South African Journal of Botany 162 (2023) 296–303

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Seed biopriming and plant growth-promoting bacteria improve nutrient absorption and dry matter production of fenugreek (*Trigonella foenum-graecum*) plants

Hessamoddin Solouki[a], Mohamad Kafi[a], Jafar Nabati[b,*], Mohammad Javad Ahmadi[a], Ahmad Nezami[a], Roshanak Shahriary Ahmady[c]

[a] *Department of Agrotechnology, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran*
[b] *Department of Legume, Research Center for Plant Sciences, Ferdowsi University of Mashhad, Iran*
[c] *Faculty of Land and Food Systems, University of British Columbia (UBC), Vancouver, Canada*

ARTICLE INFO

Article History:
Received 29 November 2022
Revised 26 August 2023
Accepted 11 September 2023
Available online xxx

Edited by: Dr S. Gupta

Keywords:
Bio-fertilizing
Dry matter
Leaf essential elements
Medicinal plant
Mineral nutrition

ABSTRACT

Legumes have a significant role in increasing soil sustainable fertility. Plant growth-promoting bacteria (PGPB) could be a significant part of agricultural sustainability. To investigate the PGPB-induced alterations in fenugreek (*Trigonella foenum-graecum*) morpho-physiological traits, nutrient absorption, and dry matter production, an experiment was carried out with two levels of seed priming (unprimed and PGPB bio-priming) and four fertilization methods (control, PGPB fertigation [B], nutrients foliar application [F], and B+F. The results indicated that the highest leaf nitrogen (5.22 %) was observed in PGPB bio-priming and nutrients foliar application treatments by 1.7 times greater than the control. The highest leaf potassium and phosphorus were observed in the B+F-treated plants, which were 1.19 and 2.14 times higher compared with the control, respectively. The B+F-treated plants showed the highest leaf K^+/Na^+ ratio which was ~3 times higher compared with the control. The seed-primed plant's leaf, stem, and total plant dry matter were 34, 13, and 24 % greater than the unprimed treatment, respectively. B+F also increased the leaf, stem, root, and total dry matter of plants by ~50, 45, 37, and 47 %, respectively, compared with the control. Generally, the combined treatment (seed bio-priming + PGPB fertigation + nutrients foliar application) more efficiently improved the fenugreek morpho-physiological traits and dry matter production.

Introduction

The Fabaceae family, with more than 730 genera and 19,000 species, is the third-largest plant family among angiosperms and the second-most important plant family in crop production systems after cereals (Graham and Vance, 2003). Legumes have a significant role in improving the sustainability of soil fertility (Stagnari et al., 2017), supplying fodder (Castro-Montoya et al., 2019), and human health and dietaries (Dove et al., 2011). The medicinal species belonging to the Fabaceae family are considered for their nutritional values, pharmaceutical aspects, and beneficial chemical compounds (Neves et al., 2017). The noticeable chemical compounds of this family, including flavonoids, alkaloids, coumarins, and other metabolites, are used in the treatment of various diseases (Wink, 2013).

Fenugreek (*Trigonella foenum-graecum*) is a well-known medicinal species belonging to the Fabaceae family with tissues containing compounds such as calcium, phosphorus, iron, protein, carotene, and vitamin C (Korat Avni et al., 2019). Fenugreek is cultivated in arid and semi-arid regions and is traditionally used as a vegetable and condiment in diets (Basu et al., 2019). In traditional medicine of various nations, including Iran, fenugreek has a long history of use, and it has important therapeutic qualities, such as analgesic, anti-inflammatory, anti-cancer, anti-diabetic, and reducing blood sugar, cholesterol, and triglyceride levels (Wani and Kumar, 2018).

Desirable growth and development of a plant in an agricultural system require supplying the nutritional requirements (Etienne et al., 2018). Applying biological fertilizers is one of the most highly-valued strategies in integrated nutritional management in a sustainable agricultural system (Akhzari et al., 2018). The bio-fertilizers, especially the plant growth-promoting bacteria (PGPB), are a functional group of free-living microorganisms (Nofal and Rezk, 2009), which can convert the nutritional elements from an inaccessible to an available form during biological processes (Veresoglou et al., 2012). Moreover, these organisms may cause an improvement in the root system and nutritional status of plants (Mandal et al., 2007). Using PGPB as a group of bio-materials for seed bio-priming is one of the methods of seed bio-enhancement (Singh et al., 2018). These bacteria influence

* Corresponding author.
E-mail address: jafarnabati@ferdowsi.um.ac.ir (J. Nabati).

https://doi.org/10.1016/j.sajb.2023.09.014
0254-6299/

different plant growth indices through various mechanisms, including the production and release of plant growth regulators, nitrogen fixation, solubilization of insoluble nutrients such as phosphate, and biological control of the plant pathogenic agents (Vejan et al., 2016).

The foliar application of nutrients has received particular attention due to the considerably increasing productivity of crops, which improves the plant-required nutrient uptake and use efficiency (Ahmad et al., 2018). The environmental pollution caused by chemical fertilizer overuse can be controlled by their foliar application, reducing the soil-applied chemical fertilizers (Rajesh and Paulpandi, 2013). The foliar application of nutrients, particularly micronutrients like Iron, Zinc, Manganese, Copper, and Boron, is considered an approach in agricultural science due to various beneficial impacts such as mitigating element deficiency, ease of fertilizer application, and preventing the elements fixation in the soil compared with their soil application (Wissuwa et al., 2008). It has been shown that the foliar application of macro and micronutrients within the range of plant growing requirements increased plant growth, the dynamics of internal and external biological activities, and the functional integrity of cellular structures of the plants (Gendy et al., 2015).

Regarding the importance of reducing the application of chemical fertilizers to reach sustainable agriculture goals, a more in-depth understanding of the biofertilizers effects such as PGPB should be developed. Therefore, the present study aimed to evaluate the morpho-physiological traits and dry matter production of fenugreek plants affected by bio-fertilizers as seed priming, fertigation, and foliar application.

Materials and methods

Experimental site and procedure

This study was carried out at the Research Greenhouse of the Faculty of Agriculture, the Ferdowsi University of Mashhad, in 2018. Fenugreek seeds (Neyshabur ecotype) were provided by the Research Center for Plan Sciences, Ferdowsi University of Mashhad, Iran. Uniform seeds were sown in 5-L plastic pots containing sterilized field soil and sand in a 1:1 (*v*/*v*) ratio as substrate in a greenhouse at 16/8 h day/night photoperiod, natural irradiance of 700±50 μmol m^{-2} s^{-1} PAR, average day/night temperature of 24/16°C, and relative humidity of 40±5 %. Seeds were sown at the 2 cm soil depth and two pots (six plants each) were considered for each replication per treatment. The physio-chemical characteristics of the soil are presented in Table 1. The plants were irrigated every 5 days to the field capacity. The chemical characteristics of irrigation water are presented in Table 2.

