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Research article

Seed priming with plant growth-promoting bacteria (PGPB) improves growth and water stress tolerance of *Secale montanum*

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A R T I C L E I N F O

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A B S T R A C T

Abiotic and biotic stresses are major global threats to food security in the 21st century. Application of plant growth-promoting bacteria (PGPB) in rangeland plants is the only possible alternative that supports plant growth and development to combat environmental stress and successfully restoring rangelands. PGPBs were also found to be a potential substitute for chemical fertilizers and pesticides. The challenge is to determine which biofertilizers can be used for *Secale montanum* in normal and under water stress conditions. We sought to determine the benefits of PGPB for *S. montanum* under water stress conditions in terms of seedling growth traits, growth indicators, and nutrient uptake in the research greenhouse. Therefore, a completely randomized factorial design was conducted with two treatments of PGPB inoculation, including the control (no PGPB inoculation), PGPBs *Bacillus cereus*, *Pseudomonas aeruginosa*, *Azospirillum lipoferm*, and *Azotobacter chroococcum*, and water stress in the research greenhouse. Overall, the results of the current study showed that water stress greatly reduced the above-ground fresh weight of above-ground plant parts and the nitrogen and potassium content of *S. montanum.* The present study confirms the positive effects of PGPB on fresh and dry weights of above- and below-ground parts and seedling, vigor index, quality index, and nitrogen and potassium content of *S. montanum*, except for below-ground parts length, compared with the controls, which shows that PGPB usually improves some indicators of plant growth and development. We suggest that restoration of *S. montanum* seed inoculation with PGPB should be supported in degraded rangelands and marginal drylands in low rainfall years, which may cause water scarcity and consequently water stress in arid and semi-arid regions.

1. Introduction

The beginning of the 21st century faces the challenge of climate change. Environmental stresses (abiotic and biotic stresses) are a major threat to future food security worldwide [1]. Rangeland ecosystems are constantly affected by environmental stresses that

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directly impact plant growth and development, soil erosion and fertility and water quality. These ecosystems occupy 50% of the land area, which are largely located in arid and semi-arid regions [2]. Water stress is one of the most important abiotic stressors associated with climate change, which also impacts biotic stress, affects plant growth and development, and leads to plant death by having a wide range of effects on morphology, physiology, biochemistry, and even gene regulation. The significant effects of this stress result in a loss of soil microbial ecology, rangeland fertility, and competition for nutrient uptake [1,3,4].

The application of mycorrhizal fungi and plant growth-promoting bacteria (PGPB) is just one possible alternative that supports plant growth and development to combat environmental stresses. This approach can help to restore degraded rangelands. Most of literatures, demonstrate this approach directly and indirectly has a positive, detrimental, and neutering effect on plant growth and survival, thereby sustainably increasing and improving rangeland and environmental stability [5–7]. Various genera of *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas*, etc. are referred to as PGPBs, which promote plant growth and development in normal and under water stress conditions. Most PGPBs are not able to tolerate environmental stress such as water stress. Selecting suitable PGPBs is a major challenge for the scientists to develop biofertilizers for such limiting conditions [8,9]. Nevertheless, some PGPBs can tolerate environmental stress and improve plant growth and development. PGPBs promote plant growth and survival through indirect and direct mechanisms by producing enzymes, antibiotics, and antifungal compounds to combat phytopathogenic bacteria, fix nitrogen, produce phytohormones, dissolve minerals, alter gene expression through sigma factors under adverse conditions, and support plant health under environmental stress conditions in the plant life cycle [1,10–12]. PGPBs have been identified as potential substitutes for chemical fertilizers and pesticides. The use of PGPB contributes to sustainable and eco-friendly farming practices and provides a sustainable alternative to reduce the negative effects of stress on plant growth and development.

Secale montanum Guss. belongs to the Poaceae family (tribe Triticeae). This plant is believed to be the ancestor of *S. céréale* L. It is a perennial, outcrossing wild grass that grows naturally in semi-arid and arid rangelands. This species is widely distributed in Europe, Africa, and Asia. This species has been grown in various rangelands around the world and can also be selected for rangeland restoration because it has a high nutritional value of protein, and is most adaptable for rangeland restoration compared to other grass species. *S. montanum* is a valuable rangeland grass species with various ecological functions in rangelands. This plant is a species with high priority in rangelands because it provides high-quality forage (nutrient richness) for livestock, protects water and soil, sequesters carbon and etc. *S. montanum* is tall, has good soil tilth, and is frost resistant. This species is suitable for various soil conditions and prefers moist and well-drained fertile soils. It grows in different pH ranges of the soil [13–15].

S. montanum is commonly cultivated in the southern Caucasia, the eastern Anatolian region of Turkey, Mediterranean countries, and Iran. The sparse and small leaves and breaking peduncles of this plant reduce its agronomic value as a forage plant. In semi-arid and arid rangeland areas, lack of water for germination is the most important growth-limiting factor for plants in these regions. Successful establishment of plants depends on the ability of seeds to germinate, emerge rapidly and uniformly, and developing under field conditions when water availability is low [14,15]. If water stress can be mitigated during the germination and growth phases, the chances of successful plant establishment are high.

In Iran, there are few studies dealing with seed priming of rangeland species with PGPB under water stress for rehabilitation of marginal drylands and degraded rangelands [5,16,17]. The main challenge is which biofertilizers can be used for *S. montanum* in normal and under water stress conditions, in Iranian rangelands. In this study, we attempted to identify favorable PGPB for *S. montanum* under water stress conditions and to investigate the effects of PGPB (*Bacillus cereus*, *Pseudomonas aeruginosa*, *Azospirillum lipoferm*, and *Azotobacter chroococcum*) under water stress (FC: Field Capacity, 0.75 FC, and 0.5 FC) on growth traits, growth indicators, and nutrient uptake of *S. montanum.* The effect of PGPB on the germination indices was investigated.

2. Methods

2.1. Study area

This study was conducted in 2021 in the research greenhouse of Shahrekord University, Iran (32° 21′ 08″ N, 50° 49′ 39″ E, at the minimum and maximum temperatures of 10–30 °C, respectively). A factorial trial with five replicates in a completely randomized design was conducted. Many PGPBs have demonstrated their efficiency in promoting plant growth, biological control, and environmental friendly behaviour such as *Bacillus*, *Pseudomonas*, *Azospirillum*, and *Azotobacter* as *B. cereus*, *P. aeruginosa*, *A. lipoferm*, and *A. chroococcum*. They are generally widely distributed in the environment (soil, water, food products, etc.). Most of these PGPBs are based on Gram-positive strains such as *B. cereus,* which are free-living and easy to formulate. However Gram-negatives strains, including *P. aeruginosa, A. chroococcum, and A. lipoferum*, are free-living and not easily handled [18,19].

