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Phytostimulation Properties of Indigenous Plant Growth-promoting Bacteria from Licorice (*Glycyrrhiza glabra* L.): Benefits for Seed Germination and Seedling Growth

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ABSTRACT

To evaluate the effects of four licorice plant growth-promoting bacteria (PGPB), i.e. *Pantoea agglomerans* (S72), *Serratia rubidaea* (S28) *Pseudomonas azotoformans* (E101), *Pseudomonas frederiksbergensis* (E56) on licorice seed germination and seedling growth, two experiments were conducted at the Research Laboratory and Greenhouse, University of Tehran in 2019. Treatments were bacterial strains in eight levels (sole or in combination) and growing media at four levels of M1 (sand + perlite (1: 1)), M2 (soil + cow manure), M3 (soil + mycorrhiza fungi) and M4 (soil + mycorrhiza fungi+ cow manure). Germination quality criteria and some morphological traits of two-month-old seedlings were evaluated. The results of seed germination showed that in most of the evaluated traits, bacterial treatments performed better than the control, although in some cases, there were no significant differences with the control. Also, in some cases, endophytic bacteria had an effective role in improving seed germination index compared to rhizospheric bacteria. According to the experiment results of seedling growth, M4 in combination with two endophytic bacteria (E101 and E56), of the Pseudomonas genus in licorice plants, had a superior performance in improving the initial growth and establishment qualities of the licorice plants. Regarding most of the traits, the co-application of mycorrhiza with S28 (M3B3) showed the lowest values. The results of this study indicated the potential use of licorice endophytic bacteria as a source of biofertilizer for the improvement of licorice seed germination and growth and, if possible, for similar applications in other plant species.

Abbreviations

M1 (sand + perlite (1: 1)), M2 (soil + cow manure), M3 (soil + mycorrhiza fungi) and M4 (soil + mycorrhiza fungi + cow manure), Plant growth-promoting bacteria (PGPB), Indole-3-acetic acid (IAA), B1 (*Pantoea agglomeransz* (S72)), B2 (*Serratia rubidaea* (S28)), B3 (*Pseudomonas azotoformans* (E101)), B4 (*Pseudomonas frederiksbergensis* (E56)

Introduction

Glycyrrhiza glabra L. is one of the oldest and most well-known medicinal plants in the Fabaceae family, with nearly 30 species reported so far (Zhang and Ye, 2009; Pastorino et al., 2018). The distribution of this plant is reported in semi-tropical and tropical regions of the world, including Southern Europe, Central Asia (Sharma et al., 2005).

Because of valuable compounds in licorice roots and rhizomes, such as saponins (glycyrrhizin and glycyrrhizinic acid) (Fuggersberger-Hein and Franz, 1984; Omar et al., 2012; Yu et al., 2015; Pastorino et al., 2018), flavones and flavonoids (glabridin) (Rizzato et al., 2017; Chouitah et al., 2011) and volatile oils (essential oils) (Chouitah et al., 2011), raw materials and root/rhizome extracts of licorice have great economic importance in global markets (Ozturk et al., 2017). The therapeutic effects of licorice are anti-inflammatory, anti-allergic, anti-cancer, anti-hypertensive, antispasmodic, laxative, and anti-depressant which have been reported in the available literature. Furthermore, the antiviral effects of licorice against a number of viruses (Hosseinzadeh and Nassiri Asl, 2007), especially in some cases against COVID-19 (Sinha et al., 2020) has been confirmed.

Due to the favorable and variable climatic conditions of Iran, Iranian licorice (*G. glabra* L. var. *violacea*) (Kokate et al., 2004) is one of the best licorice varieties in the world, with important economic values in the country. Most licorice roots and rhizomes are harvested from their natural habitats, mostly in an unsustainable manner. Due to the susceptibility of root crops that is usually actualized as a result of over-harvesting, as well as the reduction of wild populations, unsustainable harvests can be a threat to genetic extinction, especially in arid and semi-arid regions (Srivastava et al., 2013; Sharma et al., 2014). Thus, the provision of sustainable licorice raw materials, with excellent and uniform quality, can be a valuable endeavor for relevant industries. Such approaches, however, may have to take into account the domestication and introduction of this plant into agricultural lands. Important factors that tend to limit licorice cultivation include poor and slow seed germination along with growth season dependency of the seeds, as well as cuttings and rhizomes cultivation (Gupta et al., 1997). Also, in vegetative propagation, crop quality may be affected by bacterial, fungal, and systemic viruses (Sharma et al., 2010) which may waste most of the rhizomes for propagation. Furthermore, the tissue culture of licorice is expensive, so propagation by seeds and vegetative organs are still the most common methods of its cultivation.

Due to physical dormancy, which is mainly induced by a hard seed-coat, seeds should be treated before planting (Abudureheman et al., 2014). Seed priming is a strategy to increase seed germination percentage, speed and uniformity (Mousavi and Omidi, 2019) which can be used as a technical and cost-effective method to increase plant seed germination in adverse conditions (Ataei Somagh et al, 2016). Positive effects of temperature (Ghanbari et al., 2006), mechanical stresses (boiling water) (Rafieiolhossaini et al., 2014), chemical scarification (sulfuric acid) (Jin et al., 2004) and co-application of temperature and scarification (Ghadiri et al., 2000) have been reported and, as such, these effects have the ability to break the dormancy of licorice seeds. Nonetheless, it has to be stated that some treatments, such as chemical scarification with sulfuric acid in licorice seeds, can damage the seed testa (Jin et al., 2004).