Treatments and application of PGPB

Seed priming in two levels: 1) no priming as control [N] and 2) bio-priming with the mixture of plant growth-promoting bacteria [PGPB] (nitro, phosphor, and potassium power bacter, Dayan company, [P]), and fertilization in four levels: 1) control, 2) fertigation with the mixture of plant growth-promoting bacteria (nitro, phosphor, and potassium power bacter, Dayan company, [B]), 3) nutrients foliar application (silicon, calcium wafer, potassium wafer, and micronutrients, Dayan company, [F]), and 4) fertigation with the mixture of plant growth-promoting bacteria + nutrients foliar application [B+F] were considered as the experimental factors (Table 3).

For seed bio-priming, Nitro Bacter, Phospho Power Bacter, and Potash Power Bacter (5 ml each) were dissolved in 1 L of water (25°C). Then, the seeds were soaked in the solution for 10 h and were washed and spread away from each other, and dried in the air under the shadow. The seeds were then inoculated again with 15 ml of the mixture of PGPB, and ten seeds were sown in the pots. After establishment (4–6 true leaves), the seedlings were thinned to 6 plants per pot. The moisture content of pots was checked every three days to maintain the field capacity during the developmental stages.

Ten days after tinning, 2 ml of Silicon was dissolved in 1 l of water (0.2 %; 25°C), and was foliar applied. A week after the foliar application of Silicon, the Calcium Wafer (0.2 %) was foliar applied, and one week after the foliar application of the Calcium Wafer, Potash Wafer (0.2 %) along with micronutrients (0.1 %) was foliar applied. Each stage of the foliar application was performed twice over the developmental stage and before the flowering. The plans were also fertigated five times with the biological fertilizers of Khosheh Parvaran Zistfanavar Company (5 ml l^{-1} from each PGPB).

Sampling and measurements

Photosynthetic pigments

At the beginning of the flowering stage (70 days after planting; DAP), the content of the photosynthetic pigment was measured using a handheld SPAD chlorophyll meter (Minolta 502) from the second youngest fully developed leaf from the top of plants. The measurements were done three times for all plants in each pot and averaged. Leaf chlorophyll a, b, and a+b content (100 mg fresh weight) were measured using a spectrophotometer (Model SP/3000 Plus) (Arnon, 1949).

Morphological traits

Plants were harvested and separated into shoots and roots 90 DAP. Plant height and the number of lateral branches were measured. Leaf area per plant was measured using a leaf area meter (Li-1300). Roots were rinsed thoroughly, and the root characteristics such as root area, diameter, and length were measured using a scanner (Image Analysis - Delta-T Devices). The above and belowground dry matter (DM) were also calculated after 72 h oven-drying (75°C).

Leaf nutrients

Dried leaves (0.3 g) were used to determine the element's content. Leaf nitrogen, phosphorus, potassium, and sodium content were measured by the Kjeldahl method (Bremner and Mulvaney, 1982), spectrophotometer (Model SP/3000 Plus) (Olsen and Sommers,

Table 1
The physio-chemical characteristics of field soil.

Soil texture	EC (mS.m^{-1})	pH	Organic matter (%)	Organic carbon (%)	Nitrogen (%)	Phosphorus (ppm)	Potassium (ppm)
Silty-Clay-Loam	2.63	7.97	0.71	0.41	0.04	20	480

Table 2
The chemical characteristics of irrigation water.

EC (dS.m^{-1})	pH	Na (meq.l^{-1})	Ca (meq.l^{-1})	Co_3^{-2} (meq.l^{-1})	HCO_3^- (meq.l^{-1})	Cl^- (meq.l^{-1})	Mg^{++} (meq.l^{-1})	SAR
1.2	8.2	5.0	3.0	0.2	3.1	4.0	3.8	2.7

Table 3
Ingredients of the biological and chemical fertilizers (Khosheh Parvaran Zistfanavar Company).

Fertilizer	Ingredients
Dayan Nitro-Bacter	*Azotobacter* sp. + *Azospirillum* sp. + *Bacillus* sp. (10^7 per milliliter)
Dayan Phospho-Power Bacter	*Bacillus* sp. + *Pseudomonas* sp. (10^7 per milliliter)
Dayan Petas-Power Bacter	*Bacillus* sp. + *Pseudomonas* sp. (10^7 per milliliter)
Dayan Silicon	Silicon (20 %) + Potassium (15 %)
Dayan Calcium Wafer	CaO (19 %) + Nitrogen (10 %)
Dayan Potass Wafer	K_2O (54 %) + P_2O_5 (45 %)
Dayan Micro-Mix	Chelated Iron (6000 ppm), Boron (14000 ppm), Manganese (13000 ppm), Copper (3600 ppm), Molybdenum (130 ppm), Zinc (136000 ppm), Cobalt (60 ppm), Sulfur (32000 ppm), Silicon (3100 ppm), and Vitamins B1, B2, B3, B6, and c (10000 ppm)

1982), and flame photometer (Model 310C) (Tandon and Tandon, 1993), respectively.

Statistical analysis

The experiment was designed as a completely randomized design (CRD) in a factorial scheme (two seed priming and four fertilization) with three replications (n = 18). Analysis of variance (ANOVA) was performed using SAS *v*. 9.3, and means were compared by Duncan's multiple range test at a 5 % probability level (SAS, 2011).

Results

SPAD

The results showed that seed priming, fertilization, and their interaction significantly affected leaf SPAD (Table 4). Generally, the leaf SPAD of the primed seeds was higher than the N (unprimed) (Fig. 1). The highest leaf SPAD was obtained from the primed seed (P) and fertigated by PGPB (B) + nutrient foliar application (F) (P+B+F), 5.2 times higher compared with N and non-fertilized plants (Fig. 1).

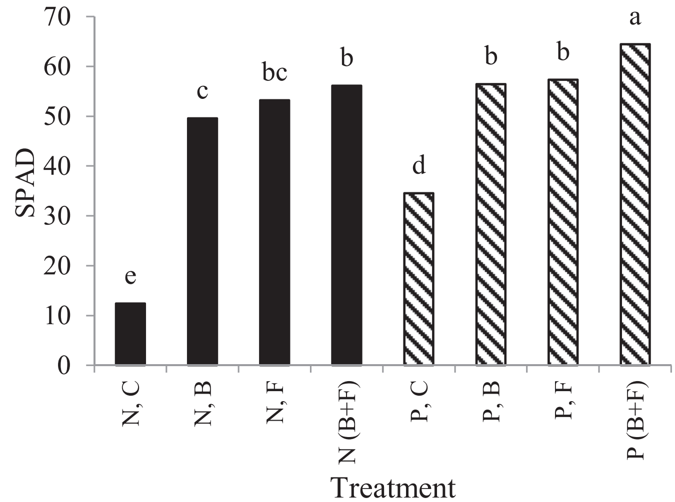

Fig. 1. Leaf SPAD index of fenugreek plants treated by seed PGPB bio-priming and fertilization. PGPB: plant growth-promoting bacteria, N: Non-priming, P: Priming, C: Control (non-fertilized), B: PGPB fertigation, F: PGPB foliar application. Means with a similar letter(s) are not significantly different (Duncan's multiple range, $p \leq 0.05$).

