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Influence of seed inoculation with promoting rhizobacteria on the germination and growth traits of *Astragalus cyclophyllon* Beck under drought stress

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Abstract

The plant growth-promoting rhizobacteria (PGPR) are one of the famous sources when applying drought stress management in rangeland plants. This research was conducted to investigate the effect of PGPRs on the germination and growth of *Astragalus cyclophyllon* Beck under drought stress. A factorial experiment in a completely randomized design with three replications, was conducted in the seed laboratory. The effects of the main factors of PGPR inoculation as well as control and drought stress at four levels and their interactions on weight, length, and germination indices were investigated. *Bacillus cereus* had the highest effect on increasing shoot, seedling dry weight, and vigour indices. *Pseudomonas aeruginosa* had the highest effect on promoting radicle length. Additionally, results revealed that -0.8 MPa drought stress had a significant effect on radicle and shoot fresh weight, shoot and seedling length, germination percentage, germination rate and mean daily germination as compared to 0.0, -0.2 and -0.4 MPa indicating *A. cyclophyllon* is a drought stress resistance species. *B. cereus* at the control level of drought stress significantly increased shoot and seedling dry weight and vigour indices, whilst, *P. aeruginosa* at the drought stress level of -0.8 MPa significantly increased shoot lengths compared to control. In general, *B. cereus* significantly increased biomass by increasing shoot and seedling dry weight but *P. aeruginosa* affected elongation growth of the plant by increasing radicle and shoot lengths of *A. cyclophyllon*. Suitable PGPR as biotic elicitors can enhance the growth of the rangeland plants.

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Introduction

Rangelands are present on all continents and occupy 30 to 50% of the Earth's land surface spreading over arid and semi-arid regions (Friedel et al., 2000). In the last few decades, desertification has increased in all arid and semi-arid rangelands of the world due to anthropogenic effects (e.g., cultivation, overgrazing, etc.) and climate change (Rowlinson et al., 2008). Drought stress can hinder germination or growth and production of plants. In addition to drought (water shortage), nitrogen scarcity is the most important factor- limiting the growth of plants in these regions. When the soil moisture is sufficient, nitrogen restricts the growth and development of plants in these regions (Kowaljow et al., 2010). The application of plant growth-promoting rhizobacteria (PGPR) is one of the approaches to deal with drought stress and nitrogen deficiency in plants. Therefore, PGPR inoculation is considered as an appropriate and natural solution for reducing drought stress and supplying nitrogen in plants (Santoyo et al., 2016). Studies have reported that PGPRs have direct and/or indirect, positive, and negative effects on the growth and development of plants. In general, PGPRs promote plant growth, produce antibiotics, enzymes, and fungicidal compounds to fight phytopathogenic microorganisms, naturally fixing nitrogen, make phytohormones, dissolve the nutrients in water, and also are known as bio-fertilizers (Ehteshami et al., 2018; Gholami et al., 2009; López-Bucio et al., 2007; Mantelin and Touraine, 2004; Numan et al., 2018; Valencia-Cantero et al., 2007; Yang et al., 2009).

Some PGPR as an alternative of chemical fertilizer also raise plant resistance against both environmental stresses (drought stress) and diseases (Gholami et al., 2009; Jahanian et al., 2012; Kumar and Verma, 2018; Numan et al., 2018).

Drought stress has a high negative impact on seed germination and plant growth, which menaces global food security (Gouda et al., 2018). However some of the plants are inherently equipped with drought resistance mechanisms such as ion exchange and hormonal stimulation (Gouda et al., 2018; Noumavo et al., 2013; Numan et al., 2018; Stamenov et al., 2018; Szczech et al., 2016).

Astragalus cyclophyllon Beck as a valuable forb species is a member of the family Papilionaceae which belongs to the largest genus of plants in the world i.e., *Astragalus* containing about 4,000 herbal and shrub species (Maassoumi, 2000). *A. cyclophyllon* is an endemic perennial species in Iran's rangelands. *Astragalus* genus contains valuable plants that have various ecological functions on rangelands but their seed production and germination are severely diminished in drought conditions and water shortage availability, specifically under grazing in drought conditions which is a commonplace phenomenon in the rangelands around the world. Like others, *A. cyclophyllon* as a palatable species is of great importance in rangelands because of providing livestock feeds with high forage quality, nutrient richness, soil conservation, facilitating water infiltration, carbon sequestration, nitrogen fixation, etc (Ardestani et al., 2015; Ghahremaninejad et al., 2016; Maassoumi, 2000). However, the regeneration of this species is restricted due to the low germination capacity, specifically in drought conditions.

This species was introduced to be at the brink of extinction due to human activities (severe grazing intensity and frequency) and climate change (Ardestani et al., 2015; Jalili and Jamzad, 1999; Maassoumi, 1998). Therefore, studies on this species is warranted and specifically its germination under water deficiency and drought stress are important issues for rehabilitation, which is lacking.

