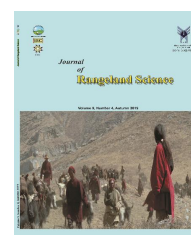


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Research and Full Length Article:

Effect of Plant Growth Promoting Rhizobacteria (PGPR) on the Seed Germination, Seedling Growth and Photosynthetic Pigments of *Astragalus caragana* under Drought Stress

Marzieh Sabeti^A, Pejman Tahmasebi^B, Elham Ghehsareh Ardestani^{C*}, Farzaneh Nikookhah^D

^A M.Sc. Graduated in Range Management, Shahrekord University, Shahrekord, Iran

^B Associate Professor, Faculty of Natural Resources and Earth Sciences, Shahrekord University, Shahrekord, Iran

^C Assistant Professor, Faculty of Natural Resources and Earth Sciences, Shahrekord University, Shahrekord, Iran, Central Laboratory, Shahrekord University, Shahrekord, Iran ^{*} (Corresponding author), Email: elham.ghehsareh@nres.sku.ac.ir

^D Assistant Professor, Faculty of Natural Resources and Earth Sciences, Shahrekord University, Shahrekord, Iran

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Abstract. Drought stress is one of the most important factors limiting plant growth all around the world. Plant Growth Promoting Rhizobacteria (PGPR) can significantly reduce drought stress effects on plants growth. This study was aimed to investigate the influence of PGPR (inoculation by *Azospirillum lipoferum*, *Azotobacter chroococcum*, *Pseudomonas aeruginosa*, and *Bacillus cereus*) on seed germination, seedling growth and photosynthetic pigments of *Astragalus caragana* under four levels of drought stress (0, -0.2, -0.4, -0.8 MPa) in laboratory conditions in Shahrekord University, Iran, 2017. Among the four PGPR treatments, *A. chroococcum* was eliminated because seeds failed to germinate and were identified as agent pathogenies. The results showed that inoculation by PGPR had a significant effect on all germination traits except shoot length. The effect of drought stress was significant on all traits expect mean germination time. The PGPR by drought stress interaction effect was significant on shoot length, embryonic leaf and seedling fresh weight, embryonic leaf and seedling dry weight, and root length. The highest and lowest seed germination indices and seedling length were related to first (control and -0.2 MPa) and second (-0.4, -0.8 MPa) levels of drought stress, respectively. It was concluded that *P. aeruginosa* was more effective in embryonic leaf fresh weight (0 and -0.2 MPa), embryonic leaf and seedling dry weight (total levels of drought stress); *B. cereus* (0 MPa) and *A. lipoferum* (0, -0.2, and -0.4 MPa) showed relatively better performance on root length and *B. cereus* (0 MPa), *A. lipoferum* (total levels of drought stress) and *P. aeruginosa* (-0.2 and -0.4 MPa; total levels of drought stress, respectively) improved growth of shoot and seedling fresh weight of the *Astragalus caragana*.

Key words: Plant germination, Plant growth, Germination indices, Chlorophyll, Carotenoid

Introduction

Rangelands have important functions in the global environmental issues nowadays. From ecological and economic aspects, they have an equal position with rainforests in terms of international attention (providing essential goods and services and forage for livestock, reducing CO₂ gas emission, providing habitat for wild animals and plants, and medicinal plants, wood and germplasm, etc.). They occupy nearly 50% of the earth's surface. The majority of rangelands are located in arid and semi-arid regions which results in drought and water shortage leading to desertification and degradation. Therefore, there is a serious need for conservation, restoration and improvement of rangelands (Friedel *et al.*, 2000; Delshadi *et al.*, 2017a; Delshadi *et al.*, 2017b). It has been shown that introducing native plant species is promising to restore degraded rangelands and leads to increase forage production and consequently livestock function.

Drought stress is one of the major constraints to plant growth and establishment that delays seed germination and reduces the plant productions (Manivannan *et al.*, 2008; Zandi Esfahan and Azarnivand, 2013; Nakhaee Nezhad Fard *et al.*, 2013; Yousefian *et al.*, 2018) and resulted in food security over the past decades. There are several ways to deal with plant drought stress including various types of germs and bacteria inclusion, especially Plant Growth Promoting Rhizobacteria (PGPR) that are considered as the desirable and natural solutions for mitigation of drought stress in plants. PGPR plays an important role in germination, and plant growth and establishment either directly or indirectly known as bio-fertilizers (Gholami *et al.*, 2009; Delshadi *et al.*, 2017a; Delshadi *et al.*, 2017b). In general, most of the studies have shown that bio-fertilizers have positive effects on germination, plant growth and development and they are suitable alternative to chemical fertilizers

(Kavandi *et al.*, 2018; Delshadi *et al.*, 2017a; Delshadi *et al.*, 2017b; Gholami *et al.*, 2009; Jahanian *et al.*, 2012). In addition, several studies showed that PGPR increases plant resistance against diseases and environmental stresses (e.g., drought stress) compared to other chemical fertilizers (Delshadi *et al.*, 2017a; Delshadi *et al.*, 2017b). More specifically, several bacteria such as *Azospirillum* spp., *Azotobacter* spp., *Pseudomonas* spp. and *Bacillus* spp. are widely used in agriculture to increase plant production and diseases control (Gholami *et al.*, 2009; Jahanian *et al.*, 2012; Shirinzadeh *et al.*, 2013; A. Noumavo *et al.*, 2013; Saric-Krsmanovic *et al.*, 2017).