Leaf chlorophyll content

Leaf chlorophyll b content was significantly affected by the seed priming and fertilization treatments (Table 4). However, leaf chlorophyll a and total chlorophyll content were only affected by the fertilization treatments (Table 4). The highest chlorophyll a and b content were observed in the B+F-treated plants by 95 and 55 % increases, respectively, compared with the control (Table 5). Also, PGPB-primed seeds showed the highest chlorophyll b content (0.25 mg g^{-1}) (Table 5). The highest total chlorophyll content (0.99 mg g^{-1}) was also obtained from B+F treatment with an increase of 80 % compared with the control (Table 5).

Leaf area

The plant leaf area was affected by the seed priming and fertilization treatments (Table 4). According to the results, the leaf area of seed-primed plants was 24 % higher than that of the unprimed (Table 5). The B+F-treated plants showed the highest leaf area which was 50 % higher compared with the control plants (Table 5).

Plant height

Seed priming and fertilization significantly affected plant height (Table 4). Seed-primed plants showed a higher plant height by 18 % than the unprimed treatment. The B+F-treated plants also showed the highest plant height, which was ~50 % higher compared with the control (Table 5).

Number of lateral branches

The number of lateral branches was significantly affected by seed priming and fertilization (Table 4). The number of lateral branches of seed-primed plants was 18 % higher than the unprimed (Table 5). The B+F-treated plants also showed the highest number of lateral branches, ~74 % higher compared with the control (Table 5).

Leaf, stem, root, and total dry matter

The results indicated that seed priming and fertilization significantly affected leaf, stem, root, and total plant dry matter (Table 4). Leaf, stem, and total plant dry matter of the seed-primed plants were 0.72, 0.99, and 2.13 g $plant^{-1}$, respectively, 34, 13, and 24 % greater

Table 4
ANOVA results of the effect of seed bio-priming, fertilization, and their interaction on morpho-physiological characteristics of fenugreek plants

S.O.V.	SPAD	Chla	Chlb	Chlt	LA	PH	LB	SDM	LDM	RDM	PDM	RA	RL
P	643.66**	0.0018^{ns}	0.0070*	0.0160^{ns}	6813.16**	206.24**	5.19*	0.61**	0.04**	0.009*	1.20**	54468.18**	337776.1**
F	1661.50**	0.1307**	0.0084**	0.2026**	4028.78**	147.55**	11.70**	0.10**	0.06**	0.02**	0.53**	44721.89**	277335.1**
P × F	96.91**	0.0013^{ns}	0.0010^{ns}	0.0028^{ns}	354.73^{ns}	0.96^{ns}	0.58^{ns}	0.01^{ns}	0.001^{ns}	0.001^{ns}	0.006^{ns}	5714.35**	35436.6**
E(T)	3.81	0.0038	0.0012	0.0044	159.62	3.34	0.70	0.003	0.002	0.001	0.008	363.35	2253.3
C.V	4.07	11.6	15.0	8.7	7.9	6.0	13.9	7.0	6.7	9.2	4.8	4.7	4.5

^{ns}, *, ** are non-significant, and Significant at 5 and 1 % of probability levels, respectively. P: seed PGPB-priming and F: PGPB fertilization. PGPB: plant growth-promoting bacteria, Chla: chlorophyll a, Chlb: chlorophyll b, Chlt: total chlorophyll, LA: leaf area, PH: plant height, LB: lateral branches, SDM: stem dry matter, LDM: leaf dry matter, RDM: root dry matter, PDM: plant dry matter, RA: root area, RL: root length

Table 5
Mean comparison of morpho-physiological characteristics of fenugreek plants affected by seed bio-priming and fertilization.

Treatment	SPAD	Chla (mg g^{-1})	Chlb (mg g^{-1})	Chlt (mg g^{-1})	LA (cm^2)	PH (cm)	LB (no.)	SDM (g)	LDM (g)	RDM (g)	PDM (g)	RA (cm^2)	RL (cm)
Priming													
N	42.81^{b}	0.52^{a}	0.21^{b}	0.73^{a}	141.63^{b}	27.53^{b}	5.54^{b}	0.65^{b}	0.63^{b}	0.40^{a}	1.71^{b}	353.68^{b}	880.78^{b}
P	53.16^{a}	0.54^{a}	0.25^{a}	0.79^{a}	175.31^{a}	33.40^{a}	6.47^{a}	0.99^{a}	0.72^{a}	0.43^{a}	2.13^{a}	448.97^{a}	1118.05^{a}
Fertilization													
C	23.45^{c}	0.37^{d}	0.18^{c}	0.55^{c}	126.73^{c}	23.75^{c}	4.53^{c}	0.66^{c}	0.53^{c}	0.35^{c}	1.55^{c}	286.18^{d}	712.66^{d}
B	53.00^{b}	0.48^{c}	0.23^{bc}	0.71^{b}	154.25^{b}	29.93^{b}	5.75^{b}	0.80^{b}	0.66^{b}	0.40^{b}	1.88^{b}	391.20^{c}	974.21^{c}
F	55.25^{b}	0.56^{b}	0.23^{b}	0.79^{b}	163.41^{b}	32.98^{a}	5.85^{b}	0.85^{b}	0.71^{b}	0.43^{b}	1.98^{b}	439.96^{b}	1095.65^{b}
B + F	60.26^{a}	0.71^{a}	0.28^{a}	0.99^{a}	189.50^{a}	35.20^{a}	7.90^{a}	0.96^{a}	0.80^{a}	0.48^{a}	2.28^{a}	487.96^{a}	1215.15^{a}

Means followed by the same letters are not significantly different at $p \leq 0.05$ probability level, based on Duncan's multiple range test ($p \leq 0.05$). N: Non-priming, P: Priming, C: Control (non-fertilized), B: PGPB fertigation, F: BGPB foliar application. PGPB: plant growth-promoting bacteria, Chla: chlorophyll a, Chlb: chlorophyll b, Chlt: total chlorophyll, LA: leaf area, PH: plant height, LB: lateral branches, SDM: stem dry matter, LDM: leaf dry matter, RDM: root dry matter, PDM: plant dry matter, RA: root area, RL: root length.

than the unprimed, respectively (Table 5). The leaf, stem, root, and total plant dry matter of the B+F-treated plants were also ~50, 45, 37, and 47 %, respectively, greater compared with the control (Table 5).

Root area and length

The results showed that the effect of seed priming, fertilization, and their interaction was significant on the root area and root length (Table 4). Generally, the seed priming and fertilization treatments enhanced root area and length compared with the untreated plants. The highest root area and length were observed in the P+B+F-treated plants by increases about 1.93 and 2.6 times, respectively, compared with the unprimed and unfertilized plants (Fig. 2A and B).

Leaf N, K, and P percentage

Leaf potassium and phosphorus were significantly affected by the seed priming and fertilization treatments (Table 6). Leaf potassium and phosphorus of the seed-primed plants were 1.74 and 0.05 percent, respectively, higher than the unprimed (Table 7). Leaf potassium and phosphorus of the B+F-treated plants were also 1.19 and 2.14 times, respectively, higher than the control plants (Table 7). The results indicated that the effects of seed priming, fertilization, and their interaction were significant on leaf nitrogen percentage (Table 6). Generally, seed priming and fertilization increased leaf nitrogen content. The results revealed the highest leaf nitrogen by 5.22 % in the P+B+F treatment, 70 % higher compared with the unprimed and the control plants (Fig. 3).