To the best of our knowledge, a few studies have been carried out on the inoculation of rangeland seeds with PGPR under drought stress for the restoration of rangeland degradation (Delshadi et al., 2017a, 2017b; Gholami et al., 2009; Radnezhad et al., 2015). Starting from these motivations, the purpose of this research is defined to study the effects of PGPR (*Bacillus cereus* (Ptcc No:1816), *Pseudomonas aeruginosa* (Atcc No:9027), *Azotobacter chroococcum* (Ptcc No: 9D) and *Azospirillum lipoferm* (Ptcc No: 12D) under drought stress on germination and growth indicators of *A. cyclophyllon*. We hypothesized that the effect of PGPR on germination and growth of *A. cyclophyllon* would increase after inoculation with PGPR due to a sustained improvement of biological traits.

Materials and methods

Preparation of Petri dishes

This study was performed in 2019 in the seed laboratory of the Faculty of Natural Resources and Earth Science, Shahrekord University, Iran. A factorial experiment in a completely randomized design with three replications was designed. To do so, four PGPR inoculation was applied including *B. cereus, P. aeruginosa, A. chroococcum,* and *A. lipoferm* with control (no PGPR inoculation). Moreover, four levels of drought stress including control (without polyethylene glycol (PEG) 6000: double-distilled water), -0.2, -0.4 and -0.8 MPa was conducted.

The seeds of *A. cyclophyllon* were prepared by Pakan Bazr Esfahan Company, Iran. The seeds were sterilized with sodium hypochlorite 10% for 30 seconds and then rinsed quickly five times in double-distilled water. Seeds were scraped using sandpaper to break plants seed coats. Then seeds were drenched for one hour at the inoculation of PGPR separately. Twenty seeds were planted in each Petri-dish (8 cm diameter).

Equation 1 was used to calculate the amount of required PEG for drought stress (osmotic pressure) (Michel and Kaufmann, 1973).

Osmotic pressure in terms of Bar (ψs):

$$\psi s = -(1.18\times10^{-2})\ C - (1.8\times10^{-4})\ C^2 + (2.67\times10^{-4})\ CT + (8.39\times10^{-7})\ C^2T$$ Equation (1)

in which: C = the concentration of PEG 6000 g/kg water (g/kg H2O), T= temperature in terms of centigrade.

The experiment was performed in Plant Growth Chamber with a temperature of 16-20 ºC (day and night), at 70% relative humidity and 8-h dark lighting, and 16-h of light conditions. Germinated seeds with root length of more than 2 mm were counted (Kaya et al., 2006). Afterwards, fresh, dry weight and length of the radicle, shoot and seedling, percentage and rate of germination, mean germination time, the coefficient velocity of germination, mean daily germination, allometric coefficient, and vigour indices (1 and 2) were determined. The studied variables were calculated based on the following equations:

Germination percentage (*GP*);

$$GP = (N/S) \times 100$$ Equation (2)

In which: N = the number of germinated seeds

S = total number of seeds

Germination rate (*GR*):

$$GR = \Sigma\ (Ni/Di)$$ Equation (3)

In which: Ni = the number of germinated seeds in daily intervals

Di = the number of days from the beginning of germination

Mean germination time (*MGT*):

$$MGT= \Sigma NiDi/N$$ Equation (4)

In which: Ni = the number of seeds in daily intervals

Di = the number of days counted from the beginning of germination

N = the number of germinated seeds

Mean daily germination (*MDG*):

$$MDG= FGP/D$$ Equation (5)

In which: FGP= final germination percentage

D = the number of days to reach the maximum final germination

The coefficient velocity of germination (*CVG*):

$$CVG = (\sum_{i=1}^{k} f_i / \sum_{i=1}^{k} f_i x_i)100$$ Equation (6)

In which: f_i = the number of seeds newly germinated on day i

x_i = the number of days from sowing

k = last day of germination

Allometric coefficient (*AC*):

Equation (7)

$$AC = MRL/\ MSL$$

In which: MRL = mean root length

MSL = mean shoot length

Vigor index (1) [*VI* (1)]:

$$VI\ (1) = (MRL+MSL) \times GP$$ Equation (8)

In which: MRL = mean root length

MSL = mean shoot length

GP = germination percentage

Vigor index (2) [*VI* (2)]:

$$VI\ (2) = (MRDW+MSDW) \times GP$$ Equation (9)

In which: $MRDW$ = mean root dry weight

$MSDW$ = mean shoot dry weight

GP = germination percentage (Abdul-Baki and Anderson, 1973; Agrawal and Dadlani, 1995; Jahanian et al., 2012; Kavandi et al., 2018).

Statistical analyses

The statistical analyses were carried out using a General Linear Model (GLM) to determine the treatment effects (inoculation with PGPR and drought stress as the main effects and their interactions) on different growth indices employing IBM SPSS statistics 25. Duncan test was performed to determine the significant differences between treatments means ($P<0.05$).

Results

Effects of PGPR

The results showed that PGPR (main effect) significantly affected on the shoot and seedling dry weight, radicle and seedling length, allometric coefficient, and vigour indices (1 and 2) ($P<0.01, 0.05$) (Table 1).