At a first factor, the seeds were primed with PGPB in five stages, including the control (no PGPB inoculation), PGPBs *B. cereus* PTCC1816, *P. aeruginosa* ATCC9027, *A. lipoferm* PTCC12D, and *A. chroococcum* PTCC9D. The PGPBs were provided by Tehran Green Biotech Co, Iran. In additional, the second factor was three levels of water stress, including field capacity (FC), 0.75 FC, and 0.5 FC on *S. montanum.*

2.2. Preparing pots

The seeds of this plant were provided by PBEC (Pakan Bazr Esfahan Company), Iran. These seeds were disinfected with NaOCl (Sodium Hypochlorite) 10% for 30 s and then washed rapidly five times in double distilled water. Then, the studied bacteria were cultured from frozen suspensions of PGPBs on Tryptic Soy Broth (TSB) culture medium for 48 h at 27 °C in an incubator. The bacterial colony was transferred to the Tryptic Soy Agar (TSA) culture medium, and the culture medium was incubated for 24–48 h at 32 °C;

thus, the bacteria were reproduced. Then the surface-sterilized seeds were separately soaked in 5 mL inoculums containing a concentration of 5×10^8 mL^{-1} bacterial cells (CFU/mL, using a spectrophotometer) at room temperature for 1 h [20,21]. The experiment was performed with sterilized soil at 120 °C for 15 min in an autoclaving. To culture the seeds of *S. montanum*, the pots were first weighted down. We places a thin layer of uniform gravel of similar weight on the bottom of the pots before planting. Then 0.675 kg of sterilized soil was poured into each pot with a top diameter of 10 cm. The pots were saturated and then watered with distilled water. Twenty seeds inoculated with PGPB were planted in each pot at a depth of 1 cm. The pots were irrigated daily at FC for a month. To measure the germination indices (Table 1) before applying water stress, the number of seedlings was recorded daily. After 30 days of germination, the number of plant stands was reduced to two plant stands in each pot, and water stress was applied for three months. At the end of the growing period (90 days of growth), the plants were harvested. Plants were washed with distilled water to measure fresh and dry biomass weight, length, number of leaves, collar diameter, growth indices (Table 1), and amount of nutrients taken up. Dry biomass weight was estimated after the fresh biomass was oven-dried at 70 °C for 72 h.

2.3. Soil study

Soil treatments were air-dried and then passed through a 2 mm sieve before being analyzed for texture, electrical conductivity (EC), soil reaction (pH), total nitrogen, available phosphorus and potassium, and organic matter. After the experiment, nitrogen, phosphorus, and potassium levels were measured in plant samples with five replicates. Soil texture was determined by the hydrometer method [24]. Soil pH and EC were estimated using a pH/EC meter. Total nitrogen was determined by the Kjeldahl method. Phosphorus was measured by the Olsen method [25]. Potassium was determined by flame photometry [26,27]. Soil organic matter (OM) was estimated using the methods described by Baranian Kabir et al. [28]. These characteristics were carried out in the laboratory of the Research Center of Agriculture and Natural Resources of Isfahan Province, Isfahan, Iran.

2.4. Statistical analyses

In the current study, the effect of PGPB on the germination indices was investigated using analysis of variance (ANOVA). Moreover, the statistical analyses were carried out using GLM (General Linear Model) to determine the effectiveness of these treatments (seed priming with PGPB and water stress) on seedling growth traits, growth indicators and nutrient uptake of *S. montanum*. The program IBM SPSS Statistics 25 for Windows was used for statistical analysis of variances between treatments. Then, to evaluate the differences between the treatments, Duncan's test was used.

3. Results

3.1. Physicochemical characteristics of soil

The soil of study area had a clay-loamy texture, EC of 0.76 dS/m, pH of 7.5, and organic matter content equal to 1.54%. The main available nutrients such as total nitrogen, phosphorus and potassium were quantified as 0.02%, 5.53 and 300 mg kg^{-1}, respectively. The soil was generally poor in phosphorus and nitrogen, but rich in potassium. The soil had a medium to heavy texture and medium organic matter. This soil was non-saline and neutral.

3.2. Effect of the PGPB on seed germination indices

It should be noted that the germination indices of the seeds were studied before the application of water stress. Table 2 shows

Table 1
Germination and growth indices were calculated using the following equations [22,23].

Index		Abbreviation	Equation	Description
Germination indices	Germination percentage	GP	$GP = (N/S) \times 100$	N = the number of germinated seeds, S = total number of seeds
	Germination rate	GR	$GR = \sum(N_i/D_i)$	Ni = the number of germinated seeds in daily, Di = the number of days from the beginning of germination
	Mean germination time	MGT	$MGT = \sum N_i D_i / N$	Ni = the number of seeds in daily, Di = the number of days counted from the beginning of germination, N = the number of germinated seeds
	Mean daily germination	MDG	$MDG = FGP/D$	FGP = final germination percentage, D = the number of days to reach the maximum final germination
	Coefficient velocity of germination	CVG	$CVG = (\sum_{i=1}^{k} f_i / \sum_{i=1}^{k} f_i x_i) \times 100$	f_i = the number of seeds newly germination on day i, x_i = the number of days from sowing, k = last day of germination
Growth indices	Vigor index	VI	$VI = (MRDW + MSDW) \times GP$	MRDW = mean root dry weight, MSDW = mean shoot dry weight, GP = germination percentage
	Quality index	QI	$QI = TDW/(H/RCD + SDW/RDW)$	TDW = Total seedling dry weight (gr), H = height (cm), RCD = diameter (mm), SDW = shoot dry weight (gr), RDW = root dry weigh (gr)

ANOVA of the data showing that the main effect of PGPB on seed germination indices (germination percentage, germination rate, mean germination time, mean daily germination, coefficient velocity of germination) had no significant differences. Our data showed that there was no significant difference between the different PGPB treatments and the control treatments. Therefore, PGPB treatments did not promote seed germination of *S. montanum*.

3.3. Effect of PGPB and water stress on plant growth traits

The results showed that the main effect of PGPB application and the different levels of water stress had significant effects on the growth traits of plants (fresh and dry weights of the above- and below-ground parts and seedling, length, number of leaves, and collar diameter) compared with the control. The interaction effect of PGPB and water stress had significant effects on fresh weight of above-ground parts, dry weight of above- and below-ground parts and seedling, length, number of leaves, and collar diameter of seedlings ($P < 0.05, 0.01$) (Table 3).

The results of the main effects of PGPB showed that the highest increase in biomass fresh and dry weights (above- and below-ground parts and seedling) was observed in seeds inoculated with *B. cereus, A. chroococcum, P. aeruginosa*, and *A. lipoferm*, respectively.

Plants treated with *A. lipoferm* had the highest above-ground part and seedling length, with an increase of 1.08 and 1.03 times, respectively, compared to the control. There was no significant difference in the length of the below-ground part of *S. montanum* between the treatments *A. lipoferm, B. cereus* and the treatment without seed preparation with PGPB. The least above- and below-ground plant parts and seedling length were observed when *P. aeruginosa* was used. Above- and below-ground parts and seedling length were negatively affected by *P. aeruginosa* compared to the control treatment.

The number of leaves of plants was significantly different from the control treatment in all treatments, except the *B. cereus* treatment, which was similar to the control. The highest number of leaves was obtained in the treatment with *A. chroococcum, A. lipoferm*, and *P. aeruginosa*, respectively. The collar diameter was significantly increased by the use of *B. cereus, A. chroococcum,* and *A. lipoferm* compared to control treatment, while no significant difference was observed between the treatment with *P. aeruginosa* and the control treatment.

The results of water stress effect showed that the fresh and dry weight of above- and below-ground parts and seedling, length, number of leaves, and collar diameter are most reduced at 0.5 FC and 0.75 FC, respectively. Despite the fact that there is no significant difference using 0.5 FC and 0.75 FC water stress levels on the length of below-ground parts and number of leaves.