Leaf Na and K^+/Na^+ ratio

The results showed that the main and interaction effects of seed priming and fertilization were significant on the Na of leaves (Table 6). The highest leaf Na was observed in the unprimed control plants by ~3 times higher Na than the N+B+F treatment (Fig. 4A). However, the lowest leaf Na was obtained from the N+B+F and P+B+F treatments (Fig. 4A). The K^+/Na^+ ratio was significantly affected by the main and interaction effects of seed priming and fertilization treatments (Table 6). The highest K^+/Na^+ ratio was observed in the N+B+F and P+B+F treatments, which was ~3 times higher compared with the unprimed and control plants (Fig. 4B).

Discussion

Among the different priming methods, bio-compounds, including plant growth-promoting bacteria, are one of the effective methods, which is called bio-priming (Ashraf and Foolad, 2005). The high efficiency and profitability of bio-priming have been reported in various research (Chakraborty et al., 2013; Hafezi Ghehestani et al., 2021; Mirshekari et al., 2012). In the present study, it was also observed that fenugreek seeds bio-priming resulted in producing more vigorous seedlings and improving seedling establishment. According to studies, the priming technique decreased seed germination time (Mirmahmood et al., 2015; Moori and Ahmadi-Lahijani, 2020; Wahid et al., 2008), resulting in an increased plant vigor, which improved the early establishment of plants and optimal utilization of food resources (Harris et al., 2001). The results of an experiment indicated that the seed bio-priming improved seed germination and seedling

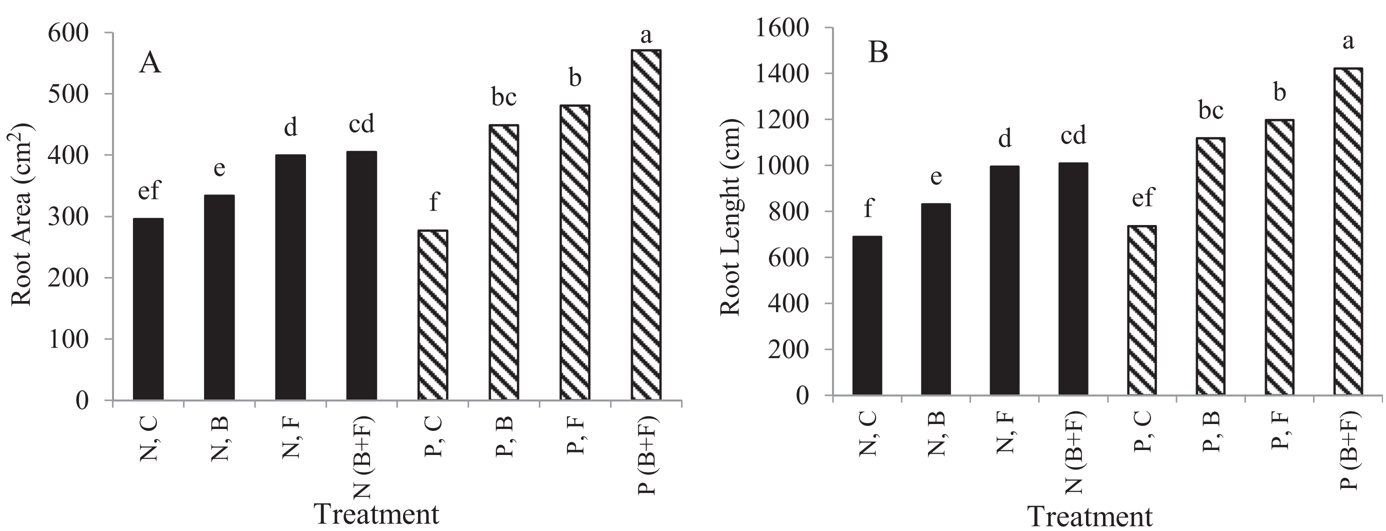

Fig. 2. Root area (A) and root length (B) of fenugreek plants treated by seed PGPB bio-priming and fertilization. PGPB: plant growth-promoting bacteria, N: Non-priming, P: Priming, C: Control (non-fertilized), B: PGPB fertigation, F: BGPB foliar application. Means with a similar letter(s) are not significantly different (Duncan's multiple range, $p \leq 0.05$).

Table 6
ANOVA results of the effect of bio-priming, fertilization, and their interaction on fenugreek leaf nutrient elements.

S.O.V.	Potassium (K)	Phosphorus (P)	Nitrogen (N)	Sodium (Na)	K^+/Na^+
P	0.0969^{**}	0.0005^{**}	0.8999^{**}	6.7734^{ns}	12.0984^{ns}
F	0.0872^{**}	0.0013^{**}	2.7517^{**}	0.0028^{**}	1095.8350^{**}
P × F	0.0021^{ns}	0.0000^{ns}	0.2685^{**}	0.0001^{*}	24.4057^{**}
E(T)	0.0015	0.0000	0.0031	0.0000	4.4762
C.V	2.3828	8.1307	1.2419	7.5467	6.7138

ns, *, ** are non-significant, and Significant at 5 and 1 % of probability levels, respectively. P: seed PGPB-priming and F: PGPB fertilization. PGPB: plant growth-promoting bacteria.

Table 7
Mean comparison of leaf nutrient elements affected by seed priming, fertilization, and their interaction.

Treatment	Potassium (%)	Phosphorus (%)
Priming		
N	1.61^{b}	0.04^{b}
P	1.74^{a}	0.05^{a}
Fertilizing		
C	1.53^{c}	0.03^{c}
B	1.65^{b}	0.03^{c}
F	1.70^{b}	0.05^{b}
B + F	1.82^{a}	0.06^{a}

Means followed by the same letters are not significantly different at $p \leq 0.05$ probability level, based on Duncan's multiple range test ($p \leq 0.05$). N: Non-priming, P: Priming, C: Control (non-fertilized), B: PGPB fertigation, F: BGPB foliar application.

establishment (Mahmood et al., 2016). It has also been reported that the seed bio-priming stimulated seed germination and improved plant growth (Moeinzadeh et al., 2010).

The positive impacts of bio-priming were reflected in the subsequent stages of plant growth, such as root length, stem length, and seedling dry weight (Sung and Chiu, 1995). Studies have indicated that the main part of biochemical, molecular, and physiological processes, including cell cycles, endosperm softening, synthesis of proteins, transcriptome, and re-regulation of DNA to continuing germination process, is related to the seed imbibition stage (Gallardo et al., 2001; Yu et al., 2014). DNA reconstruction pathways to