The highest enhancement of shoot and seedling dry weight and vigour indices (1 and 2) were observed in the treatment of *B. cereus* that was 4, 2.33, 2.56, and 10 times more than the control treatment, respectively (Table 2) whilst no significant difference was found in other PGPR treatments. *P. aeruginosa* treatment significantly increased radicle length by 1.24 in comparison of the other treatments with the control treatment. There was no significant difference among *B. cereus*, *A. chroococcum*, and control treatments on radicle length of *A. cyclophyllon*. The lowest radicle length was observed when *A. lipoferum* treatment was applied (Table 2). There was a negative effect on radicle length by *A. lipoferum* compared to control treatment. *A. chroococcum* and *A. lipoferum* negatively affected seedling length relative to control. *B. cereus* had the highest effects on the allometric coefficient compared to *P. aeruginosa*, *A. chroococcum*, and control but not with *A. lipoferum* (Table 2).

Table 1. Analysis of variance of measured parameters of seed germination indicators of *A. cyclophyllon* under different PGPR (Plant growth-promoting rhizobacteria) inoculation and drought stress levels (Statistically significant values are indicated: *P, 0.05; **P, 0.01).

<table>
<tr><th colspan="3" rowspan="2">Source of Variation</th><th colspan="3">Mean square</th></tr>
<tr><th>PGPR</th><th>Drought stress</th><th>PGPR × Drought stress</th></tr>
<tr><td rowspan="10">Growth traits</td><td></td><td>df</td><td>4.000</td><td>3.000</td><td>12.000</td></tr>
<tr><td rowspan="3">Fresh weight</td><td>Radicle</td><td>0.002^{ns}</td><td>0.009**</td><td>0.002 ns</td></tr>
<tr><td>Shoot</td><td>0.007 ns</td><td>0.027**</td><td>0.004 ns</td></tr>
<tr><td>Seedling</td><td>0.025 ns</td><td>0.331**</td><td>0.023 ns</td></tr>
<tr><td rowspan="3">Dry weight</td><td>Radicle</td><td>0.000 ns</td><td>7.306E-6 ns</td><td>6.382E-5 ns</td></tr>
<tr><td>Shoot</td><td>0.002*</td><td>0.002*</td><td>0.002**</td></tr>
<tr><td>Seedling</td><td>0.003**</td><td>0.004**</td><td>0.004**</td></tr>
<tr><td rowspan="3">Length</td><td>Radicle</td><td>0.670**</td><td>1.654**</td><td>0.161 ns</td></tr>
<tr><td>Shoot</td><td>0.198 ns</td><td>0.952**</td><td>0.267*</td></tr>
<tr><td>Seedling</td><td>1.018*</td><td>3.827**</td><td>0.605 ns</td></tr>
<tr><td rowspan="6">Germination indices</td><td colspan="2">Germination percentage</td><td>282.917 ns</td><td>2194.444**</td><td>199.306 ns</td></tr>
<tr><td colspan="2">Germination rate</td><td>0.231 ns</td><td>1.791**</td><td>0.163 ns</td></tr>
<tr><td colspan="2">Mean germination time</td><td>0.247 ns</td><td>1.099 ns</td><td>0.411 ns</td></tr>
<tr><td colspan="2">Coefficient of velocity of germination</td><td>0.002 ns</td><td>0.021 ns</td><td>0.006 ns</td></tr>
<tr><td colspan="2">Mean daily germination</td><td>5.773 ns</td><td>44.783**</td><td>4.068 ns</td></tr>
<tr><td colspan="2">Allometric coefficient</td><td>0.583*</td><td>0.488 ns</td><td>0.352 ns</td></tr>
<tr><td rowspan="2">Growth indices</td><td colspan="2">Vigour index 1</td><td>0.035**</td><td>0.061**</td><td>0.041**</td></tr>
<tr><td colspan="2">Vigour index 2</td><td>0.000**</td><td>0.000**</td><td>0.000**</td></tr>
</table>

Main effect of drought stress

The drought stress significantly affects on the fresh weight, shoot and seedling dry weight, length, germination percentage, germination rate, mean daily germination, and vigour indices (1 and 2) (Table 1).

Table 2. Traits and germination indices stages of germination of *A. cyclophyllon* under different treatments of PGPR (Plant growth-promoting rhizobacteria) inoculation and different levels of drought stress (Statistically significant values are indicated: *P, 0.05; **P, 0.01).