Astragalus sp. is one of the largest Angiosperm genera. It is a large genus of about 3,000 species of herbs and small shrubs, belonging to the legume family (Fabaceae) and the largest genus of plants in the world. *Astragalus caragana* is endemic in Iran's rangelands and some neighboring countries. The species is classified as perennial range species which has been threatened and endangered because of human activities and climate change (Maassoumi, 1998; Ardestani *et al.*, 2015). It is known as a moderately palatable plant resistant to livestock grazing, rich in nutrients and plays an important role in soil conservation and animal feeding (Ardestani *et al.*, 2015), so it deserves consideration.

A few researches concern the use of PGPR for the native rangeland plants in particular a species from *Astragalus* genus to restore degraded rangeland (Delshadi *et al.*, 2017a; Delshadi *et al.*, 2017b). Considering the fact that the most of the rangelands are located in arid and semi-arid regions across the world, and regarding that drought stress prevents seed germination or leads to reduction of plant growth (Gholami *et al.*, 2009; Radnezhad *et al.*, 2015; Delshadi *et al.*, 2017a; Delshadi *et al.*, 2017b), the current research was conducted to investigate the effect of PGPR (*Pseudomonas aeruginosa*,

Azospirillum lipoferum, *Bacillus cereus*, *Azotobacter chroococcum* and control) on seed germination, seedling growth and photosynthetic pigments of *A. caragana* under drought stress.

Materials and Methods

Research Method

This study was conducted as a factorial experiment in a completely randomized design with three replications in the seed laboratory in Department of Natural Resources and Earth Sciences Shahrekord University, Iran in 2017. Five levels of inclusion by PGPR including *Pseudomonas aeruginosa*, *Azospirillum lipoferum*, *Bacillus cereus*, *Azotobacter chroococcum* and control were used as the first factor plus drought stress as the second factor with four levels of -0.2, -0.4 and -0.8 Mpa and control (without the use of polyethylene glycol 6000). The seeds were disinfected with sodium hypochlorite 10% for 30 seconds and then washed five times with double-distilled water. Then, the seeds were scratched using a sandpaper to break seed dormancy; after that, *P. aeruginosa*, *A. lipoferum*, *B. cereus* and *A. chroococcum* bacteria were cultured from frozen suspension of PGPR on TSA (Tryptic Soy Agar) culture medium for 48 h at 27°C incubator. The bacterial colony was moved into the TSB (Tryptic Soy Broth) culture medium and the culture medium was put on the shaker incubator at 32°C for 24-48 h; therefore, the bacteria were reproduced. Then seeds were soaked in 5 ml of inoculums at concentration $5 \times 10^8 \text{ ml}^{-1}$ bacterial cell (CFU/ml) at room temperature for 1 h separately (Bahmani et al., 2016; Saric-Krsmanovic et al., 2017). For each replication, twenty seeds were put in a petri dish (8 cm in diameter).

Polyethylene glycol (PEG) 6000 was applied as the drought stress at the stage of germination and creation of various levels of water potential including -0.2, -0.4, and -0.8 MPa. The required amount of PEG for osmotic pressure (drought stress) (Michel

and Kaufman, 1973) was calculated as follows:

$$\psi_s = -(1.18 \times 10^{-2})C - (1.8 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T \quad (\text{Equation 1})$$

Where

Ψ_s = Osmotic pressure in terms of bars,

C = Concentration of PEG 6000 g/kg water (g/kg H₂O), and

T = Temperature (°C)

Double-distilled water was used to prepare zero water potential (control). Then, 5 ml of various levels of water potential was added to each petri dish, and petri dishes were transferred to Plant Growth Chamber at temperature of 20-16 °C (day and night). The seed germination was observed daily for three weeks. Germinated seeds with root length of more than 2 mm were considered (Kaya et al., 2006). Then, the seed germination percent, rate of germination, mean germination time, vigor index, chlorophyll a and b, total chlorophyll, carotenoids, the fresh and dry weight of the root, shoot, embryonic leaf, seedling and lengths of root, shoot and seedling l were determined.

In this experiment, variables were calculated using the following Equations:

$$GP = (N/S) \times 100 \quad (\text{Equation 2})$$

$$GR = \Sigma(N_i/D_i)$$

$$MGT = \Sigma N_i D_i / N$$

$$VI = (MRL + MSL) \times GP$$

Where:

GP is germination percent,

GR is germination rate,

MTG is mean germination time,

VI is vigour index,

N is the number of germinated seeds,

S is the total number of seeds,

N_i is the number of germinated seeds per day,

D_i is the number of days from the beginning of germination,

MRL is mean root length,

MSL is mean shoot length

(Abdul-Baki and Anderson, 1973; Agrawal and Dadlani, 1995).

Measuring chlorophyll content

In order to measure chlorophyll a and b and carotenoid content, 0.25 g of fresh tissue was pulverized inside a porcelain mortar with 0.1 g calcium carbonate, and then was mixed with 10 ml of 80% acetone. The solution was intensely mixed by the shaker for 20 minutes. Then, the solution was transferred to centrifuge tubes. The tubes were placed in the centrifuge and were centrifuged for 2 min at 1000 rpm and the solution passed through the filter paper. The absorption intensity of the solutions was read at 470, 845, and 663 nm wavelengths using the spectrophotometer (DR6000 Hach). The chlorophylls and carotenoids contents were estimated (Lichtenthaler and Wellburn, 1983) as follows:

$$\text{Chl a} = (19.3 \times A_{663} - 0.86 \times A_{645})V/100w$$

) (Equation 3)

$$\text{Chl b} = (19.3 \times A_{645} - 3.60 \times A_{663})V/100w$$

$$\text{Ca} = 100 (A_{470}) - 3.27 (\text{mg Chl a}) - 104 (\text{mg Chl b}) / 227$$

Where:

Chl a, Chl b, and Ca are chlorophyll a, chlorophyll b, and carotenoids, respectively.

