV		**13,08**	**13,79**	**16,77**	**15,51**

*P<0.05 level, ** P<0.01 level significances: ns: not significance

However, it was determined that there was no statistically significant difference with the control applications and it was in the same Duncan group. The highest value in the interaction of rhizobacteria × deficit irrigation was measured from DW1 applications of R2 applications with 22.43 cm (Table 1). Dolgun and Çifci, (2018) reported that after 5 bar drought stress level, shoot length decreased significantly in durum wheat in laboratory conditions as the stress levels increased. In the study, it was determined that the rhizobacteria, deficit irrigation and interaction of rhizobacteria × deficit irrigation had a statistically 1% effect on root fresh weight. Deficit irrigation applications; While the highest value was resulted in DW1 treatments with 1.37 g, the lowest data was observed from DW3 applications with 0.97 g. In rhizobacteria applications, the highest result was1.34 g with R2 applications, and the lowest was 0.92 g and resulted in the control group. The highest value in the interaction of rhizobacteria × deficit irrigation was obtained from DW2 applications of R2 applications with 1.70 g (Table 1). In a similar study it has been reported that as the amount of irrigation decreased, root fresh weight of soybean and decreased as well in the fully controlled climate (Dehkordi et al., 2021; Oral et al., 2021). In rhizobacteria applications, the lowest value was obtained from the control group with 0.92 g, and the highest value was obtained from R2 application with 1.34 g. In the deficit water × rhizobacteria interaction, the lowest value was obtained with 0.54 g from the DW3 deficit water application of the R0 rhizobacteria application, and the highest value was obtained from the DW2 deficit water application of the R2 rhizobacteria application with 1.70 g (Tablo 1). Dehkordi et al. (2021), observed that while water deficit affected root fresh weight negatively, bio stimulants showed a positive effect and enhanced it. The increase by bio stimulants is because IAA increases water efficiency and water retention capacity (Blum, 2009; Gururani et al., 2013). According to the study data; while the effect of rhizobacteria and rhizobacteria × deficit irrigation interaction on shoot fresh weight was not statistically significant, the effect of deficit water applications on shoot fresh weight was found to be significant at a rate of 1%. In terms of rhizobacteria applications, the results were determined between 2.38-2.75 g. In terms of deficit water applications, the highest value was obtained from DW1 with 3.39 g, and the lowest was 1.74 g in DW3 treatments (Table 1).It has been observed that different water deficit levels (5.0, 7.5, and 10.0 bar) affected these shoot wet weight of durum wheat negatively in laboratory conditions in 2018 (Dolgun and Çifci, 2018). The decrease in shoot wet weight is because water deficit decelerates improvement of wheat seedling (Dolgun and Çifci, 2018). It has been determined that rhizobacteria and deficit irrigation applications have a statistically significant 1% effect on root dry weight. The effect of rhizobacteria × deficit irrigation interaction on root dry weight was found to be significant at the rate of 5%. In terms of rhizobacteria applications, the highest root dry weight was observed from R2 applications with 0.18 g, and the lowest was obtained from R1 treatments with 0.14 g. However, it was determined that there was no statistically significant difference with the R3 application sand it was determined in the same Duncan group. In deficit water applications, DW1 had the highest root dry weight with 0.19 g, and DW3 was resulted as the lowest in root dry weight with 0.13 g. However, it was observed that there was no statistically significant difference with the DW2 treatment sand it was determined in the same Duncan group. The highest value of rhizobacteria × deficit irrigation interaction with 0.24 g was found in the DW1 applications of *Basillus megaterium* (Table 1). Oral et al. (2021) determined that as the water level decreased from 100% to 25%, the root dry weight of soybean was affected negatively. Similarly, Dolgun and Çifci, (2018), found that increasing drought stress from 2.5 bar to 10.0 bar decreased root dry weight in different durum wheat varieties.