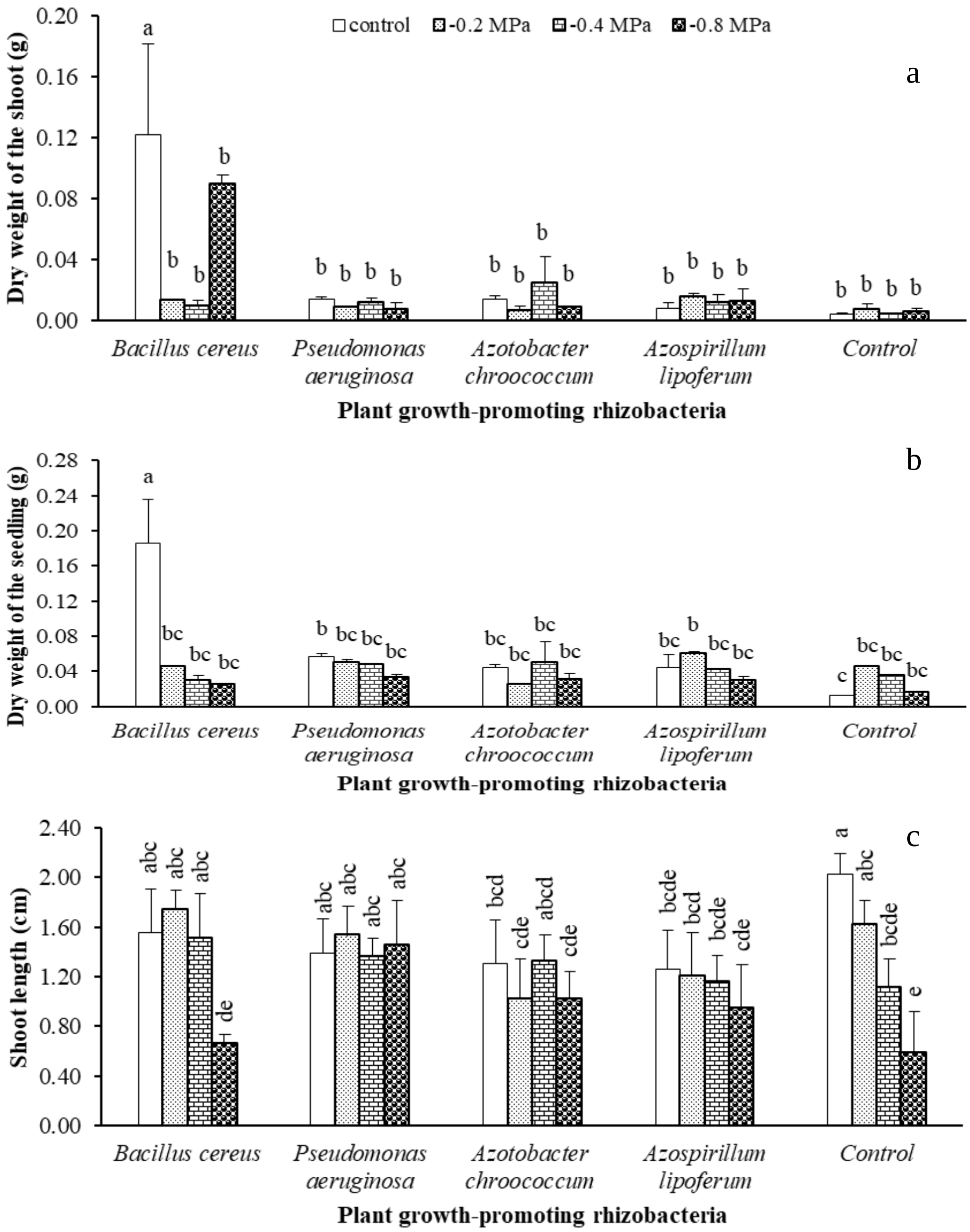
Trait and index		PGR (5×10^8 CFU/ml)					Drought stress (MPa)			
		B. cereus	*P. aeruginosa*	*A. chroococcum*	*A. lipoferm*	Control	0	-0.2	-0.4	-0.8
Fresh weight (g)	Radicle	0.08±0.03a	0.07±0.01a	0.07±0.02a	0.05±0.01a	0.05±0.01a	0.07±0.02a	0.09±0.01a	0.07±0.01a	0.03±0.005b
	Shoot	0.17±0.09a	0.17±0.03a	0.13±0.07a	0.13±0.04a	0.12±0.02a	0.18±0.04a	0.17±0.02a	0.14±0.03a	0.09±0.03b
	Seedling	0.47±0.13a	0.49±0.07a	0.43±0.11a	0.44±0.09a	0.37±0.07a	0.57±0.08a	0.50±0.05ab	0.45±0.06b	0.23±0.06c
Dry weight (g)	Radicle	0.01±0.004a	0.01±0.007a	0.01±0.001a	0.01±0.002a	0.01±0.002a	0.01±0.003a	0.01±0.002a	0.01±0.002a	0.01±0.005a
	Shoot	0.04±0.002a	0.01±0.002b	0.01±0.002b	0.01±0.004b	0.01±0.002b	0.03±0.004a	0.01±0.002b	0.01±0.002b	0.01±0.003b
	Seedling	0.07±0.01a	0.05±0.01b	0.04±0.01b	0.04±0.007b	0.03±0.005b	0.07±0.01a	0.04±0.005b	0.04±0.008bc	0.03±0.004c
Length (cm)	Radicle	1.24±0.34b	1.57±0.24a	1.23±0.22b	0.91±0.13c	1.27±0.17b	1.42±0.17ab	1.54±0.24a	1.23±0.18b	0.78±0.21c
	Shoot	1.37±0.29a	1.44±0.16a	1.17±0.14a	1.15±0.14a	1.34±0.30a	1.51±0.24a	1.43±0.17a	1.30±0.12a	0.94±0.24b
	Seedling	2.64±0.48ab	3.01±0.40a	2.38±0.25b	2.45±0.26b	2.97±0.44a	2.96±0.72a	3.10±0.31a	2.74±0.28a	1.97±0.35b
Germination percentage (%)		64.61±8.08a	64.58±4.53a	74.58±10.2a	71.67±10.2a	63.75±7.17a	72.08±6.32a	73.67±5.20a	77.33±6.45a	51.33±8.21b
Germination rate (no/day)		1.84±0.46a	2.131±0.27a	2.047±0.61a	1.82±0.57a	2.06±0.41a	2.10±0.36a	2.2±0.30a	2.14±0.39a	1.46±0.46b
Mean germination time (day)		3.01±0.19a	3.01±0.25a	3.15±0.47a	3.35±0.36a	3.10±0.30a	3.00±0.41a	2.79±0.24a	3.19±0.27a	3.44±0.40a
Coefficient velocity of germination		0.35±0.005a	0.35±0.002a	0.32±0.042a	0.32±0.006a	0.35±0.003a	0.35±0.006a	0.39±0.002a	0.32±0.003a	0.31±0.003a
Mean daily germination		9.23±1.15a	9.23±0.006a	10.66±1.03a	10.24±1.40a	9.11±1.00a	10.3±0.90a	10.52±0.75a	11.05±0.90a	7.33±1.15b
Allometric coefficient		1.46±0.91a	0.97±0.31b	1.02±0.26b	1.31±0.28ab	0.99±0.32b	1.09±0.30a	0.98±0.30a	1.12±0.32a	1.40±0.82a
Vigour index 1		0.21±0.10a	0.14±0.05b	0.09±0.07b	0.11±0.06b	0.082±0.05b	0.21±0.10a	0.14±0.05ab	0.11±0.06bc	0.05±0.02c
Vigour index 2		0.01±0.004a	0.002±0.001b	0.002±0.001b	0.002±0.001b	0.001±0.0005b	0.009±0.005a	0.002±0.001b	0.002±0.001b	0.001±0.0005b