V = Volume of filtrate solution;

A = Absorption of light at wavelengths of 663, 645 and 470 nm, and

W = Wet weight of sample (g)

Data analysis

The statistical analyses were carried out using SPSS₂₅ statistics software. Data were analyzed using General Linear Model (GLM). Duncan test was performed to statistically determine significant difference between treatments means. A probability of 0.05 and 0.01 was considered as significant.

Results

In this study, among the four PGPR treatments, *Azotobacter chroococcum* was eliminated because the seeds failed to germinate and was identified as agent pathogenic.

The results (Tables 1 and 2) showed that the main effects of PGPR were

significant on the fresh and dry weight, root and seedling length, germination indices (GP, GR, MGT and VI) and photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid) ($P < 0.05$). Similarly, the main effects of drought stress were significant on the fresh and dry weight, root, shoot, and seedling length, germination indices (GP, GR and VI) and photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid) (Tables 1 and 2).

The results also showed that the PGPR by drought stress interaction effects were significant for shoot, embryonic leaf and seedling fresh weight and embryonic leaf and seedling dry weight, root length (Table 1), but there were no significant interaction effects on germination indices and photosynthetic pigments ($P < 0.05$) (Table 2).

Effects of Rhizobacteria

Means comparison between Rhizobacteria treatments showed that the highest embryonic leaf fresh weight, root, embryonic leaf and seedling dry weight were observed for inclusion by *Pseudomonas aeruginosa* with 1.38, 2.14, 1.18, and 1.20 times higher than the control, respectively (Table 3). *A. lipoferum* significantly enhanced shoot and seedling fresh weight by 3.86 and 1.95 times higher than that of control, respectively (Table 3). Both *A. lipoferum* and *P. aeruginosa* treatments produced the highest root fresh weight. In contrast, *Bacillus cereus* and *A. lipoferum* had a negative effect on embryonic leaf fresh weight, shoot, embryonic leaf and seedling dry weight (Table 3).

For root and seedling length, the highest values were recorded when seeds were treated with *A. lipoferum*. The results showed that *A. lipoferum* had significantly increased the root and seedling length compared to control by 1.37 and 1.27 times, respectively (Table 3).

For GP, GR, and VI, the highest values were related to *B. cereus* and *P. aeruginosa* with no significant difference between them (Table 4). For MGT, a higher value was obtained in control indicating that both *B. cereus* and *A. lipoferum* had a negative effect on MGT (Table 4).

For photosynthetic pigments, *A. lipoferum* had significantly increased chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid as compared to control by 2.17, 2.21, 2.82, and 2.40 times, respectively (Table 4).

Main effect of Drought Stress

The results of the main effect of drought stress (Table 3) showed that the highest values of shoot, embryonic leaf and seedling fresh weight, and embryonic leaf and seedling dry weight were observed at control (0 MPa). Similarly, the highest value of length was observed at control and -0.2 MPa, with no significant difference between them (Table 3). The lowest values of lengths were observed at -0.4 and -0.8 MPa (Table 3).

Similar to root length, the highest GP, GR and VI were observed in -0.2 MPa and control. In contrast, the lowest values of GP, GR, and VI were observed at levels of -0.4 and -0.8 MPa with no significant difference between them (Table 4).

The highest chlorophyll a, chlorophyll b, and carotenoid were observed at -0.2 MPa and control with no significant difference between them, and the maximum value of total chlorophyll was obtained in control (Table 4).

PGPR by Drought Stress interaction effects

Interaction effects of PGPR inoculation and drought stress showed that higher values of root length were observed by application of *B. cereus* at the level of -0.2 MPa and *A. lipoferum* at the levels of -0.2 MPa and control (0 MPa) as compared to without inoculation treatment at the same level of drought (Fig. 1a). The higher value of shoot fresh weight was observed in *B. cereus* treatment at control level (0 MPa), and higher value of shoot fresh weight was observed in *A. lipoferum* treatment at all levels of drought stress (Fig. 1b). For embryonic leaf fresh weight, higher values were observed by application of *P. aeruginosa* treatment at drought stress of -0.2 MPa, and control (Fig. 1c). Similarly, embryonic leaf dry weight was increased significantly by application of *P. aeruginosa* treatment at the levels of -0.2 MPa and control as compared to without inoculation treatment at the same level of drought (Fig. 2a).

The higher values of seedling fresh weight were observed by application of *B. cereus* treatments at no stress. Similarly, higher values of seedling fresh weight were observed for *A. lipoferum*, and *P. aeruginosa* at all levels of drought stress compared to control (without inoculation) treatment at the same level of drought stress (Fig. 2b).

The seedling dry weight was significantly increased in *P. aeruginosa* treatment at the levels of -0.2 and -0.8 MPa in comparison with control (without inoculation) treatment at the same level of drought (Fig. 2c).