Table 1. Effects of deficit water and some rhizobacteria, microalgae on some growth and physiological parameters in fenugreek

APPLICATION		**Root Dry Weight (g)**	**Shoot Dry Weight (g)**	**Leaf Area (cm^2)**	**Leaf Temp. (°C)**
Rhizobacteria (R)	**Deficit Water (DW)**				
Control (R0)	**DW1 (control)**	0,24 a	0,4	1,39	26,99
	DW2 (½ reduced)	0,17 c	0,28	1,26	27,07
	DW (³/₄ reduced)	0,14 c	0,22	1,23	27,14
Average		**0,18 A**	**0,3**	**1,3**	**27,07**
***Azospirillum lipoferum* (R1)**	**DW1 (control)**	0,18 c	0,41	1,45	27,32
	DW2 (½ reduced)	0,12 c	0,4	1,62	27,72
	DW (³/₄ reduced)	0,12 c	0,25	1,14	27,83
Average		**0,14 B**	**0,35**	**1,4**	**27,62**
***Bacillus megaterium* (R2)**	**DW1 (control)**	0,21 b	0,45	1,61	27,52
	DW2 (½ reduced)	0,15 c	0,34	1,5	27,66
	DW (³/₄ reduced)	0,13 c	0,34	1,38	27,83
Average		**0,16 AB**	**0,38**	**1,49**	**27,67**
***Chlorella saccharophilia* (R3)**	**DW1 (control)**	0,14 c	0,39	1,26	27,11
	DW2 (½ reduced)	0,16 c	0,36	1,64	27,58
	DW (³/₄ reduced)	0,16 c	0,3	1,37	27,66
Average		**0,15 B**	**0,35**	**1,42**	**27,45**
Deficit Water Average	**DW1 (control)**	0,19 *A*	0,41 *A*	1,42 *AB*	27,23
	DW2 (½ reduced)	0,15 *B*	0,34 *B*	1,50 *A*	27,5
	DW (³/₄reduced)	0,13 *B*	0,27 *C*	1,28 *B*	27,61
LSD (%5) (R)		**	ns	ns	ns
LSD (%5) (DW)		**	**	*	ns
R×DW		*	ns	ns	ns
CV		**15,11**	**17,38**	**13,76**	**2,75**

*P<0.05 level, ** P<0.01 level significances: not significance

According to the study data; The effect of rhizobacteria and rhizobacteria × deficit irrigation interaction on shoot dry weight was found to be statistically insignificant. The effect of deficit irrigation practices on shoot dry weight was found to be statistically insignificant at the rate of 1%. In terms of rhizobacteria applications, shoot dry weight was resulted between 0.30-0.38 g. In terms of deficit irrigation applications, the highest value was determined from DW1 with 0.41 g, and the lowest value was determined from DW3 with 0.27 g (Table 1). Similar insignificant results shoot dry weight of different durum wheat varieties were found by Dolgun and Çifci, (2018). As a result of the research; The effect of rhizobacteria and rhizobacteria × deficit irrigation interaction on leaf area was not statistically significant. The effect of deficit irrigation practices on leaf area was found to be statistically significant at the rate of 5%. According to the rhizobacteria applications, the leaf area was determined between 1.3-1.49 cm^2. In deficit irrigation applications, the highest leaf area was obtained from DW2 applications with 1.50 cm^2, and the lowest leaf area was obtained from DW3 with 1.28 cm^2 (Table 1).Similarly, in a two-year experiment, leaf area index of fenugreek decreased as the water stress level increased from 100% to 40% at a research field in Iran (Baghbani-Arani et al., 2017). On the other hand, the application of PGPR had a positive impact on the leaf area of fenugreek in green house conditions in Iran (Bolandnazar et al., 2018). According to the study data, the effect of rhizobacteria, deficit irrigation and R x KS interaction on leaf temperature was not found statistically significant. According to the deficit irrigation applications, the leaf temperature ranged between 27.23-27.61 ^{o}C, while it was found between 27.07-27.67 ^{o}C in the rhizobacteria applications (Table 1).

CONCLUSION

According to the results of the study, growth retardation was detected in all plant growth parameters under deficit irrigation conditions. Water restriction compared to control had been negative results on growth parameters. In rhizobacteria and microalgae applications, the best results from parameters such as root length, root age and stem weight were obtained from *Bacillus megaterium* rhizobacteria application. According to the results of the study, it was concluded that deficited water applications cause negative results in all growth parameters, rhizobacteria and microalgae applications can be effective in reducing the negative effects of drought stress. The use of rhizobacteria is great importance in order to reduce the damage caused by adverse environmental conditions, especially drought, to plant growth and development. Thanks to the studies to be carried out with rhizobacteria in agricultural products, the damage caused by many negative ecological factors, especially drought, to plant production will be decreased.

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