The interaction of PGPB and water stress showed that the four treatments *B. cereus, A. chroococcum*, *P. aeruginosa*, and *A. lipoferm*, significantly increased the fresh weight of above-ground parts and dry weight of above and below-ground parts and seedling compared to the control treatment (no seed priming with PGPB) at the different water stress levels (Fig. 1).

The length of above-ground parts increased with *A. lipoferm* treatment under the three water stress levels, while *P. aeruginosa* treatment at a level of 0.50 FC reduced the length of above-ground parts compared to no seed priming with PGPB treatment (control) at the same water stress level (Fig. 2).

The length of below-ground parts of *P. aeruginosa* and *A. chroococcum* was reduced compared to no seed priming with PGPB (control treatment) at the different water stress levels. Seedling length was reduced in the treatments *A. chroococcum* at FC and *P. aeruginosa* under three water stress levels compared to no seed priming with PGPB treatment (control) at the same water stress levels. The greatest number of leaves was observed in the treatments *A. chroococcum* at the different water stress levels, *A. lipoferm* at FC, and *P. aeruginosa* at 0.75 FC compared to no seed priming with PGPB (control treatment) at the same water stress levels. Treatments with *B. cereus* and *A. chroococcum* at the different water stress levels and with *A. lipoferm* at FC and 0.75 FC resulted in an increase in collar diameter compared to no seed priming with PGPB (control treatment) at the same water stress levels (Fig. 2).

Table 2
Analysis of variance (ANOVA) of germination indices at different PGPB treatments (See other abbreviations as in Table 1).

Germination indices		df	Mean square	F	Sig.
GP	Between Groups	4	317.833	1.105	0.361^{ns}
	Within Groups	70	287.524		
	Total	74			
GR	Between Groups	4	1.143	0.110	0.979^{ns}
	Within Groups	70	10.438		
	Total	74			
MGT	Between Groups	4	0.764	0.754	0.559^{ns}
	Within Groups	70	1.013		
	Total	74			
CVG	Between Groups	4	0.022	0.611	0.656^{ns}
	Within Groups	70	0.035		
	Total	74			
MDG	Between Groups	4	0.721	1.104	0.362^{ns}
	Within Groups	70	0.653		
	Total	74			

ns., not significant.

Table 3
Effects of PGPB and water stress on growth traits of *Secale montanum* (*B. ce, Bacillus cereus*, *P. ae, Pseudomonas aeruginosa A. li, Azospirillum lipoferm; A. ch*, *Azotobacter chroococcum*; Cont, Control; WS, Water-stress; IA, Interaction; AG, Above-ground part; BG, Below-ground part; S, Seedling; NL, Number of leaves; CD, Collar diameter).

	Fresh weight			Dry weight			Length			NL	CD
PGPB	AG	BG	S	AG	BG	S	AG	BG	S		
B. ce	3.727 ± 0.320a	2.477 ± 0.150a	6.206 ± 0.462a	2.590 ± 0.178a	1.000 ± 0.037a	3.590 ± 0.187a	18.706 ± 0.525c	15.606 ± 0.711a	34.310 ± 1.184b	8.133 ± 1.06bc	2.172 ± 0.367a
P. ae	2.414 ± 0.281c	1.568 ± 0.197c	3.982 ± 0.471c	1.638 ± 0.190c	0.683 ± 0.065c	2.320 ± 0.249c	17.692 ± 1.869d	9.719 ± 2.560c	27.412 ± 4.411d	9.000 ± 2.725b	1.354 ± 0.296c
A. ch	2.879 ± 0.347b	1.789 ± 0.166b	4.668 ± 0.506b	1.926 ± 0.237b	0.719 ± 0.112b	2.646 ± 0.329b	19.578 ± 0.514b	13.372 ± 0.535b	32.950 ± 0.816c	10.600 ± 0.910a	2.066 ± 0.099a
A. li	2.171 ± 0.185d	1.344 ± 0.190d	3.516 ± 0.348d	1.417 ± 0.167d	0.520 ± 0.089d	1.936 ± 0.244d	20.866 ± 0.491a	15.560 ± 0.860a	36.427 ± 1.031a	9.066 ± 1.980b	1.668 ± 0.258b
Cont	0.806 ± 0.167e	0.484 ± 0.073e	1.290 ± 0.228e	0.476 ± 0.051e	0.180 ± 0.018e	0.658 ± 0.063e	19.270 ± 0.433b	15.900 ± 0.973a	35.170 ± 1.362b	7.733 ± 0.593c	1.400 ± 0.084c
WS											
FC	2.635 ± 1.036a	1.680 ± 0.697a	4.317 ± 1.732a	1.761 ± 0.761a	0.679 ± 0.282a	2.440 ± 1.039a	19.887 ± 0.854a	14.910 ± 2.087a	34.796 ± 2.596a	9.600 ± 2.179a	1.877 ± 0.513a
0.75 FC	2.412 ± 1.034b	1.557 ± 0.665b	3.968 ± 1.698b	1.630 ± 0.720b	0.606 ± 0.277b	2.236 ± 0.992b	19.265 ± 0.997b	13.820 ± 2.065b	33.084 ± 2.566b	8.600 ± 1.290b	1.753 ± 0.321b
0.50 FC	2.152 ± 0.900c	1.360 ± 0.633c	3.513 ± 1.524c	1.437 ± 0.652c	0.576 ± 0.281c	2.013 ± 0.929c	18.515 ± 1.816c	13.365 ± 3.524b	31.881 ± 5.208c	8.520 ± 1.960b	1.566 ± 0.336c
Sig.											
PGPB	***	***	***	***	***	***	***	***	***	***	***
WS	***	***	***	***	***	***	***	***	***	**	***
IA	***	ns	ns	***	***	***	***	**	***	**	***

■Values represent means, and different letters indicate significant difference $P < 0.05$ level. ns., not significant, * significant at $P < 0.1$, ** significant at $P < 0.05$, *** significant at $P < 0.01$.