Applying the -0.8 MPa level of drought stress caused the lowest radicle and shoot fresh weight, shoot and seedling length, germination percentage, germination rate and mean daily germination while there was no significant difference in applying 0, -0.2, -0.4 MPa drought stress on these indicators (Table 2). The highest shoot and seedling dry weight, and vigour index 2 observed when control drought stress was applied (Table 2). The highest seedling fresh weight, radicle length, and vigour index 1 were observed at -0.2 MPa and controls but no significant differences were found between these two indicators at -0.2 and control (Table 2).

PGPR by drought stress interaction effects

The interaction of PGPR and drought stress significantly affects on the shoot and seedling dry weight, shoot length, and vigour indices (1 and 2) (Table 1).

Shoot dry weight, seedling dry weight, and vigour indices (1 and 2) significantly increased by implementing *B. cereus* at the level of control when compared with all the other treatments including PGPR and drought stress (Figure 1a, b, d, e).



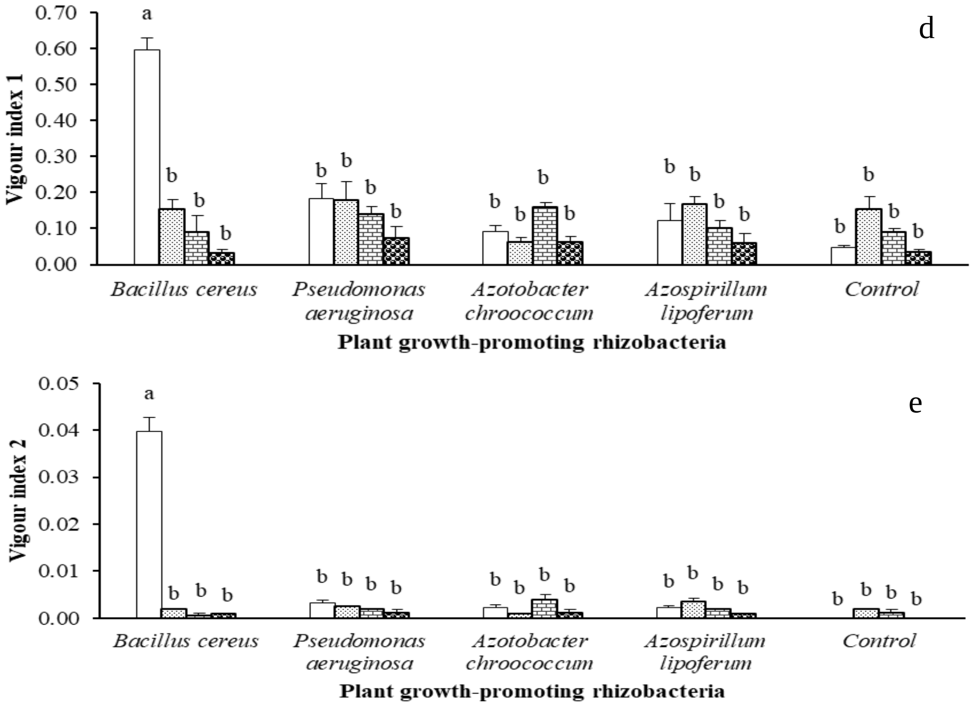


Figure 1. Shoot dry weight (a), seedling dry weight (b), shoot length (c), vigour index 1 (d), and vigour index 2 (e) of *A. cyclophyllon*, Duncan test (*P< 0.05*). Different small letters indicate significant differences between means of the plant growth-promoting rhizobacteria under drought conditions.