Table 1. The results of analysis of variance of the effects of PGPR inoculation and drought stress on seedling growth variables in *Astragalus caragana*

Source of variation	Df	Mean square										
		Fresh weight				Dry weight				Length		
		Root	Shoot	Embryonic leaf	Seedling	Root	Shoot	Embryonic leaf	Seedling	Root	Shoot	Seedling
PGPR	3	49.50**	428.9**	76.91**	209.73**	22.89**	9.99**	22.52**	45.84**	23.31**	4.28 ^{ns}	33.65**
Drought stress	3	3.56*	89.82**	33.25**	130.58**	1.89**	4.37*	8.54**	14.13**	17.43**	19.46**	72.94**
PGPR× Drought stress	9	1.67 ^{ns}	51.1**	9.21**	24.78**	0.78 ^{ns}	2.55 ^{ns}	3.68**	3.65**	4.13*	3.38 ^{ns}	8.74 ^{ns}
Error	32	0.81	6.04	1.36	5.03	0.35	1.50	0.56	0.81	1.89	2.02	5.77
CV (%)		22.95	25.06	17.61	19.32	24.54	17.64	16.09	14.80	25.73	27.00	22.92

(* = p < 0.05, ** = p < 0.01, ns = not significant)

Table 2. The results of analysis of variance of the effects of PGPR inoculation and drought stress on germination traits and photosynthetic pigments in *Astragalus caragana*

Source of variation	Df	Mean square							
		Germination percentage	Germination rate	vigor index	Mean germination time (MGT)	carotenoid	Chlorophyll a	chlorophyll b	Total chlorophyll
PGPR	3	3030**	3.40**	3.23**	0.09**	0.23**	0.28**	0.23**	0.51**
Drought stress	3	1043**	1.20**	2.520**	0.03 ^{ns}	0.08**	0.08**	0.04**	0.12**
PGPR× Drought stress	9	195.8 ^{ns}	0.21 ^{ns}	0.09 ^{ns}	0.01 ^{ns}	0.01 ^{ns}	0.02 ^{ns}	0.01 ^{ns}	0.02 ^{ns}
Error	32	202.6	0.23	0.23	0.01	0.01	0.01	0.01	0.01
CV (%)		27.22	27.41	20.81	19.69	22.54	23.11	27.27	23.68

(* = p < 0.05, ** = p < 0.01, ns = not significant)

Table 3. Traits measured at germination stages of *Astragalus caragana* under different treatments of PGPR inoculation and different levels of drought stress

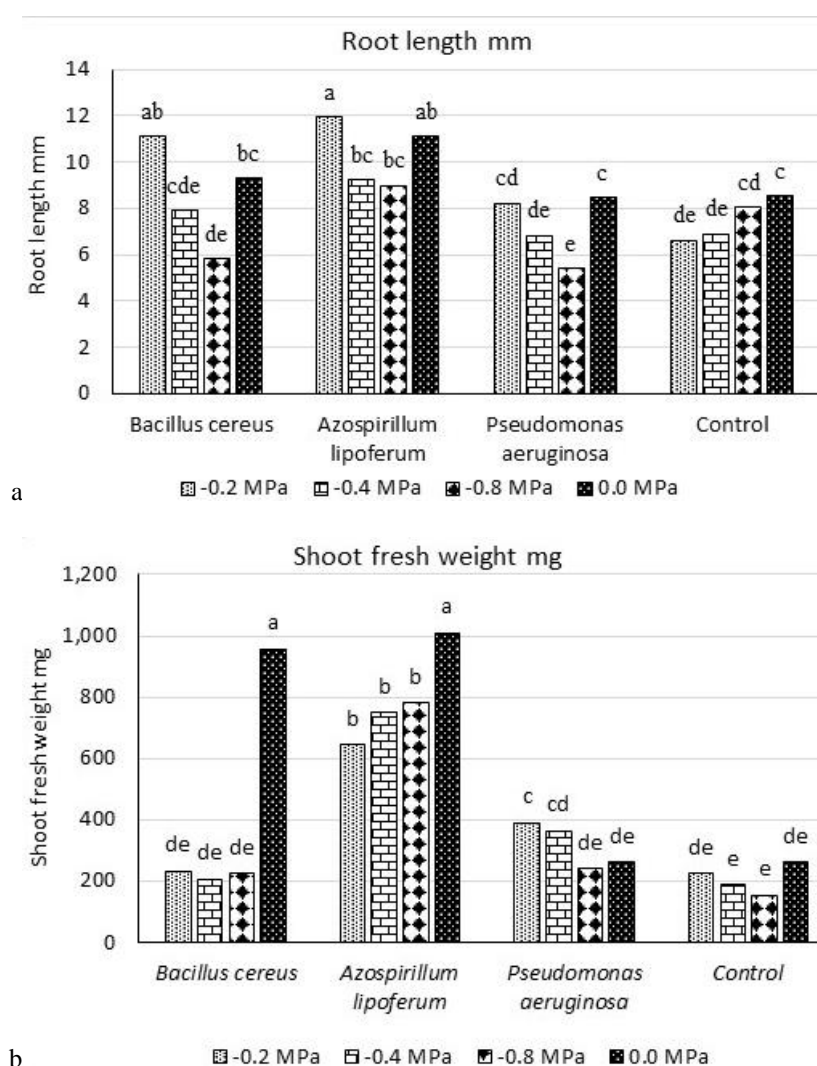
Treatments	Fresh weight(mg)				Dry weight(mg)				Length(mm)		
	Root	Shoot	Embryo leaf	Seedling	Root	Shoot	Embryo leaf	Seedling	Root	Shoot	Seedling
PGPR											
<i>B. cereus</i>	52.5±15.4b	405±3397b	257.8±54.7c	733.3±351.4c	22.5±9.6c	64.2±24.6b	95±21.9c	180.8±33.7c	8.5±2.3b	6.5±2.1a	15±3.8b
<i>A. lipoferum</i>	115±30.6a	796±168.3a	234.2±59d	1145.8±231.2a	27.5±6.2b	69.2±23.1b	96.6±13.7c	193.3±30.8c	10.3±1.6a	7.5±1.7a	17.8±3a
<i>P. aeruginosa</i>	118.3±15.3a	314.2±84.3c	453.3±126a	885.8±166.5b	60±4.3a	90.8±19.7a	159.2±43.9a	311.6±50.9a	7.2±1.9c	7.5±1.8a	14.7±3.3b
Control	54.2±14.4b	260±84.8d	327.5±81.1b	588.3±160.3d	28.3±8.3b	130±43.6a	135±33.2b	260.8±68.3b	7.5±1.5bc	6.4±1.9a	13.9±3.1b
Drought stress (MPa)											
-0.2	85.8±33.9ab	374.2±201.9b	345.8±139b	805.8±241.3b	35.8±15ab	78.3±31.3ab	130±45.3b	245±85b	9.4±2.6a	7.7±1.4a	17.2±3.5a
-0.4	84.6±45ab	376.6±246.6b	290±69.1c	750.8±272.2bc	30.8±17.3b	76.7±25.3ab	122.5±21.8c	220±53.6c	7.7±1.5b	6.2±2.2b	13.9±2.8b
-0.8	74.2±38.5b	349.2±267.1b	255±49.3d	678.3±269.9c	31.6±18b	70±18.6b	98.3±14.7d	200±34.9c	7.1±2b	5.6±1.2b	12.6±2.8b
Control	95.8±32.9a	622.5±383.9a	400±136.2a	1118.3±296.7a	40±16.5a	96.6±34.2a	145±52.8a	281±78.7a	9.4±1.5a	8.3±1.2a	17.6±2.3a