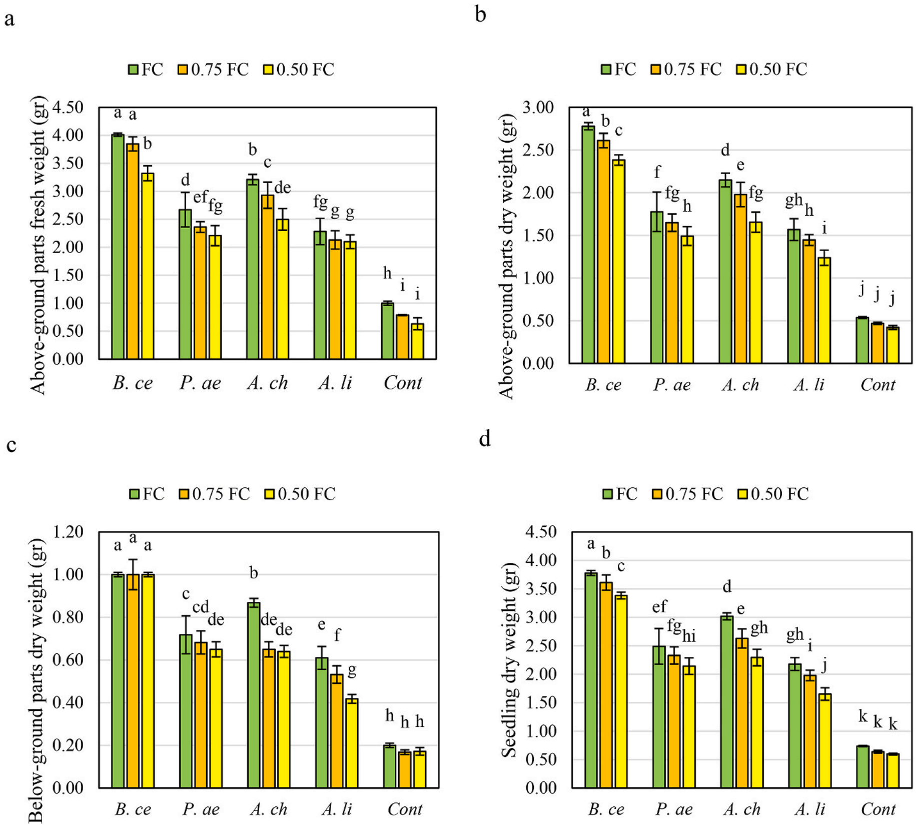

Fig. 1. Effects of PGPB inoculation and water stress on above-ground plant part fresh weight (a), above- and below-ground plant parts and seedling dry weight (b, c and d, respectively) of *S. montanum.* The error bars represent the standard deviation of the mean value (*B. ce, Bacillus cereus*, *P. ae, Pseudomonas aeruginosa A. li, Azospirillum lipoferm; A. ch, Azotobacter chroococcum*; Cont, Control).

3.4. The effect of PGPB and water stress on growth indices

The results showed that the main effect of PGPB application, different levels of water stress, and the interaction effect of PGPB and water stress had significant effects on growth indices (vigor index and seedling quality index) (Table 4).

The results of the main effect of PGPB showed that the best vigor and quality indices were obtained from seeds inoculated with *B. cereus*, followed by those inoculated with *A. chroococcum*, *P. aeruginosa*, and *A. lipoferm,* while the vigor index was not significant in the treatments with *A. chroococcum* and *P. aeruginosa*.

The results of the water stress effect showed that the growth indices decreased the most at 0.5 FC and 0.75 FC. The vigor index was not significant at 0.5 FC and 0.75 FC.

The interaction between PGPB and water stress showed that the four treatments *B. cereus, A. chroococcum, P. aeruginosa* and *A. lipoferm* had maximum vigor indices at different water stress levels compared to no seed priming with PGPB (control treatment) at the different water stress levels (Fig. 3).

3.5. The effect of PGPB and water stress on plant nutrient uptake

The results showed that the main effect of PGPB application and the different levels of water stress had significant effects on plant nutrient uptake (plant nitrogen, phosphorus, and potassium levels) ($P < 0.01$). The interaction effect of PGPB and water stress had significant effects on nitrogen and potassium levels (Table 4).

The results of the main effects of PGPB showed that the highest nitrogen and potassium levels, respectively, were observed in the *B. cereus, A. chroococcum, P. aeruginosa*, and *A. lipoferm* treatments, while the highest phosphorus level was observed in the *B. cereus, A. chroococcum*, *A. lipoferm*, and *P. aeruginosa* treatments.

The results of the water stress effect expressed that the nutrient uptake of the plants was most reduced at 0.5 FC and 0.75 FC (Table 3).

The interaction of PGPB and water stress showed that the four treatments *B. cereus, A. chroococcum, P. aeruginosa,* and *A. lipoferm*, at the different water stress levels, had the highest nitrogen and potassium levels compared to the control treatment at the different water stress levels, while nitrogen level were not significant in the *A. chroococcum* and *P. aeruginosa* treatments at the FC and 0.5 FC levels,

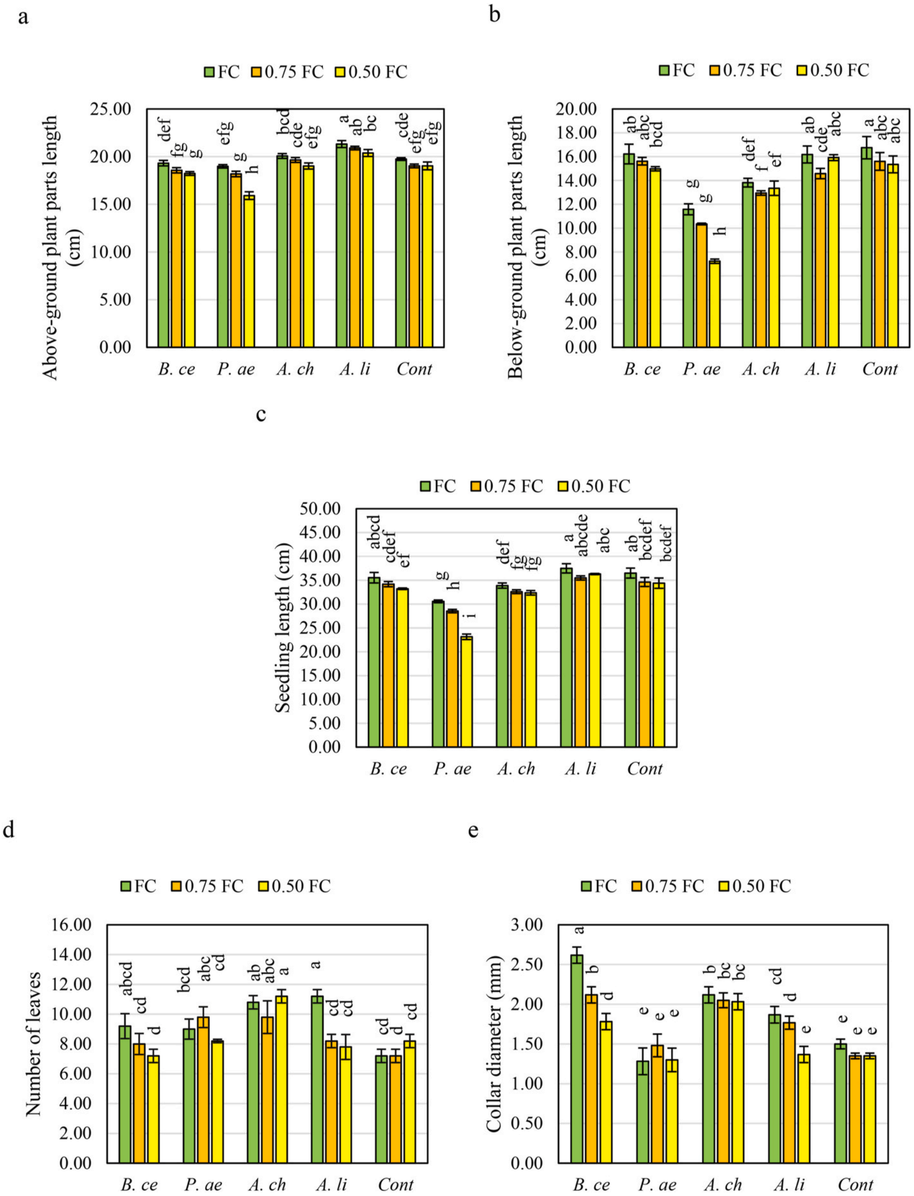

Fig. 2. Effects of PGPB inoculation and water stress on above- and below-ground plant parts and seedling length (a, b, and c, respectively), number of leaves (d), and collar diameter (e) of *S. montanum* (see the error bars and other abbreviations as in Fig. 1).

and potassium levels were not significant in the same treatments at the FC level (Fig. 4).