P. aeruginosa significantly enhanced seedling dry weight in comparison to no PGPR inoculation at the control drought stress level (Figure 1b). Moreover, *P. aeruginosa* inoculation at the level of -0.8 MPa drought stress significantly raised shoot length in comparison to control at the same level of drought stress (Figure 1c).

Application of *A. lipoferum* and *A. chrooccum* had a negative effect on shoot length at control (without PGPR) when no drought stress applied (Figure 1c).

Correlation between traits

There was a significant and positive correlation between radicle fresh weight and seedling fresh weight (*r*= 0.812). Shoot fresh weight had a significant positive correlation with seedling fresh weight (*r*= 0.852). There was a significant and positive correlation between shoot dry weight and seedling dry weight (*r*= 0.931), vigour index 1 (*r*= 0.920), vigour index 2 (*r*= 0.966). Seedling dry weight had a significant positive correlation with vigour index 1 (*r*= 0.980) and vigour index 2 (*r*= 0.952). There was a significant and positive correlation between radicle length and seedling length (*r*= 0.800). Germination percentage had a significant positive correlation with mean daily germination (*r*= 0.999). There was a significant and positive correlation between germination rate and mean daily germination (*r*= 0.999). Mean germination time had a significant negative correlation coefficient with the velocity of germination (*r*= -0.941). There was a significant and positive correlation between vigour index 1 and vigour index 2 (*r*= 0.941) (Table 3).

Discussion

Drought stress, grazing, and anthropogenic effects have put some of the valuable rangeland species of the *Astragalus* genus in the red book as the species at the brink of extinction (Jalili and Jamzad, 1999). *A. cyclophyllon*, amongst other species of this genus, is a valuable rangeland species and its germination and reproduction have been subject of much concern in recent years. Therefore, increasing germination and growth of the seeds of this species through inoculation of rhizobacteria as biotic promoting plant germination and growth is important from an ecological point of view, which is studied in this research.

Table 3. Pearson's correlation coefficient between measured parameters stages of germination of *A. cyclophyllon* under different treatments of PGPR inoculation and different levels of drought stress (FW= Fresh weight, DW= Dry weight, L= Length, GI= Germination Indices, GP= Germination percentage, GR= Germination rate, MGT= Mean germination time, CVG= Coefficient velocity of germination, MDG= Mean daily germination, AC= Allometric coefficient, VI1= Vigour index 1 and VI2= Vigour index). Note: if two parameters are >±0.8, they have been investigated. Correlations were significant at $P< 0.05$ and 0.01 calculated by IBM SPSS statistics 25.0.

		FW			DW			L			GI							
		Radicle	Shoot	Seedling	Radicle	Shoot	Seedling	Radicle	Shoot	Seedling	GP	GR	MGT	CVG	MDG	AC	VI1	VI2
FW	Radicle	1.000																
	Shoot	0.681**	1.000															
	Seedling	0.812**	0.852**	1.000														
DW	Radicle	0.278*	0.289*	0.248	1.000													
	Shoot	0.355**	0.466**	0.432**	0.320*	1.000												
	Seedling	0.419**	0.549**	0.546**	0.339**	0.931**	1.000											
L	Radicle	0.663**	0.688**	0.688**	0.274*	0.211	0.293*	1.000										
	Shoot	0.533**	0.655**	0.660**	0.202	0.354**	0.340**	0.702**	1.000									
	Seedling	0.546**	0.588**	0.646**	0.213	0.190	0.239	0.800**	0.795**	1.000								
GI	GP	0.335**	0.418**	0.475**	0.022	0.092	0.146	0.519**	0.470**	0.538**	1.000							
	GR	0.335**	0.418**	0.475**	0.022	0.092	0.146	0.519**	0.470**	0.538**	0.999**	1.000						
	MGT	-0.021	-0.123	-0.207	0.077	-0.114	-0.106	-0.084	-0.245	-0.113	0.006	0.006	1.000					
	CVG	0.033	0.165	0.238	0.009	0.241	0.217	0.070	0.231	0.099	-0.060	-0.060	-0.941**	1.000				
	MDG	0.335**	0.418**	0.475**	0.022	0.092	0.146	0.519**	0.470**	0.538**	0.999**	0.999**	0.006	-0.060	1.000			
	AC	-0.327*	-0.305*	-0.305*	-0.188	0.007	-0.073	-0.561***	-0.010	-0.344**	-0.342**	-0.342**	-0.116	0.080	-0.342**	1.000		
	VI1	0.494**	0.611**	0.615**	0.372**	0.920**	0.980**	0.405**	0.451**	0.401**	0.216**	0.215**	-0.135	-0.244	0.215**	-0.113	1.000	
	VI2	0.322**	0.439**	0.427**	0.335*	0.966**	0.952**	0.196	0.319*	0.180	0.078	0.078	-0.169	0.309*	0.078	0.007	0.941**	1.000

This study revealed that under laboratory conditions, seed inoculation of *A. cyclophyllon* with some PGPR strains improved shoot and seedling dry weight, radicle length, allometric coefficient, and vigour indices relative to the controls indicating that PGPRs generally improve some indicators of plant growth. By enhancing the volume and root development, The PGPR increased the plant's access to nutrients and water, thereby increasing absorption of the nutrients by the plant. The plant nutrient uptake enhanced the plant shoot growth (Delshadi et al., 2017a, 2017b).