Means of column followed by same letters has no significant differences based on Duncan method (P< 0.05)

Table 4. Germination traits and photosynthetic indices of *Astragalus caragana* under different treatments of PGPR inoculation and different levels of drought stress

Treatments	germination	Germinate	vigour	MGT	Carotenoid	Chl a	Chl b	Chl a+b
	%	Rate no/day	index	Day				
PGPR								
<i>B. cereus</i>	86.2±10.7a	2.9±0.4a	13.1±4.4b	0.3±0.1b	0.7±0.2b	0.7±0.2b	0.4±0.1b	1.1±0.4b
<i>A. lipoferum</i>	69.6±20.8b	3.1±0.2a	16.6±3.5a	0.3±0.0b	1.0±0.3a	0.9±0.3a	0.5±0.1a	1.5±0.4a
<i>P. aeruginosa</i>	92.9±6.2a	2.3±0.7b	13.8±3.6b	0.5±0.2ab	0.5±0.1c	0.5±0.1c	0.2±0.1c	0.6±0.2c
Control	57.9±21.1b	1.9±0.7b	8.5±4.1c	0.6±0.3a	0.5±0.1c	0.4±0.1c	0.2±0.1c	0.6±0.2c
Drought stress (MPa)								
-0.2	85.4±15.3a	2.8±0.5a	15.0±4.5a	0.4±0.1a	0.7±0.3ab	0.7±0.3ab	0.3±0.2ab	1.0±0.4b
-0.4	70.8±22.6b	2.3±0.8b	11.3±3.8b	0.5±0.3a	0.5±0.3b	0.5±0.3b	0.3±0.1b	0.8±0.4b
-0.8	66.6±21.0b	2.2±0.7b	9.4±3.7b	0.5±0.2a	0.6±0.3b	0.6±0.3b	0.3±0.2b	0.8±0.5b
Control	83.7±19.7a	2.8±0.6a	16.2±3.9a	0.4±0.1a	0.8±0.3a	0.8±0.3a	0.4±0.2a	1.2±0.4a

Means of column followed by same letters has no significant differences based on Duncan method ($P < 0.05$)