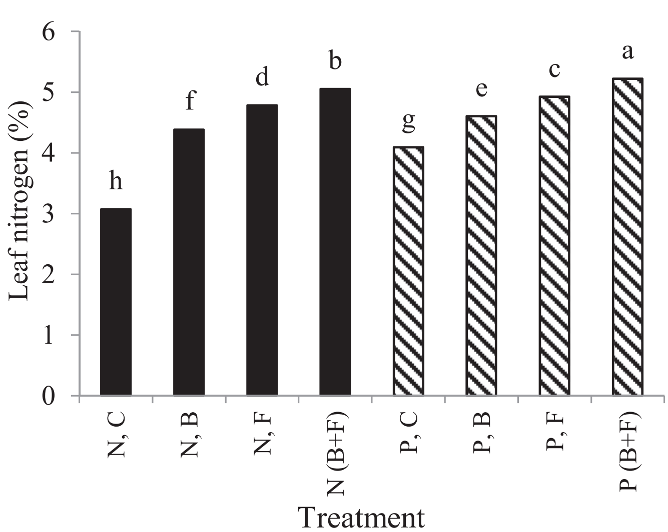

Fig. 3. Leaf nitrogen content of fenugreek plants treated by seed PGPB bio-priming and fertilization. PGPB: plant growth-promoting bacteria, N: Non-priming, P: Priming, C: Control (non-fertilized), B: PGPB fertigation, F: BGPB foliar application. Means with a similar letter(s) are not significantly different (Duncan's multiple range, $p \leq 0.05$).

maintain the integrity of the plant genome are associated with the seed imbibition stage (Balestrazzi et al., 2015). Therefore, it can induce synthesizing of the rootlet cell's DNA, which can effectively raise the rootlet length (Riha et al., 1998). In the present study, seed bio-priming enhanced root area and root length compared with the unprimed seed treatment. Seed priming can improve the gene expression of antioxidant enzymes, including catalase as an essential enzyme in the seed germination stage (Kibinza et al., 2011), which is expressed as a critical factor in improving germination under stressful conditions (Liu et al., 2007). Rouhi et al. (2011) also reported that priming tall fescue (*Festuca arundinacea* Schreb.) seeds significantly increased the root length compared with the control.

The availability of minerals is inevitable due to their crucial role in the plant life cycle, such as metabolism, growth and development, protection against various stresses, and yield quantity and quality (Morgan and Connolly, 2013). Using PGPB is one of the advanced scientific techniques to improve nutrient availability for plants (Fasciglione et al., 2015). A combination of *Azotobacter* and *Azospirilum* increased the chlorophyll content of some species of mint (*Mentha sp.*) plants due to their ability to increase plant nitrogen absorption (El-Hadi et al., 2009). Our results also showed that the application of PGPB enhanced the leaf chlorophyll content of fenugreek plants. Nadeem et al. (2006) also reported an increased chlorophyll content in maize (*Zea maize*) plants treated with PGPB. Working on the cumin (*Cuminum cyminum* L.) ecotype, Hafezi Ghehestani et al. (2021) found that foliar application of bacterial siderophore increased the leaves and grain nutrient status, leaf photosynthetic pigments, and essential oil content, leading to a higher grain yield.

The results of the present study showed that the application of PGPB enhanced leaf nutrient elements, *i.e.,* N, P, and K. Regarding the improvement of nutrient absorption affected by PGPB, it was reported that there was a significantly positive correlation between plant biomass and the N and P content in plants (Rana et al., 2012). Han and Lee (2006) also reported that applying symbiotic nitrogen-fixing bacteria increased nitrogen absorption in pepper (*Capsicum annum* L.) plants. The plant nutrient use efficiency was enhanced with PGPR and AMF in an integrated nutrient management system (Adesemoye et al., 2008). Wheat (*Triticum aestivum* L.) seed bio-priming reduced the plant nitrogen and phosphorus requirements during the growth and development process compared with the unprimed plants (Saber et al., 2012). It was also reported that N, P, and K content were significantly higher in wheat (*Triticum* spp.) leaves treated with PGPB (Upadhyay and Singh, 2015).

The availability of nutrients resulting from PGPB application improved the physiological, growth, and developmental processes of fenugreek plants, which was in agreement with other studies that evaluated the effect of bio-compounds on plant productivity (Amtmann et al., 2008; Rashid et al., 2016). It seems that the foliar application of potassium wafer (54 % K_2O and 45 % P_2O_5) used in our study played a critical role in the plant physiological processes as an essential element in DNA regulation and protein synthesis. Furthermore, the calcium wafer (19 % CaO and 10 % nitrogen) probably helped in the organic acids balance, activating the plant's enzymatic system,

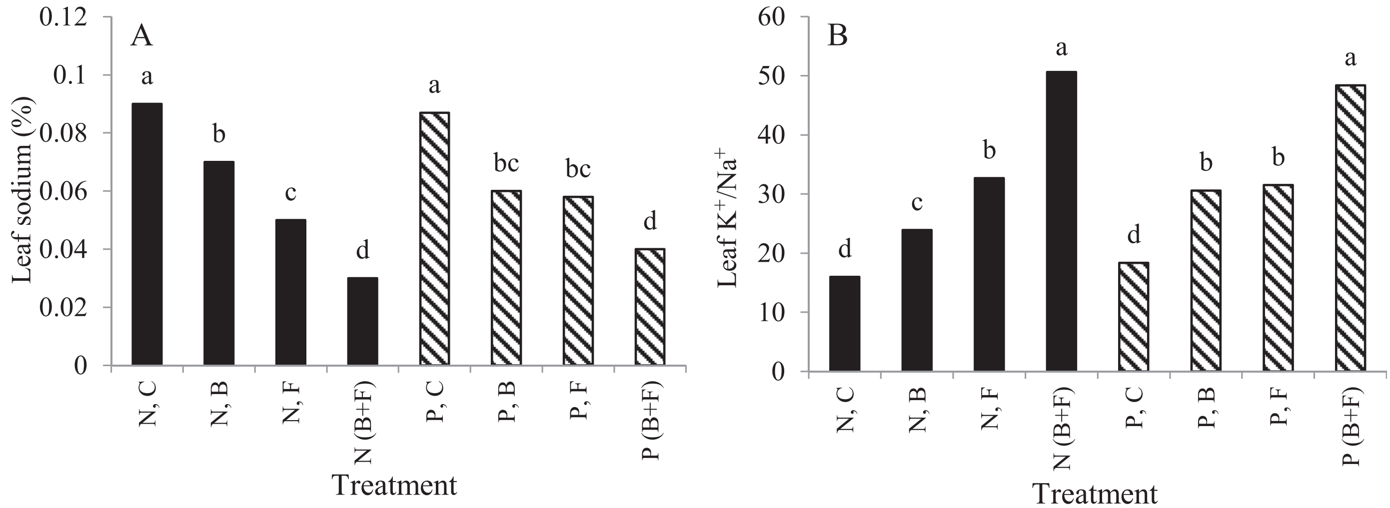

Fig. 4. Leaf sodium content (A) and K^+/Na^+ ratio (B) of fenugreek plants treated by seed PGPB bio-priming and fertilization. PGPB: plant growth-promoting bacteria, N: Non-priming, P: Priming, C: Control (non-fertilized), B: PGPB fertigation, F: BGPB foliar application. Means with a similar letter(s) are not significantly different (Duncan's multiple range, $p \leq 0.05$).

and improving growth. Calcium, as another important element in physiological processes, has a vital role in cell membrane stability and integrity, protein synthesis, improvement of the cellular water status, and acting as a co-factor in many enzymatic activities (Demidchik and Shabala, 2017).