Similar improvement of plant traits by PGPRs has been reported (Delshadi et al., 2017a, 2017b; Gholami et al., 2009; Jahanian et al., 2012; Noumavo et al., 2013).

Inoculation of seeds of *A. cyclophyllon* with *B. cereus* significantly increased shoot and seedling dry weight relative to control (no PGPR) at different drought stress levels. Increasing shoot and seedling dry weight as two indicators of biomass assured that *B. cereus* affected directly on biomass indicators of plants as also reported by Jetiyanon et al. (2008), Jetiyanon and Plianbangchang (2010) and Widnyana and Javandira (2016). They demonstrated that the indoleacetic acid (IAA) (the plant growth regulators) extracted from *B. cereus* would be one of the substances for plant growth enhancement and it can, directly and indirectly, provide favorable conditions for plant growth (Glick et al., 2007; Nadeem et al., 2014).

P. aeruginosa increased radicle length suggesting the radicle length elongation may increase water accessibility. Existing literature reveals that inoculation of some plants with PGPR provided plants with more access to water and nutrients by increasing the length and volume of roots (Davoodifard et al., 2012; Gholami et al., 2009; Widnyana and Javandira, 2016). Therefore, we expect that a significant increase of *A. cyclophyllon* radicle length by inoculation of *P. aeruginosa* will consequently increase plant resistance to drought via root increment and more water accessibility, though, reactions of plant growth to inoculation with PGPR depend on PGPR strain, plant genotype, and growth conditions (Gholami et al., 2009; Khalid et al., 2004).

In contrast to *P. aeruginosa,* inoculation of *A. cyclophyllon* seeds with *A. lipoferum* had a significant negative effect on radicle length. Moreover, the inoculation of *A. chrooccum* and *A. lipoferum* also resulted in a significant negative effect on the plant seedling length. This could be due to the ability of some PGPR to produce hydrogen cyanide gas, which inhibits the germination and growth of plant roots (Stamenov et al., 2018), these PGPR deal with pathogens decreased access to iron, resulting in decreased plant growth (Alstrom and Burns, 1989). On the other hand, Khosravi and Mohmoudi (2013) demonstrated a similar reduction in wheat growth in the presence of Azotobacter. One of the materials that can prevent and reduce plant height and the transfer of auxin is ethylene acetic acid (Vacheron et al., 2013). Drought stress increases the ethylene concentration of plant species. Also, the decreased plant height, is a result of applying drought stress conditions. This can be displayed to damage photosynthesis due to the dehydration and decreased production of materials for submission to the plant growing parts (Jamshidi et al., 2012). Moreover, in contrast to the current study, Shaukat et al. (2006) reported that the introduction of *Azospirillum* and *Azotobacter* in the rhizosphere of sunflower and wheat had a positive effect on the germination and the length of the seedlings whilst, Stamenov et al. (2018) also reported a negative effect of PGPR on the germination and growth of *Allium cepa* L., therefore, considering this contradictory results, we may conclude that applied treatments of PGPR had a different effect on the germination and growth indicators of different species.

The drought stress significantly effects on the fresh weight, shoot and seedling dry weight, length, germination percentage and rate, mean daily germination, and vigour indices (1 and 2) are all negatively related with drought stress, indicating that this species needs more conservative management decisions in the drought condition, which is a commonplace phenomenon of the arid rangelands. However, comparison of means revealed that shoot and seedling dry weight and vigour index 2 significantly decreased with reducing available water (drought stress), but radicle and shoot fresh weight, shoot and seedling length, percentage and rate of germination and mean daily germination at the level of 0.2, -0.4 MPa and control (without drought stress) were not affected significantly. However, applying the -0.8 MPa level of drought stress caused the lowest the above mentioned traits. Therefore, it can be stated that *A. cyclophyllon* fairly exhibits drought resistance mechanisms. Also, *A. cyclophyllon* reveals drought tolerance in seedling fresh weight, radicle length and vigour index 1 in low drought stress level (-0.2 MPa). These results proved that *A. cyclophyllon* is moderately resistant to drought stress as stated by Zandi Esfahani and Azarnivand (2013). The findings suggest that rehabilitation of this species in abandoned rangelands should be prevented in low rainfall years with water deficiency and consequently drought stress in the arid rangelands.