4. Discussion

Increasing the success rate of rangeland restoration is critical, as these ecosystems continue to be lost in the face of climate change and environmental stress. Ecological restoration practitioners should aim to maximize plant growth in the shortest time possible under existing conditions using PGPB, with short-term plant and bacterial coexistence enabling achievement of long-term restoration goals.

Overal, the results of the current study showed that water stress greatly reduced the fresh and dry weights, above-ground part length, seedling length, collar diameter, quality index, and nutrient uptake of *S. montanum*, while the below-ground part length, number of leaves, and vigor index were not significant at 0.5 FC and 0.75 FC, respectively, as noted by Bouremani et al. [29]. All values are negatively affected by water stress, indicating that *S. montanum* need to make wise management decisions when faced with water scarcity, which is common in semi-arid and arid regions. This suggests the restoration of this plant in low rainfall years, as this leads to water scarcity and consequently water stress in arid and semi-arid regions, as noted by Zandi Esfahani and Azarnivand [30].

Seeds of *S. montanum* inculated with PGPB grew better and larger than the control treatment (uninoculated seeds) during the

Table 4
Effects of PGPB and water stress on growth indices, and nutrients uptake of *Secale montanum.*

PGPB	Vigor index	Quality index	Nitrogen	Phosphorus	Potassium
B. cereus	2.594 ± 0.428a	0.194 ± 0.030a	6.937 ± 0.424a	1.164 ± 0.072a	10.530 ± 0.643a
P. aeruginosa	1.570 ± 0.598b	0.102 ± 0.019c	4.201 ± 0.526c	0.595 ± 0.073d	5.691 ± 0.713c
A. chroococcum	1.694 ± 0.509b	0.142 ± 0.019b	4.704 ± 0.434b	0.840 ± 0.076b	6.708 ± 0.620b
A. lipoferm	1.271 ± 0.398c	0.079 ± 0.018d	3.912 ± 0.555d	0.659 ± 0.091c	5.377 ± 0.762d
Control	0.396 ± 0.140d	0.023 ± 0.003e	1.272 ± 0.194e	0.295 ± 0.045e	1.877 ± 0.285e
Water stress					
FC	1.775 ± 0.784a	0.122 ± 0.071a	4.612 ± 1.952a	0.780 ± 0.306a	6.615 ± 2.979a
0.75 FC	1.381 ± 0.838b	0.109 ± 0.057b	4.270 ± 1.866b	0.722 ± 0.295b	6.128 ± 2.855b
0.50 FC	1.360 ± 0.841b	0.093 ± 0.051c	3.734 ± 1.766c	0.631 ± 0.282c	5.366 ± 2.711c
Sig.					
PGPB	***	***	***	***	***
Water stress	***	***	***	***	***
Interaction	***	***	*	ns	*

See ■ as in Table 3.

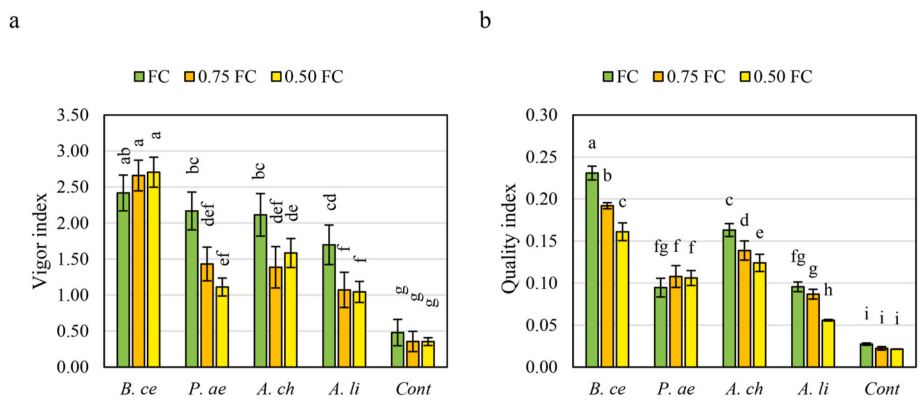

Fig. 3. Effects of PGPB inoculation and water stress on growth indices (vigor index: a, quality index: b) of *S. montanum* (see the error bars and other abbreviations as in Fig. 1).

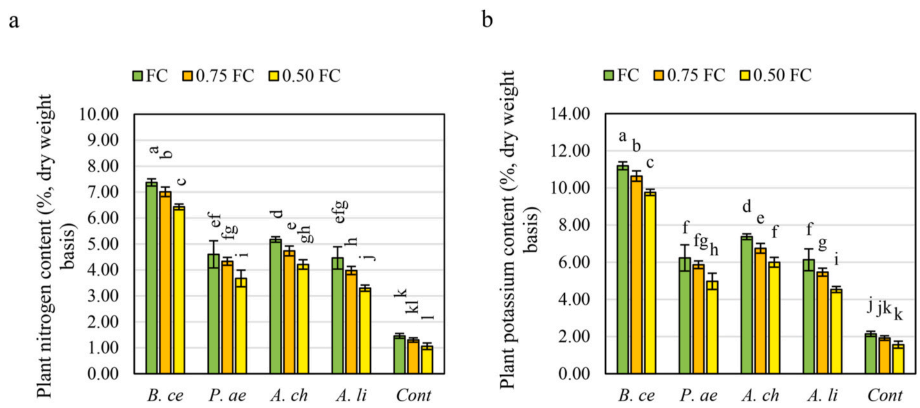

Fig. 4. Effects of PGPB inoculation and water stress on nutrient uptake (plant nitrogen content: a, plant potassium content: b) of *S. montanum* (see the error bars and other abbreviations as in Fig. 1).

greenhouse experiment. The present studyconfirms the positive effects of some PGPBs on growth traits, growth indices, and nutrient uptake of *S. montanum*, except for the length of the below-ground part, compared with the uninoculated seeds, which shows that some PGPBs improve overall plant growth and survival. In agreement with the results, previous reports [5,16,17,31–36] have also shown the positive influence of biopriming in terms of plant growth traits, growth indices, and nutrient uptake.

Inoculation of *S. montanum* seeds with *B. cereus, A. chroococcum, P. aeruginosa*, and *A. lipoferm* resulted in significant increase in the fresh weight and dry weights of above- and below-ground parts and seedling, vigor index, quality index, and nitrogen and potassium content. The increase in the fresh weight and dry weights of above- and below-ground parts and seedling as biomass agents assure us that PGPB act directly on species biomass agents as stated by e.g., Ahmad et al. [37]; Jetiyanon and Plianbanchang [38]; Widnyana and Javandira [39]. They proved regulators extracted from PGPB such as indoleacetic acid (IAA) are one of the approaches to improve plant growth and quality. The PGPBs can create favorable conditions for plant growth and development through direct and/or indirect mechanisms [40]. *S. montanum* inoculated with biofertilizers showed a significant increase in fresh and dry biomass compared to the control group. The highest increase in the dry weight of above- and below-ground plant parts by 5.44 and 5.55 times, respectively, compared to the control was observed in plants treated with *B. cereus*.