Silicon, one of the most effective elements in the soil, is another influential element in physiological processes (Epstein, 1994). However, the accessible form of silicon is deficient in the soil for plants (Mali and Aery, 2009). The results of different studies have shown that foliar application of silicon could significantly affect the physiological performance of plants (Merwad et al., 2018; Sapre and Vakharia, 2017). Kafi et al. (2021) reported that the plant water status was improved by the application of silicon. They found that the leaf osmotic potential was increased by the application of $NaSiO_3$-NPs. Silicon wafers (20 % silicon and 15 % potassium) might increase the plant's potential to effectively use light by influencing the stomata status (Putra et al., 2012) and enhancing the photosynthetic efficiency (Al-aghabary et al., 2005). Silicon, through a decrease in cell sap concentration, leads to preserving water in the plant (Liang, 1999).

The results of studies suggested that the improvement of ion selectivity and reduction of toxic ions such as sodium is one of the impacts of using PGPB (Ilangumaran and Smith, 2017; Shah et al., 2017). In the present study, it was also observed that PGPB application reduced the Na content of fenugreek leaves. Alamri and Mostafa (2009) also observed that the application of PGPB reduced the Na concentration and increased N, P, K, and Ca concentrations. Silicon may improve plant growth and productivity by preventing Na^+ ions entry and accumulation. In the present study, silicon application might reduce leaf Na content and the Na^+/K^+ ratio of plant leaves. Silicon increases the activity of the ATPase pump and facilitates sodium removal from cells (Zhu and Gong, 2014). The Na^+/H^+ antiporters, by removing sodium from the cytosol or moving it into the vacuole, play a vital role in maintaining low sodium concentrations. Kafi et al. (2021) also observed that foliar application of silicon compounds improved leaf gas exchange variables, soluble carbohydrates and proline content, antioxidant enzyme activities, and reduced malondialdehyde content and Na/K ratio in potato (*Solanum tuberosum* L.) plants under saline conditions.

Leaves, as the main plant organ to perform photosynthesis, have a vital role in producing photoassimilates. Plants can utilize light to produce nutrients by increasing leaf area (Weraduwage et al., 2015). The results of studies suggested that using plant nutrients as a foliar application can improve the plant's physiological characteristics during plant growth and development (Thalooth et al., 2006; Vojodi Mehrabani et al., 2017). The results of the present study also indicated that foliar application of nutrients enhanced leaf chlorophyll content and leaf area, which was an important factor in improving the photosynthesis and photoassimilates production of fenugreek plants.

Greater leaf area and plant above- and below-ground biomass in fenugreek plants were observed in the present study when PGPB was applied. The PGPB application was also reported in increasing leaf area by induction of indole acetic acid production in a plant (Spaepen et al., 2009). Supplying ~48 % of the total required nitrogen through nitrogen fixation during the fenugreek growth inoculated with PGPB increased leaf protein content (Deserrier et al., 1986). The inoculation of fenugreek plants with PGPB also showed that these bacteria increased seed and plant vigor index, the number of nodules, above and below-ground biomass, and grain yield (Kumar et al., 2011). It is been reported that biological fertilizers such as *Azotobacter, Pseudomonas,* and *Azospirillium* genus and their combinations significantly increased the dry weight of fennel (*Foeniculum vulgare* Mill.) plants due to the higher availability of nutrients for the plants (Mahfouz and Sharaf-Eldin, 2007). Hegab (2018) also observed that the foliar application of micronutrients significantly increased fenugreek yield and improved its nutritional values.

Conclusion

The results indicated that seed bio-priming by the plant growth-promoting bacteria and application of nutrients significantly impacted the fenugreek plants. These bacteria improved the plant's physiological metabolism and nutrient uptake by increasing the availability of nutrients for the plant. The results showed that the leaf nitrogen content was continuously higher in PGPB-primed plants than in the unprimed ones. Plant biomass, root area, root length, and leaf N, P, and K content were positively affected by the PGPB treatments. Leaf sodium content was decreased in the PGPB-treated plants, while the leaf K^+/Na^+ ratio was enhanced. To reduce the application of chemical fertilizers to reach sustainable agriculture, utilizing biofertilizers such as PGPB seems applicable and efficient. Comparing the PGPB application methods revealed that the combined treatment (seed bio-priming + foliar application of silicon, potassium, calcium, and micronutrients + PGPB fertigation) was more efficient in improving fenugreek morpho-physiological traits, nutrient absorption, and dry matter production.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosure statement

The authors report there are no competing interests to declare.

Declaration of Competing Interest

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

Acknowledgments

This research was supported by the Ferdowsi University of Mashhad. We are also grateful to Dr. Morteza Goldani and Dr. Mehdi Parsa for their advice in the implementation of this research.