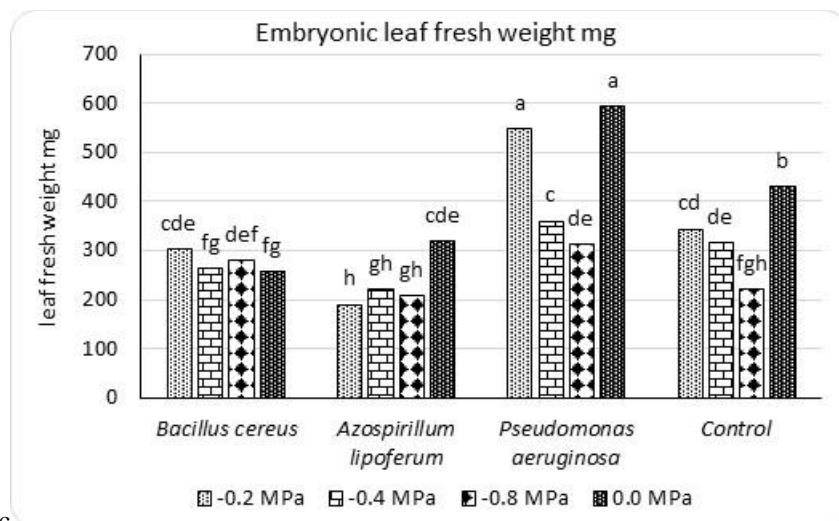
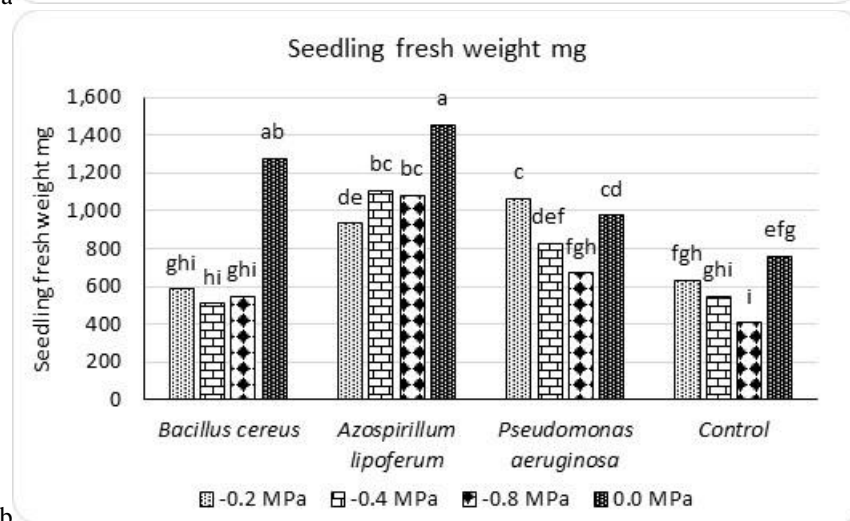
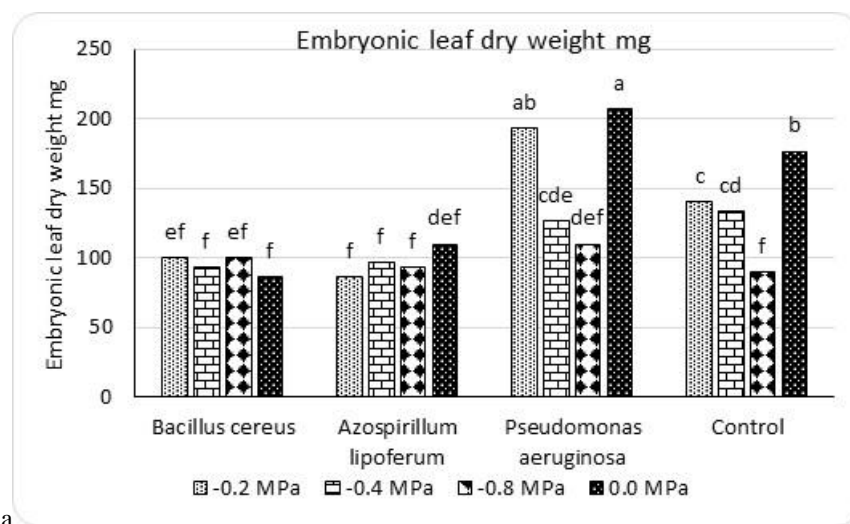


Fig. 1. Means of Root length (a), shoot fresh weight (b), Embryonic leaf fresh weight (c) of *Astragalus caragana* under three levels of drought stress (Means of column followed by same letters has no significant differences based on Duncan method ($P < 0.05$))



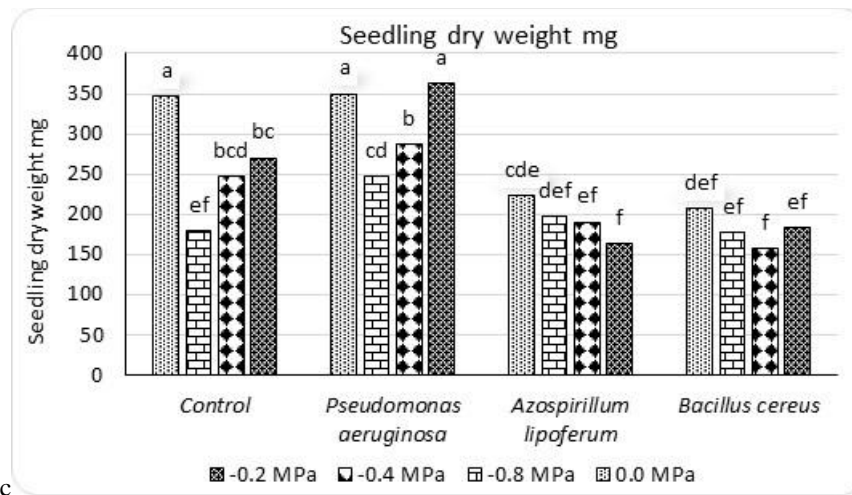


Fig. 2. Means of dry weight of the embryonic leaf (a), fresh weight of the seedling (b) and dry weight of the seedling (c) of *Astragalus caragana*, under three levels of drought stress (Means of column followed by same letters has no significant differences based on Duncan method ($P < 0.05$))

Correlation between traits

Result of Pearson's correlation coefficient between traits at germination stages of *Astragalus caragana* is presented in Table 5, showing strong positive correlation of shoot fresh weight and embryonic leaf fresh weight with seedling fresh weight ($r=0.90^{**}$), chlorophyll b ($r=0.80^{**}$), and total chlorophyll ($r=0.82^{**}$), respectively (Table 5). The parameters of root length, shoot length, and seedling length had strong positive and significant correlation with seedling length ($r=0.88^{**}$), seedling length ($r=0.85^{**}$), vigor index ($r=0.81^{**}$), and vigor index ($r=0.89^{**}$), respectively (Table 5). Germination rate index had strong negative and significant correlation with mean germination time ($r=-0.87^{**}$) (Table 5). The measured parameters of carotenoid, chlorophyll a, and chlorophyll b had a strong positive and significant correlation with chlorophyll a ($r=0.94^{**}$), chlorophyll b ($r=0.83^{**}$), and total chlorophyll ($r=0.95^{**}$); chlorophyll b ($r=0.80^{**}$), and total chlorophyll ($r=0.97^{**}$); and total chlorophyll ($r=0.91^{**}$), respectively (Table 5).