PGPB has been reported to increase fresh and dry weight, length, and volume of roots in numerous research studies. These changes in root morphology and physiology are closely related to IAA and cytokinins (plant hormones) produced by PGPB that cause cell division and elongation [41–43]. PGPB appears to improve the availability of nutrients and water to plants by promoting root development and thus the uptake of plant nutrients and water. As a result, plant nutrient uptake may improved growth traits and indices [16,44].

The significant dry weight of the below-ground parts of PGPB indicates that the water availability of this plant species has been improved. Many studies have reported that seed priming with PGPB increase plants availability of nutrients and water and consequently increases fresh and dry weight of roots [5,39,45,46]. Therefore, we expect that increasing the weight of the below-ground plant parts of *S. montanum* by seed priming with PGPB will ultimately improve the plant's resistance to water stress through the growth of below-ground plant parts and further water uptake.

The positive effects of bio-fertilizers from our research are results of agents confirmed in other studies. The increase in growth from the application of PGPB to plants such as *Triticum aestivum* [47], *Glycine max* [48], *Beta vulguris* [49], *Zea mays* [50], *Onobrychis sativa* [17], and *Bromus tomentellus* [16] is due to the production of phytohormones, nitrogen fixation, and phosphate solubilization. Burd et al. [51] showed that the increase in plants inoculated with biofertilizers was triggered by a local increase in available nutrients and increased access to nutrient uptake by biofertilizers. Other studies have documented increases in growth traits and yield of various plants after seed inoculation with biofertilizers [52–54].

The tallest plants were observed in seeds inoculated with *A. lipoferum* treatment. Studies in which seeds were inculated with biofertilizers resulted in greater plant height compared to the control. However, the percent increase in plant height after inoculation of seeds with diffrerent PGPB indicates that the response to PGPB may vary among plant species.

Inoculation of *S. montanum* seeds with *P. aeruginosa* had a significantly negative effect on above- and below-ground parts and seedling length, in contrast to the control. In addition, inoculation with *P. aeruginosa* had a significant negative effect on the size of species. This could be due to the fact that *P. aeruginosa* significantly increases biomass by increasing cell division; therefore, *P. aeruginosa* affects the biomass growth of the plant by increasing the weight of *S. montanum*. Some PGPB inhabit plant root growth by producing HCN (hydrogen cyanide) gas [55]. These PGPBs control pathogens and decrease access to iron, resulting in reduced plant growth and development [56]. Ethylene acetic acid can also inhibit species size and reduce auxin movement [57]. Water stress increases ethylene levels in plant species. The reduced plant height may then show up as damaged photosynthesis due to desiccation and reduced production of materials for production of growing parts of the species [16]. In contrast to the results of the present study, Shaukat et al. [58] demonstrated that the presence of *Azospirillum* and *Azotobacter* around the wheat root environment (*T. aestivum*) had a beneficial effects on plant sprouting. However, Stamenov and Jafari [55] found a negative effect of these biofertilizers on the sprouting of *Allium cepa*. Considering these contrasting results, we can conclude that seed priming with PGPB has different effects on sprouting of other species. Therefore, it is important to test effects of PGPB on different plant species before applying it.

In our study, the highest number of leaves was obtained in seeds treated with *Azotobacter*, *Azospirillum*, and *Pseudomonas* compared to the control. In many other studies where seeds were inoculated with *Pseudomonas*, *Azospirillum*, and *Azotobacter* species, the results were similar to our study [33,58,59].

Seed inoculated with PGPB of *B. cereus* had the highest vigor and quality indices, which were 6.55 and 8.45 times higher than the control treatment, respectively. The results of Gholami et al. [5] and Govindappa et al. [60] on *Carthamus tinctorius* and *Zea mays* confirmed the similar results of our study. They obtained similar results with *Azospirillum* and *Pseudomonas.* The increase in these indices could be due to better metabolism and production of auxin and cytokinin, hormones that stimulate cell elongation and division by inoculation with PGPB [61,62].

The results of the current study showed that the use of PGPB had positive effects on nutrient intake compared with the control treatment. The interaction effect of PGPB and water stress showed that PGPB on FC and/or 0.75 FC improved nutrient uptake, while the use of PGPB did not reduce the negative effects of water stress on nutrient uptake. Delshadi et al. [16,17], Gashash et al. [63] and Shah et al. [8] also reported that PGPBs had a positive effect on nutrient uptake by plants. The results of the current study showed higher production of *B. cereus* treatment in uptake of materials such as nitrogen and potassium in *S. montanum* compared to no PGPB inoculation.

5. Conclusions

Increasing water stress resulted in reduced growth traits, growth indices, and nutrient uptake. Seed biopriming is one of the approaches to neutralize the negative effects of abiotic stress. This study showed that seed priming of *S. montanum* with PGPB strains, especially *Bacillus cereus*, improved the fresh weight and dry weights of above- and below-ground parts and seedling, growth indices, and nutrient uptake compared to unprimed seeds, indicating that PGPB usually improves plant sprouting. However, the biopriming treatments in this experiment improved growth characteristics. At the end of the experiment, PGPB inoculated plants were heavier than control plants (uninoculated). Plant response to seed sprouting with PGPB depend on the PGPB species, the genetic constitution of the plant, and the conditions required for growth.

Thus, we emphasize the role of PGPBs as a strategy to address the challenges of global food security and climate change to increase the success rate of rangeland restoration. PGPB is thus a promising approach to increase *S. montanum* resilience to climate change for plants. In addition, the application of PGPBs can reduce the use of pesticides and artificial growth regulators, which contribute to climate change through greenhouse gas emissions, as an eco-friendly alternative to increase crop growth and development.

Author contribution statement

Ebrahimi and Ghehsareh Ardestani: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data. Rahnama, Ebrahimi, Ghehsareh Ardestani and Nikookhah: Performed the experiments; Wrote the paper. Rahnama, Ebrahimi and Ghehsareh Ardestani: Analyzed and interpreted the data.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Additional information

No additional information is available for this paper.