Shoot dry weight, seedling dry weight and vigour indices (1 and 2) significantly increased by inoculation of *B. cereus* at the control level when compared with all the other treatments including PGPR and drought stress, suggesting that *B. cereus* significantly increased biomass indicators in no-drought stress conditions. Therefore, we recommend facilitating *B. cereus* increment through seed inoculation in the absence of drought conditions or rehabilitation practices. *P. aeruginosa* also significantly enhanced seedling dry weight in comparison to no PGPR inoculation at the control drought stress level (Fig. 1b). In a natural environment without stress, many of the mechanisms used by PGPR for growth increase are common. One of the mechanisms of increasing the plant growth and yield by PGPR is the ability to decrease siderophore and raise the level of iron in the plant (Bhattacharyya and Jha, 2012). However, increased plant dry weight can be displayed as the ability of the PGPR. Under difficult and stressful conditions, some species cannot react with the host plant and, the PGPR are not efficient on the plant growth and development. Moreover, *P. aeruginosa* inoculation at the level of -0.8 MPa drought stress significantly raised shoot length in comparison to control (without inoculation) at the same level of drought stress (Figure 1c). These results convinced us that both *B. cereus* and *P. aeruginosa* are promising PGPR that mutually increase biomass (i.e., shoot dry weight, seedling dry weight) and establishment indicators of plants (shoot length and vigour indices). Nevertheless, their reciprocal effects should be studied in future research.

Increasing shoot length of *A. cyclophyllon* by inoculation of *P. aeruginosa* at the level of -0.8 MPa reveals that *P. aeruginosa* is one of the best choices for increasing rehabilitation chance of the species in drought condition due to increasing water accessibility through shoot length increment. We should underline that both biomass (shoot and seedling dry weight, shoot length) and plant vigour (vigour indices) are simultaneously affected by mutual effects of PGPR and drought.

Considering the results of PGPR inoculations and correlation between germination indicators reveals that PGPRs do not significantly increase the inter-correlated indicators of germination (i.e., germination percentage *with* mean daily germination; germination rate and mean daily germination; mean germination time coefficient *with* the velocity of germination) (Table 3). In other words, PGPRs affects germination indicators of seeds and not seed dormancy indicators.

Positive and significant correlation between (i) radicle and shoot fresh weight with seedling fresh weight and (ii) shoot dry weight with seedling dry weight were observed indicating a direct relationship between the seedling growth and seedling components. Also, a strong and positive relationship between shoot and seedling dry weight with vigour index 1 was seen representing seedling strength and growth rate that consequently affects seedling efficiency. The highest correlation was observed between mean germination time with the percentage and rate of germination which can be an indication of rapid seedling growth and plant reestablishment.

Enhanced plant growth through seed treatment with PGPR can be due to the impact of the PGPR on the metabolic and physiological activities of the plant species. As well as nitrogen fixation, another part of this additive impact is on the increased plant efficiency by the hormones cytokinin and auxin produced by these PGPR, stimulating the uptake of water and nutrients (Delshadi et al., 2017a, 2017b). The results of the study showed that PGPR can be regarded as an organic approach to improve seed establishment and efficiency. Taking into account climatic changes and drought stress conditions, which is commonplace phenomena of arid lands, enforce us to conduct further research on the effects of PGPRs on the plant seed germination and establishment indicators, specifically, for those plant species which are on the brink of extinction such as *A. cyclophyllon*. It should be noted that selecting appropriate PGPR may depend on the desired seed indicator to be promoted or restricted, climatic conditions, and the species under consideration. Therefore, mutual interactions of the PGPRs, as well as responses of different species to PGPRs' inoculation, need to be studied further, specifically, in the field.

Conclusions

In the coming years, land degradation will be the greatest threat to food safety. Accordingly, the critical issue is how to feed the growing population of the Earth where the universal loss of plant production due to degradation of arid rangeland soils lowers to as little as 5% due to its extensive use.

Seed inoculation with PGPR could have a capability for the rehabilitation of degraded soils because PGPR can create an interdependent effect for nutrient absorption as well as formation and stabilization of soil aggregate.

The major aim of this study was to understand the effect of seed inoculation with PGPR for restoration of degraded rangelands. In the process, a mixture of management practices and decisions were found to be essential. These include reintroduction of rangeland plant species and inoculation of their seeds with bio-fertilizers and other associated components. The present study showed that the use of some PGPR increased the production of *A. cyclophyllon*. The fertilizers were efficient in raising plant growth and nutrient acquisition. Therefore, drought stress had little influence on the studied plant. These impacts can be made better when the plants are strong in terms of nutrients and carbon materials.

Furthermore, our study exhibits the likelihood of replacing chemical fertilizers with PGPR, which in turn will help to prevent the considerable global problem of biosphere pollution. Also, as a result of low costs in the production of elements required by plants (N and P), some tested PGPR in our study may well be fitted to obtain sustainable of rangeland plants production without reducing yield.

The results could be applied to decrease the use of chemical fertilizers. Therefore, the question as to at what range can the PGPR raise the host plant’s resistance to drought stress effects requires further research. In general, scientists should focus more on examination of what strains of the PGPR are suitable for increasing plant growth and decreasing drought stress in arid rangelands. However, this research emphasizes PGPRs and rangeland plants to increase the speed of the rehabilitation of degraded rangelands.

In general, it can be stated that PGPR had both a positive and negative effect on seed germination indicators. *B. cereus* and *P. aeruginosa* rhizobacteria increased plant seeds indicator that may help in higher production and establishment of the rangeland plant. In Contrast *A. chroococcum* and *A. lipoferm* had a negative effect on shoot length and outlined that these last two PGPR should not be implemented.

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