Table 5. Pearson's correlation coefficient between measured parameters at germination stages of *Astragalus caragana* under different treatments of PGPR inoculation and different levels of drought stress

Traits	Fresh weight				Length			Germination indices				Photosynthetic pigments		
	Root	Shoot	Embryo	Seedling	Root	Shoot	Seedling	GP	GR	VI	MGT	Ca	Chl a	Chl b
Shoot Fresh weight	0.46**													
Embryo Fresh weight	0.32*	-0.26												
Seedling Fresh weight	0.68**	0.90**	0.16											
Root Length	0.17	0.50**	-0.11	0.45**										
Shoot Length	0.33*	0.36**	0.30*	0.50**	0.51**									
Seedling Length	0.28*	0.50**	0.09	0.54**	0.88**	0.85**								
Germination percentage	0.23	0.05	0.36*	0.21	0.15	0.47**	0.34*							
Germination rate	0.30*	0.59**	-0.07	0.57**	0.52**	0.55**	0.59**	0.49**						
Vigor index	0.48**	0.61**	0.14	0.69**	0.75**	0.81**	0.89**	0.60**	0.76**					
Mean germination time	-0.26	-0.41**	-0.03	-0.42**	-0.46**	-0.46**	-0.53**	-0.53**	-0.87**	-0.60**				
Carotenoid	0.24	0.75**	-0.22	0.66**	0.58**	0.29*	0.51**	0.07	0.62**	0.57**	-0.49			
Chl a	0.33*	0.76**	-0.23	0.67**	0.55**	0.27	0.48**	0.02	0.62**	0.56**	-0.50**	0.94**		
Chl b	0.25	0.80**	-0.31*	0.67**	0.62**	0.27	0.53**	0.18	0.68**	0.62**	-0.52**	0.83**	0.80**	
Chl a+b	0.30*	0.82**	-0.27	0.71**	0.68**	0.28*	0.52**	0.08	0.68**	0.61**	-0.53**	0.95**	0.97**	0.91**

Significant at P< 0.05 (*) and P< 0.01 (**)

Discussion

Bacterial inclusion by *Pseudomonas aeruginosa* and *Azospirillum lipoferum* resulted in a significant increase in root fresh weight compared to control and other Rhizobacteria treatments. It is likely that these two treatments improved water absorption and uptake of nutrients. The same results for *Pseudomonas* strain were also reported on the weight of some plants by Jahanian *et al.* (2012). Moreover, inclusion by *A. lipoferum* and *P. aeruginosa* increased the seedling fresh and dry weight of the plant compared to control, respectively. Therefore, an increase in the seedling weight can be attributed to the ability of the PGPR to improve the absorption of water and nutrients by roots through the development itself (Delshadi *et al.*, 2017b).

The results also showed that embryonic leaf fresh and dry weight decreased as water potential reduced, but no significant difference was observed between root fresh weight in control (0 MPa), -0.2 and -0.4 MPa, root dry weight in control (0 MPa) and -0.2 MPa, and shoot dry weight in control (0 MPa), -0.2 and -0.4 MPa. The results also showed that the root length increased at -0.2 MPa (Table 3). Similar to other research studies, our study showed that *A. caragana* is relatively resistant to drought (Zandi Esfahani and Azarnivand, 2013; Delshadi *et al.*, 2017b). The results showed that under the initial level of drought stress, *B. cereus* and *A. lipoferum* could enhance drought stress tolerance in *A. caragana* with an increase in root length. On the other hand, they improved drought tolerance of plant under drought stress conditions (Fig. 1a). Therefore, this indicates that *Astragalus caragana* has drought resistance mechanisms (Zandi Esfahani and Azarnivand, 2013).

The interaction effects of PGPR and drought stress showed that *P. aeruginosa* improved some of the measured parameters (shoot fresh weight in -0.2 and -0.4 MPa, embryonic leaf fresh weight at all levels of drought stress, seedling fresh

weight at all levels of drought stress, embryonic leaf dry weight in 0, -0.2 MPa and seedling dry weight at all levels of drought stress) compared to other Rhizobacteria treatments.

A. lipoferum treatment significantly increased root length compared to control and other Rhizobacteria treatments. It seems that this treatment leads to better absorption of water and uptake of nutrients. Similar result was reported by Delshadi *et al.* (2017b).

There was a significant positive correlation between shoot and seedling length with vigor index (vigor index shows the power and seedling growth), indicating the direct relationship between vigor index and seedling efficiency (rapid seedling growth and plant establishment).

Our results showed that the use of one of the PGPR treatments was effective in GP, GR, VI and MGT. Their application led to a significant improvement in germination indices. Advantages of PGPR seed bio-inoculation include the improvement of different indices such as germination, photosynthetic pigments, biomass production, etc (Lucy *et al.*, 2004; Jahanian *et al.*, 2012). The results of germination indices were similar to results of drought stress on plant length, showing that *A. caragana* is relatively resistant to drought stress. The results revealed the greater effect of *B. cereus* and *A. lipoferum* on improvement of photosynthetic pigments in *A. caragana* than that of control treatment (no inoculation) Which is consistent to with those results obtained by Efeoglu *et al.* (2009) and Delshadi *et al.* (2017b), showing that *A. caragana* chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid were reduced under high levels of drought stress.