References

Adesemoye, A., Torbert, H., Kloepper, J., 2008. Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. Can. J. Microbiol. 54, 876–886.
Ahmad, Z., Anjum, S., Waraich, E.A., Ayub, M.A., Ahmad, T., Tariq, R.M.S., Ahmad, R., Iqbal, M.A., 2018. Growth, physiology, and biochemical activities of plant responses with foliar potassium application under drought stress−a review. J. Plant Nutr. 41, 1734–1743.
Akhzari, D., Kalantari, N., Mahdavi, S., 2018. Studying the effects of mycorrhiza and vermicompost fertilizers on the growth and physiological traits of Vetiver Grass (*Chrysopogon zizanioides* L.). Desert 23, 57-62.
Al-aghabary, K., Zhu, Z., Shi, Q., 2005. Influence of silicon supply on chlorophyll content, chlorophyll fluorescence, and antioxidative enzyme activities in tomato plants under salt stress. J. Plant Nutr. 27, 2101–2115.
Alamri, S.A., Mostafa, Y.S., 2009. Effect of nitrogen supply and *Azospirillum brasilense* Sp-248 on the response of wheat to seawater irrigation. Saudi J. Biol. Sci. 16, 101–107.
Amtmann, A., Troufflard, S., Armengaud, P., 2008. The effect of potassium nutrition on pest and disease resistance in plants. Physiol. Plant. 133, 682–691.
Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24, 1.
Ashraf, M., Foolad, M.R., 2005. Pre-sowing seed treatment—a shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. Adv. Agron. 88, 223–271.
Balestrazzi, A., Donà, M., Macovei, A., Sabatini, M.E., Pagano, A., Carbonera, D., 2015. DNA repair and telomere maintenance during seed imbibition: correlation of transcriptional patterns. Telomere Telomerase 2.
Basu, S., Cetzal-Ix, W., Zandi, P., Możdżeń, K., Siavoshi, M., 2019. Rich potential for fenugreek (*Trigonella foenum*-graecum L.) cultivation in Iran. The 2nd International Conference on Medicinal Plants, Organic Farming, Natural and Medicinal Materials, pp. 13–14.
Bremner, J., Mulvaney, C., 1982. Salicylic acid-thiosulphate modification of Kjeldahl method to include nitrate and nitrite. Agronomy 9, 621–622.
Castro-Montoya, J., Gownipuram, R., Mendoza, M., Solano, N., López, F., Dickhöfer, U., Corea, E., 2019. Effects of feeding tropical forage legumes on nutrients digestibility, nitrogen partitioning and performance of crossbred milking cows. Anim. Feed Sci. Technol. 247, 32–40.
Chakraborty, U., Chakraborty, B., Chakraborty, A., Dey, P., 2013. Water stress amelioration and plant growth promotion in wheat plants by osmotic stress tolerant bacteria. World J. Microbiol. Biotechnol. 29, 789–803.
Demidchik, V., Shabala, S., 2017. Mechanisms of cytosolic calcium elevation in plants: the role of ion channels, calcium extrusion systems and NADPH oxidase-mediated 'ROS-Ca^{2+} Hub'. Funct. Plant Biol. 45, 9–27.
Deserrier, N., Baccou, J., Sauvaire, Y., 1986. Nitrogen fixation and nitrate assimilation in field-grown fenugreek (*Trigonella foenum graecum* L.). Plant Soil.
Dove, E.R., Mori, T.A., Chew, G.T., Barden, A.E., Woodman, R.J., Puddey, I.B., Sipsas, S., Hodgson, J.M., 2011. Lupin and soya reduce glycaemia acutely in type 2 diabetes. Br. J. Nutr. 106, 1045–1051.
El-Hadi, N., El-Ala, H.A., El-Azim, W., 2009. Response of some Mentha species to plant growth promoting bacteria (PGPB) isolated from soil rhizosphere. Aust. J. Basic Appl. Sci. 3, 4437–4448.
Epstein, E., 1994. The anomaly of silicon in plant biology. Proc. Natl Acad. Sci. USA 91, 11–17.
Etienne, P., Diquelou, S., Prudent, M., Salon, C., Maillard, A., Ourry, A., 2018. Macro and micronutrient storage in plants and their remobilization when facing scarcity: the case of drought. Agriculture 8, 14.
Fasciglione, G., Casanovas, E.M., Quillehauquy, V., Yommi, A.K., Goni, M.G., Roura, S.I., Barassi, C.A., 2015. Azospirillum inoculation effects on growth, product quality and storage life of lettuce plants grown under salt stress. Sci. Hortic. 195, 154–162.
Gallardo, K., Job, C., Groot, S.P., Puype, M., Demol, H., Vandekerckhove, J., Job, D., 2001. Proteomic analysis of Arabidopsis seed germination and priming. Plant Physiol. 126, 835–848.
Gendy, A., Soliman, A., Mohammed, N.S., 2015. Response of growth, productivity and oil composition of fenugreek plants to foliar application of complete fertilizer, dry yeast and L-tryptophan under sandy soil conditions. Curr. Top. Forensic Sci. Proc. Meet. Int. Assoc. Forensic Sci., 14th 4, 736–749.
Graham, P.H., Vance, C.P., 2003. Legumes: importance and constraints to greater use. Plant Physiol. 131, 872–877.
Hafezi Ghehestani, M.M., Azari, A., Rahimi, A., Maddah-Hosseini, S., Ahmadi-Lahijani, M.J., 2021. Bacterial siderophore improves nutrient uptake, leaf physiochemical characteristics, and grain yield of cumin (*Cuminum cyminum* L.) ecotypes. J. Plant Nutr. 1–13.
Han, H.-S., Lee, K., 2006. Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant Soil Environ. 52, 130.
Harris, D., Pathan, A., Gothkar, P., Joshi, A., Chivasa, W., Nyamudeza, P., 2001. On-farm seed priming: using participatory methods to revive and refine a key technology. Agric. Syst. 69, 151–164.
Hegab, R., 2018. Effect of foliar application of micronutrients and compost tea on chemical composition and the productivity of fenugreek plants under baloza-north sinai conditions. J. Soil Sci. Agric. Eng. 9, 403–410.
Ilangumaran, G., Smith, D.L., 2017. Plant growth promoting rhizobacteria in amelioration of salinity stress: a systems biology perspective. Front. Plant Sci. 8, 1768.
Kafi, M., Nabati, J., Ahmadi-Lahijani, M.J., Oskoueian, A., 2021. Silicon compounds and potassium sulfate improve salinity tolerance of potato plants through instigating the defense mechanisms, cell membrane stability, and accumulation of osmolytes. Commun. Soil Sci. Plant Anal. 52, 843–858.
Kibinza, S., Bazin, J., Bailly, C., Farrant, J.M., Corbineau, F., El-Maarouf-Bouteau, H., 2011. Catalase is a key enzyme in seed recovery from ageing during priming. Plant Sci. 181, 309–315.
Korat Avni, N., Kavathiya, Y., Lakhani, N., Kandoliya, U., BA, G., Golakiya, B., 2019. Nutritional and antioxidant components of fenugreek (*Trigonella foenum-graecum* L.) seedlings. J. Pharmacog. Phytochem. 8, 443–447.
Kumar, H., Dubey, R., Maheshwari, D., 2011. Effect of plant growth promoting rhizobia on seed germination, growth promotion and suppression of Fusarium wilt of fenugreek (*Trigonella foenum-graecum* L.). Crop Prot. 30, 1396–1403.
Liang, Y., 1999. Effects of silicon on enzyme activity and sodium, potassium and calcium concentration in barley under salt stress. Plant Soil 209, 217–224.
Liu, X., Xing, D., Li, L., Zhang, L., 2007. Rapid determination of seed vigor based on the level of superoxide generation during early imbibition. Photochem. Photobiol. Sci. 6, 767–774.
Mahfouz, S., Sharaf-Eldin, M., 2007. Effect of mineral vs. biofertilizer on growth, yield, and essential oil content of fennel [*Foeniculum vulgare* Mill.]. Int. Agrophys. 21, 361–366.
Mahmood, A., Turgay, O.C., Farooq, M., Hayat, R., 2016. Seed biopriming with plant growth promoting rhizobacteria: a review. FEMS Microbiol. Ecol. 92, fiw112.
Mali, M., Aery, N., 2009. Effect of silicon on growth, biochemical constituents, and mineral nutrition of cowpea. Commun. Soil Sci. Plant Anal. 40, 1041–1052.
Mandal, A., Patra, A.K., Singh, D., Swarup, A., Masto, R.E., 2007. Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. Bioresour. Technol. 98, 3585–3592.
Merwad, A.-R.M., Desoky, E.-S.M., Rady, M.M., 2018. Response of water deficit-stressed *Vigna unguiculata* performances to silicon, proline or methionine foliar application. Sci. Hortic. 228, 132–144.
Mirmahmood, S.J., Ahmadi-Lahijani, M.J., Emam, Y., 2015. Effect of cycocel seed priming on osmotic stress tolerance in rapeseed germination (*Brassica napus*). Iran. J. Seed Res. 1, 33–44.
Mirshekari, B., Hokmalipour, S., Sharifi, R.S., Farahvash, F., Ebadi-Khazine-Gadim, A., 2012. Effect of seed biopriming with plant growth promoting rhizobacteria (PGPR) on yield and dry matter accumulation of spring barley (*Hordeum vulgare* L.) at various levels of nitrogen and phosphorus fertilizers. J. Food Agric. Environ. 10, 314–320.
Moeinzadeh, A., Sharif-Zadeh, F., Ahmadzadeh, M., Tajabadi, F., 2010. Biopriming of Sunflower ('*Helianthus annuus*' L.) Seed with'Pseudomonas fluorescens' for Improvement of Seed Invigoration and Seedling Growth. Aust. J. Crop Sci. 4, 564–570.
Moori, S., Ahmadi-Lahijani, M.J., 2020. Hormopriming instigates defense mechanisms in Thyme (*Thymus vulgaris* L.) seeds under cadmium stress. J. Appl. Res. Med. Aromat. Plants 19, 100268.
Morgan, J.á., Connolly, E.á., 2013. Plant-soil interactions: nutrient uptake. Nat. Educ. Knowl. 4 (2).
Nadeem, S., Hussain, I., Naveed, M., Asghar, H., Zahir, Z., Arshad, M., 2006. Performance of plant growth promoting rhizobacteria containing ACC-deaminase activity for improving growth of maize under salt-stressed conditions. Pakistan J. Agric. Sci. 43, 114–121.
Neves, A.M., Costa, P.S., Coutinho, M.G.S., Souza, E.B., dos Santos, H.S., Silva, M.G., Fontenelle, R.O., 2017. Chemical characterization and the antimicrobial potential of species of the *Genus Senna* Mill.(Fabaceae). Revista Virtual de Química 9, 2506-2538.
Nofal, O., Rezk, A., 2009. Role of fertilization in improving quality of some agricultural crops. International Journal of Academic Research 1.
Olsen, S., Sommers, L., 1982. Phosphorus in AL Page, (Ed). Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties.