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References

[1] A. Kumar, J.P. Verma, Does plant—microbe interaction confer stress tolerance in plants: a review? Microbiol. Res. 207 (2018) 41–52.
[2] M.H. Friedel, W.A. Laycock, G. Bastin, Field and Laboratory Methods for Grassland and Animal Production Research, CABI, Wallingford, 2000.
[3] M. Bekele, S. Demissew, T. Bekele, F. Woldeyes, Soil seed bank distribution and restoration potential in the vegetation of Buska Mountain range, Hamar district, southwestern Ethiopia, Heliyon 8 (2022), e11244.
[4] M. Chodak, M. Golebiewski, J. Morawska-Płoskonka, K. Kuduk, M. Niklinska, Soil chemical properties affect the reaction of forest soil bacteria to drought and rewetting stress, Ann. Microbiol. 65 (2015) 1627–1637.
[5] A. Gholami, S. Shahsavani, S. Nezarat, The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize, World Acad. Sci. Eng. Technol. Int. J. Biol. Biomol. Agric. Food Biotechnol. Eng. 3 (2009) 9–14.
[6] S.I.A. Pereira, D. Abreu, H. Moreira, A. Vega, P.M.L. Castro, Plant growth-promoting rhizobacteria (PGPR) improve the growth and nutrient use efficiency in maize (Zea mays L.) under water deficit conditions, Heliyon 6 (2020), e05106.
[7] E. Valencia-Cantero, E. Hernandez-Calderon, C. Velazquez-Becerra, J.E. Lopez-Meza, R. Alfaro-Cuevas, J. Lopez-Bucio, Role of dissimilatory fermentative iron-reducing bacteria in Fe uptake by common bean (Phaseolus vulgaris L.) plants grown in alkaline soil, Plant Soil 291 (2007) 263–273.
[8] A. Shah, M. Nazari, M. Antar, L.A. Msimbira, J. Naamala, D. Lyu, M. Rabileh, J. Zajonc, D.L. Smith, PGPR in agriculture: a sustainable approach to increasing climate change resilience, Front. Sustain. Food Syst. 5 (2021).
[9] S.R. Vimal, J.S. Singh, N.K. Arora, S. Singh, Soil-plant-microbe interactions in stressed agriculture management: a review, Pedosphere 27 (2017) 177–192.
[10] D. Dasgupta, A. Paul, K. Acharya, T. Minkina, S. Mandzhieva, A.V. Gorovtsov, N. Chakraborty, C. Keswani, Bioinoculant mediated regulation of signalling cascades in various stress responses in plants, Heliyon 9 (2023), e12953.
[11] O.M. Finkel, G. Castrillo, S. Herrera Paredes, I. Salas Gonzalez, J.L. Dangl, Understanding and exploiting plant beneficial microbes, Curr. Opin. Plant Biol. 38 (2017) 155–163.
[12] S.P. Sandilya, B. Jeevan, G. Subrahmanyam, K. Dutta, N. Vijay, N. Bhattacharyya, M. Chutia, Co-inoculation of native multi-trait plant growth promoting rhizobacteria promotes plant growth and suppresses Alternaria blight disease in castor (Ricinus communis L.), Heliyon 8 (2022), e11886.
[13] E.J. Daly, K. Kim, G. Hernandez-Ramirez, K. Klimchuk, The response of soil physical quality parameters to a perennial grain crop, Agric. Ecosyst. Environ. 343 (2023), 108265.
[14] H. Khazaie, H. Earl, S. Sabzevari, J. Yanegh, M. Bannayan, Effects of osmo-hydropriming and drought stress on seed sermination and seedling growth of rye (Secale Montanum), ProEnvironment/ProMediu 6 (2013) 496–507.
[15] T. Miedaner, Breeding wheat and rye for resistance to Fusarium diseases, Plant Breed. 116 (1997) 201–220.
[16] S. Delshadi, M. Ebrahimi, E. Shirmohammadi, Effectiveness of plant growth promoting rhizobacteria on Bromus tomentellus Boiss seed germination, growth and nutrients uptake under drought stress, South Afr. J. Bot. 113 (2017) 11–18.
[17] S. Delshadi, M. Ebrahimi, E. Shirmohammadi, Influence of plant-growth-promoting bacteria on germination, growth and nutrients' uptake of Onobrychis sativa L. under drought stress, J. Plant Interact. 12 (2017) 200–208.
[18] J. Liu, J. Zhang, M. Zhu, H. Wan, Z. Chen, N. Yang, J. Duan, Z. Wei, T. Hu, F. Liu, Effects of plant growth promoting rhizobacteria (PGPR) strain Bacillus licheniformis with biochar amendment on potato growth and water use efficiency under reduced irrigation regime, Agronomy 12 (2022) 1031.
[19] B. Tabassum, A. Khan, M. Tariq, M. Ramzan, M.S. Iqbal Khan, N. Shahid, K. Aaliya, Bottlenecks in commercialisation and future prospects of PGPR, Appl. Soil Ecol. 121 (2017) 102–117.