It indicates that the reduction of photosynthetic pigments under high levels of drought stress is due to increased activity of some enzymes in stress conditions; which in turn reduces the amount of photosynthetic pigments under

drought stress (Ahmadi and Baker, 2000; Ebrahimi *et al.*, 2016).

The photosynthetic pigments had a positive correlation with each other. This means that any change in any of the photosynthetic pigments will affect the rest of the photosynthetic pigments which affect plant growth.

Conclusion

The PGPR may be appropriate in developing bio-inoculation in order to improve germination, growth, development and yield of plants. In recent decades, a large number of bacteria such as *Bacillus* spp., *Azospirillum* spp., *Pseudomonas* spp. and *Azotobacter* spp. have been reported to enhance the plant growth and development. Although some researches note that the use of PGPR had a negative or no effect on seed germination, and plant growth and development; in present research, we confirmed the positive effect of *B. cereus*, *A. lipoferum* and *P. aeruginosa* on seedling fresh weight and negative effect of *B. cereus* and *A. lipoferum* on seedling dry weight of *Astragalus caragana*. with overall effect of *P. aeruginosa* on seedling dry weight of *A. caragana*.

The results showed no effect of PGPR on some traits compared to the control. Also, the results showed that *A. caragana* had relatively drought resistance mechanisms. It will be improved using PGRP. It was concluded that the most important approach to restore and improve rangelands and soil fertility is the use of seed inoculation with PGPR.

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تأثیر ریزوباکترهای محرک رشد گیاهی روی جوانه‌زنی، رشد گیاهچه و رنگدانه‌های فتوسنتز در گونه *Astragalus caragana* تحت تنش خشکی

مرضیه ثابتی^{الف}، پژمان طهماسبی^ب، الهام قهساره اردستانی^ج، فرزانه نیکوخواجه^د
^{الف} فارغ التحصیل کارشناسی ارشد رشته مرتع و آبخیزداری، دانشکده منابع طبیعی و علوم زمین، دانشگاه شهرکرد، شهرکرد، ایران
^ب دانشیار، دانشکده منابع طبیعی و علوم زمین، دانشگاه شهرکرد، شهرکرد، ایران
^ج استادیار، دانشکده منابع طبیعی و علوم زمین، دانشگاه شهرکرد، شهرکرد، ایران؛ آزمایشگاه مرکزی، دانشگاه شهرکرد، شهرکرد، ایران* (نگارنده مسئول)، پست الکترونیک: elham.ghesareh@nres.sku.ac.ir
^د ستادیار، دانشکده منابع طبیعی و علوم زمین، دانشگاه شهرکرد، شهرکرد، ایران

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چکیده. تنش خشکی یکی از مهمترین عوامل محدود کننده رشد گیاه در بسیاری از نقاط جهان است. ریزوباکترهای محرک رشد گیاهی (PGPR) می‌تواند تاثیر مهمی در کاهش اثر تنش خشکی در گیاهان داشته باشند. این مطالعه به بررسی تاثیر ریزوباکترهای محرک رشد (*Azotobacter Azospirillum lipoferum*) و *Bacillus cereus* و *Pseudomonas aeruginosa* و *chroococcum* و شاهد: بدون تلقیح) روی جوانه‌زنی و رشد گیاهچه در گونه *Astragalus caragana* تحت تنش خشکی (شاهد: ۰، ۰/۲، ۰/۴ و ۰/۸ MPa) در طول سال ۱۳۹۷ تحت شرایط آزمایشگاهی در دانشگاه شهرکرد پرداخته است. از میان چهار تیمار PGPR، تیمار *Azotobacter chroococcum* به دلیل عدم موفقیت در جوانه‌زنی بذر حذف گردید و به عنوان عامل بیماری‌زا شناسایی شد. نتایج نشان داد که اثر تلقیح بذر با سایر ریزوباکتری PGPR روی هم‌رفته صفات به جز طول ساقچه معنی‌دار بود. اثر تنش خشکی روی همه صفات بجز میانگین مدت جوانه‌زنی معنی‌دار بود. اثر متقابل PGPR در تنش خشکی روی وزن تر و خشک ساقچه‌چه، برگ اولیه، گیاهچه، و طول ریشه‌چه معنی‌دار بود ($P < 0.05$). بیشترین و کمترین شاخص‌های جوانه‌زنی و طول گیاهچه به ترتیب با سطوح اول (شاهد و ۰/۲ MPa) و دوم (۰/۴ و ۰/۸ MPa) تنش خشکی مرتبط بودند. در مجموع *P. aeruginosa* روی وزن تر برگ اولیه (۰ و ۰/۲ MPa) و وزن خشک برگ اولیه و گیاهچه (کل سطوح تنش خشکی) کارآمدتر بود، *B. cereus* (صفر مگاپاسکال) و *A. lipoferum* (۰، ۰/۲ و ۰/۴ MPa) به طور نسبی عملکرد بهتری روی طول ریشه‌چه نشان دادند، *B. cereus* (صفر مگاپاسکال)، *A. lipoferum* (کل سطوح تنش خشکی) و *P. aeruginosa* (۰/۲ و ۰/۴ MPa؛ کل سطوح خشکی به ترتیب) رشد وزن تر ساقچه‌چه و گیاهچه گونه گیاهی *A. caragana* بهبود بخشیدند.

کلمات کلیدی: جوانه‌زنی گیاه، رشد گیاه، شاخص‌های جوانه‌زنی، کلروفیل، کاروتنوئید