Putra, E., Zakaria, W., Abdullah, N., Saleh, G., 2012. Stomatal morphology, conductance and transpiration of *Musa* sp. *cv*. Rastali in relation to magnesium, boron and silicon availability. J. Plant Physiol. 7, 84–96.
Rajesh, N., Paulpandi, V., 2013. Review of foliar nutrition in Redgram enhancing the growth and yield characters. Am. Int. J. Res. Formal Appl. Natur. Sci. 13, 9–14.
Rana, A., Saharan, B., Nain, L., Prasanna, R., Shivay, Y.S., 2012. Enhancing micronutrient uptake and yield of wheat through bacterial PGPR consortia. Soil Sci. Plant Nutr. 58, 573–582.
Rashid, M.M., Jahan, M., Islam, K.S., 2016. Impact of nitrogen, phosphorus and potassium on brown planthopper and tolerance of its host rice plants. Rice Sci. 23, 119–131.
Riha, K., Fajkus, J., Siroky, J., Vyskot, B., 1998. Developmental control of telomere lengths and telomerase activity in plants. Plant Cell 10, 1691–1698.
Rouhi, H.R., Sharif-Zadeh, F., Aboutalebian, M.A., 2011. Seed priming improves the germination traits of tall fescue (*Festuca arundinacea*). Not. Sci. Biol. 3, 57–63.
Saber, Z., Pirdashti, H., Esmaeili, M., Abbasian, A., Heidarzadeh, A., 2012. Response of wheat growth parameters to co-inoculation of plant growth promoting rhizobacteria (PGPR) and different levels of inorganic nitrogen and phosphorus. World Appl. Sci. J. 16, 213–219.
Sapre, S., Vakharia, D., 2017. Silicon induced physiological and biochemical changes under polyethylene glycol-6000 water deficit stress in wheat seedlings. J. Environ. Biol. 38, 313.
SAS, 2011. SAS/STAT® 9.3 user's guide, Care. SAS Institute Inc., NC.
Shah, G., Jan, M., Afreen, M., Anees, M., Rehman, S., Daud, M., Malook, I., Jamil, M., 2017. Halophilic bacteria mediated phytoremediation of salt-affected soils cultivated with rice. J. Geochem. Explor. 174, 59–65.
Singh, V., Maharshi, A., Singh, D.P., Upadhyay, R.S., Sarma, B.K., Singh, H.B., 2018. Role of microbial seed priming and microbial phytohormone in modulating growth promotion and defense responses in plants. Advances in Seed Priming. Springer, pp. 115–126.
Spaepen, S., Vanderleyden, J., Okon, Y., 2009. Plant growth-promoting actions of rhizobacteria. Adv. Bot. Res. 51, 283–320.
Stagnari, F., Maggio, A., Galieni, A., Pisante, M., 2017. Multiple benefits of legumes for agriculture sustainability: an overview. Chem Biol Technol Agric 4, 1–13.
Sung, J.M., Chiu, K.Y., 1995. Hydration effect on seedling emergence strength of watermelon seeds differing in ploidy. Plant Sci. 110, 21–26.
Tandon, H.L.S., Tandon, H., 1993. Methods of Analysis of Soils, Plants, Waters, and Fertilisers. Fertiliser Development and Consultation Organisation, New Delhi.
Thalooth, A., Tawfik, M., Mohamed, H.M., 2006. A comparative study on the effect of foliar application of zinc, potassium and magnesium on growth, yield and some chemical constituents of mungbean plants grown under water stress conditions. World J. Agric. Sci. 2, 37–46.
Upadhyay, S., Singh, D., 2015. Effect of salt-tolerant plant growth-promoting rhizobacteria on wheat plants and soil health in a saline environment. Plant Biol. 17, 288–293.
Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., Nasrulhaq Boyce, A., 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability—a review. Molecules 21, 573.
Veresoglou, S.D., Chen, B., Rillig, M.C., 2012. Arbuscular mycorrhiza and soil nitrogen cycling. Soil Biol. Biochem. 46, 53–62.
Vojodi Mehrabani, L., Kamran, R.V., Hassanpouraghdam, M.B., Pessarakli, M., 2017. Zinc sulfate foliar application effects on some physiological characteristics and phenolic and essential oil contents of *Lavandula stoechas* L. under sodium chloride (NaCl) salinity conditions. Commun. Soil Sci. Plant Anal. 48, 1860–1867.
Wahid, A., Noreen, A., Basra, S.M., Gelani, S., Farooq, M., 2008. Priming-induced metabolic changes in sunflower (*Helianthus annuus*) achenes improve germination and seedling growth. Botan. Stud. 49, 343–350.
Wani, S.A., Kumar, P., 2018. Fenugreek: A review on its nutraceutical properties and utilization in various food products. J. Saudi Soc. Agric. Sci. 17, 97–106.
Weraduwage, S.M., Chen, J., Anozie, F.C., Morales, A., Weise, S.E., Sharkey, T.D., 2015. The relationship between leaf area growth and biomass accumulation in *Arabidopsis thaliana*. Front. Plant Sci. 6, 167.
Wink, M., 2013. Evolution of secondary metabolites in legumes (Fabaceae). S. Afr. J. Bot. 89, 164–175.
Wissuwa, M., Ismail, A.M., Graham, R.D., 2008. Rice grain zinc concentrations as affected by genotype, native soil-zinc availability, and zinc fertilization. Plant Soil 306, 37–48.
Yu, Y., Guo, G., Lv, D., Hu, Y., Li, J., Li, X., Yan, Y., 2014. Transcriptome analysis during seed germination of elite Chinese bread wheat cultivar Jimai 20. BMC Plant Biol. 14, 1–19.
Zhu, Y., Gong, H., 2014. Beneficial effects of silicon on salt and drought tolerance in plants. Agron. Sustain. Dev. 34, 455–472.