[20] M. Bahmani, G.A. Jalali, A. Asgharzadeh, M. Tabari Kouchaksaraei, Effect of inoculation growth promotion bacterium Pseudomonas putida on tolerance to salinity of Carotropis procera Ait. Seedlings, Arid Biom Sci. Res. J. 6 (2016) 81–94.
[21] M. Saric-Krsmanovic, D. Bozic, L. Radivojevic, J. Gajic-Umiljendic, L. Santric, S. Vrbnicanin, Effects of plant growth promoting rhizobacteria (PGPR) and cover crops on seed germination and early establishment of field dodder (Cuscuta campestris Yunk.), Pestic. i Fitomedicina. 32 (2017) 105–111.
[22] A.A. Abdul-Baki, J.D. Anderson, Vigor determination in soybean seed by multiple criteria 1, Crop Sci. 13 (1973) 630–633.
[23] R.L. Agrawal, M. Dadlani, Seed the Technology, second ed., Oxford and IBH publishing co. PVT LTD, India, 1995.
[24] G.J. Bouyoucos, Hydrometer method improved for making particle size analyses of soils 1, Agron. J. 54 (1962) 464–465.
[25] C.S. De Silva, I.S.A. Koralage, P. Weerasinghe, N.R.N. Silva, The determination of available phosphorus in soil: a quick and simple method, OUSL J 8 (2015) 1.
[26] J. Ryan, G. Estefan, A. Rashid, Soil and Plant Analysis Laboratory Manual, ICARDA, 2001.
[27] M. Zarinkafsh, Soil Survey, Methods of Assessment Morphologic and Analysis for Soil, Water & Plant, Tehran University Publication, Tehran, Iran, 1993.
[28] E. Baranian Kabir, H. Bashari, M.R. Mosaddeghi, M. Bassiri, Soil aggregate stability and organic matter as affected by land-use change in central Iran, Arch. Agron Soil Sci. 63 (2017) 1823–1837.
[29] N. Bouremani, H. Cherif-Silini, A. Silini, A.C. Bouket, L. Luptakova, F.N. Alenezi, O. Baranov, L. Belbahri, Plant growth-promoting rhizobacteria (PGPR): a rampart against the adverse effects of drought stress, Water 15 (2023) 418.
[30] E. Zandi Esfahan, H. Azarnivand, Effect of water stress on seed germination of Agropyron elongatum, Agropyron desertourm & Secale montanum, Desert 17 (2013) 249–253.
[31] O. Ansari, M.S. Azadi, E. Younesi, O. Ansari, M.S. Azadi, E. Younesi, Effect of hormone priming on germination characteristics and enzyme activity of mountain rye (Secale montanum) seeds under drought stress conditions, J. Stress Physiol. Biochem. 9 (2013) 61–71.
[32] A. Jahanian, M.R. Chaichi, K. Rezaei, K. Rezayazdi, K. Khavazi, The effect of plant growth promoting rhizobacteria (PGPR) on germination and primary growth of artichoke (Cynara scolymus), Agric. Crop Sci. 4 (2012) 923–929.
[33] P.A. Noumavo, E. Kochoni, Y.O. Didagbé, A. Adjanohoun, M. Allagbé, R. Sikirou, E.W. Gachomo, S.O. Kotchoni, L. Baba-Moussa, Effect of different plant growth promoting rhizobacteria on maize seed germination and seedling development, Am. J. Plant Sci. 4 (2013) 1013–1021.
[34] H.R. Rouhi, M.A. Aboutalebian, F. Sharif-zadeh, Effects of hydro and osmopriming on drought stress tolerance during germination in four grass species, Int. J. AgriScience. 1 (2011) 107–114.
[35] P.V. Yadav, M. Kumari, Z. Ahmed, Seed priming mediated germination improvement and tolerance to subsequent exposure to cold and salt stress in Capsicum, Res. J. Seed Sci. 4 (2011) 125–136.
[36] R. Kumar, P. Swapnil, M. Meena, S. Selpair, B.G. Yadav, Plant growth-promoting rhizobacteria (PGPR): approaches to alleviate abiotic stresses for enhancement of growth and development of medicinal plants, Sustainability 14 (2022), 15514.
[37] H.M. Ahmad, S. Fiaz, S. Hafeez, S. Zahra, A.N. Shah, B. Gul, O. Aziz, Mahmood-Ur-Rahman, A. Fakhar, M. Rafique, Y. Chen, S.H. Yang, X. Wang, Plant growth-promoting rhizobacteria eliminate the effect of drought stress in plants: a review, Front. Plant Sci. 13 (2022).
[38] K. Jetiyanon, P. Plianbangchang, Dose-responses of Bacillus cereus RS87 for growth enhancement in various Thai rice cultivars, Can. J. Microbiol. 56 (2010) 1011–1019.
[39] I.K. Widnyana, C. Javandira, Activities Pseudomonas spp. and Bacillus sp. to stimulate germination and seedling growth of tomato plants, Agric. Agric. Sci. Procedia. 9 (2016) 419–423.
[40] S.M. Nadeem, M. Ahmad, Z.A. Zahir, A. Javaid, M. Ashraf, The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments, Biotechnol. Adv. 32 (2014) 429–448.
[41] C.L. Patten, B.R. Glick, Role of Pseudomonas putida indoleacetic acid in development of the host plant root system, Appl. Environ. Microbiol. 68 (2002) 3795–3801.
[42] G.L.O.D. Souza, S. Nietsche, A.A. Xavier, M.R. Costa, M.C.T. Pereira, M.A. Santos, Triple combinations with PGPB stimulate plant growth in micropropagated banana plantlets, Appl. Soil Ecol. 103 (2016) 31–35.
[43] J. Yuan, Y. Ruan, B. Wang, J. Zhang, R. Waseem, Q. Huang, Q. Shen, Plant growth-promoting rhizobacteria strain Bacillus amyloliquefaciens NJN-6-enriched bio-organic fertilizer suppressed fusarium wilt and Ppromoted the growth of banana plants, J. Agric. Food Chem. 61 (2013) 3774–3780.
[44] H. Chandran, M. Meena, P. Swapnil, Plant growth-promoting rhizobacteria as a green alternative for sustainable agriculture, Sustainability 13 (2021), 10986.
[45] M. Davoodifard, D. Habibi, F. Davoodifard, Effect of plant growth promoting rhizobacteria and foliar application of amino acids and silicic acid on some physiological traits of wheat (Triticum aestivum) under drought stress, Iran, J. Agron. Plant Breed. 8 (2012) 101–114.
[46] Z. Najafi Vafa, Y. Sohrabi, G. Mirzaghaderi, G. Heidari, The effect of rhizobia in improving the protective mechanisms of wheat under drought and supplementary irrigation conditions, Front. Sustain. Food Syst. 6 (2022).
[47] A. Salantur, A. Ozturk, S. Akten, Growth and yield response of spring wheat (Triticum aestivum L.) to inoculation with rhizobacteria, Plant Soil Environ. 52 (2011) 111–118.
[48] A.J. Cattelan, P.G. Hartel, J.J. Fuhrmann, Screening for plant growth-promoting rhizobacteria to promote early soybean growth, Soil Sci. Soc. Am. J. 63 (1999) 1670–1680.
[49] R. Cakmakci, F. Donmez, A. Aydin, F. Sahin, Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions, Soil Biol. Biochem. 38 (2006) 1482–1487.
[50] D. Egamberdiyeva, The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils, Appl. Soil Ecol. 36 (2007) 184–189.
[51] G.I. Burd, D.G. Dixon, B.R. Glick, Plant growth-promoting bacteria that decrease heavy metal toxicity in plants, Can. J. Microbiol. 46 (2000) 237–245.
[52] V. Gravel, H. Antoun, R.J. Tweddell, Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with Pseudomonas putida or Trichoderma atroviride: possible role of indole acetic acid (IAA), Soil Biol. Biochem. 39 (2007) 1968–1977.
[53] J. Kozdroj, J.T. Trevors, J.D. van Elsas, Influence of introduced potential biocontrol agents on maize seedling growth and bacterial community structure in the rhizosphere, Soil Biol. Biochem. 36 (2004) 1775–1784.
[54] B. Shahroona, M. Arshad, Z. Zahir, A. Khalid, Performance of Pseudomonas spp. containing ACC-deaminase for improving growth and yield of maize (Zea mays L.) in the presence of nitrogenous fertilizer, Soil Biol. Biochem. 38 (2006) 2971–2975.
[55] D. Stamenov, T.H. Jafari, Effect of Pgpr on the Germination and Early Growth of Onion, Allium Cepa, 2018, pp. 6–9.
[56] S. Alstrom, R.G. Burns, Cyanide production by rhizobacteria as a possible mechanism of plant growth inhibition, Biol. Fertil. Soils 7 (1989) 232–238.
[57] J. Vacheron, G. Desbrosses, M.-L. Bouffaud, B. Touraine, Y. Moenne-Loccoz, D. Muller, L. Legendre, F. Wisniewski-Dyé, C. Prigent-Combaret, Plant growth-promoting rhizobacteria and root system functioning, Front. Plant Sci. 4 (2013).
[58] K. Shaukat, S. Affrasayab, S. Hasnain, Growth responses of Triticum aestivum to plant growth promoting rhizobacteria used as a biofertilizer, Res. J. Microbiol. 1 (2006) 330–338.
[59] I. Siddiqui, S. Shaukat, Mixtures of plant disease suppressive bacteria enhance biological control of multiple tomato pathogens, Biol. Fertil. Soils 36 (2002) 260–268.
[60] M. Govindappa, R.V. Ravishankar, S. Lokesh, Screening of Pseudomonas fluorescens isolates for biological control of macrophomina phaseolina root-rot of safflower, Afr. J. Agric. Res. 6 (2011) 6256–6266.
[61] F. Anzala, Contrôle de la vitesse de germination chez le maïs (Zea mays) : étude de la voie de biosynthèse des acides aminés issus de l'aspartate et recherche de QTLs, Université d'Angers, Français, 2006.
[62] R. Bharathi, R. Vivekananthan, S. Harish, A. Ramanathan, R. Samiyappan, Rhizobacteria-based bio-formulations for the management of fruit rot infection in chillies, Crop Protect. 23 (2004) 835–843.
[63] E.A. Gashash, N.A. Osman, A.A. Alsahli, H.M. Hewait, A.E. Ashmawi, K.S. Alshallash, A.M. El-Taher, E.S. Azab, H.S. Abd El-Raouf, M.F.M. Ibrahim, Effects of plant-growth-promoting rhizobacteria (PGPR) and cyanobacteria on botanical characteristics of tomato (Solanum lycopersicon L.) plants, Plants 11 (2022) 2732.