Seeds bio-priming with plant growth-promoting bacteria (PGPB) can affect seed germination characteristics and seedling growth positively, negatively, and neutrally, depending on the variety of bacterial strains (Ghorbanpour and Hatami, 2014). PGPB affected plant growth, plant physiology and plant metabolism through production of plant hormones such as auxin and ethylene and their regulation, facilitating the absorption of nutrients such as phosphorus and iron through solubilization, siderophore production, biological stabilization of nitrogen and increasing plant resistance to biotic and abiotic stress (Aarab et al., 2015; Ayyaz et al., 2016; Islam et al., 2016; Kesaulya et al., 2015; Majeed et al., 2015; Scagliola et al., 2016). The positive effects of PGPB on seed germination as bio-priming was reported in the case of *Celosia cristata* L. (Dutta et al., 2019), *Glycyrrhiza inflata* Bat. (Zhao et al., 2018) and *Dracocephalum moldavica* L. (Khosravi et al., 2018). In this regard, the positive effects of rhizospheric and endophytic growth-promoting bacteria in the initial establishment of seedlings in greenhouse conditions were reportedly diverse (Deepa et al., 2010; Majeed et al., 2015; Das et al., 2016). For example, the application of *Pseudomonas aeruginosa* (MCC 3198) isolated from the rhizosphere of the medicinal plant *Celosia cristata* L. showed significant improvements in

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seedling growth criteria and some plant defense enzymes (Dutta et al., 2019). It was reported that the ability of these bacteria to produce IAA, a hormone with positive effects on root growth and development, led to an increase in plant nutrient uptake (Khalid et al., 2004) and, subsequently, improved plant growth. Although these microorganisms show a wide range of plant growth stimuli in vitro, in some cases, however, researchers have suggested that PGPB can negatively affect seed germination under stress and non-stress conditions through the production of plant hormones such as IAA and gibberellins. Also, there could be direct effects of these hormones on the activity of α-amylase (Brimecombe et al., 2007; McPhail et al., 2010; Lee et al., 2013: Tabatabaei et al., 2016). Preventing seed germination usually comes into contradiction with the growth promoting effects of these microorganisms. Therefore, it is necessary to conduct more experiments to understand the nature of these bacteria.

Regarding the importance of licorice domestication programs, while considering poor seed germination and slow patterns of initial establishment by this plant, the aim of the present study was to evaluate the behavior of seed germination criteria and seedling growth of licorice through an eco-friendly method by the application of its indigenous, superior, endophytic and rhizophytic bacteria that were isolated from its natural habitat in semi-arid regions.

Materials and Methods

Effect of bacterial seed treatment on germination quality criteria of licorice

In order to evaluate the selected rhizospheric and endophytic bacteria, which were previously isolated from the rhizospheric soil and rhizome of licorice plants (Table 1), their effects were measured on seed germination characteristics. Thus, an experiment was performed in a completely randomized design with three replications at the Soil Science Biology Laboratory, University of Tehran in 2019. The treatments included bacterial isolates, B1 (*Pantoea agglomerans* (S72)), B2 (*Serratia rubidaea* (S28)), B3 (*Pseudomonas azotoformans* (E101)), B4 (*Pseudomonas frederiksbergensis* (E56)), B5 (B1 + B2), B6 (B3 + B4), B7 (B1 + B2 + B3 + B4) and B8 (no bacteria).

Table 1. Characterization of selected bacterial strains isolated from the rhizosphere and rhizome of licorice (G. glabra L.)

Characteristics	Isolates			
	S72	**S28**	**E101**	**E56**
Morphological characteristics				
Colony color	Bright yellow	Red	Milky	Cream
Gram reaction	-	-	-	-
PGP traits				
Phosphate solubilization	+	+	+	+
IAA production	+	+	+	-
Siderophore production	+	-	+	+
Water stress resistance	+	+	+	+
Salinity stress resistance	-	+	+	-
Growth on nitrogen free medium	+	+	+	+
16 S rRNA identification	*Pantoea agglomerans*	*Serratia rubidaea*	*Pseudomonas azotoformans*	*Pseudomonas frederiksbergensis*
Identity (%)	98.23	97.19	99.64	99.36
Gene Bank Accession number	MN698728	MN698726	MN698729	MN636330
Geographic origin	30° 0′ 36.9″ N 52°1′ 28.04″ E	30°1′9.99″ N 51°58′59.16″E	30° 0′ 44.52″ N 51° 55 ′27.13″ E	30° 0′ 46.91 ″ N 52° 0′ 32.41″ E

To prepare the bacterial suspension, a pure bacterial colony was first cultured in a plate containing NA and, after the growth of bacteria, a clone of the bacterium was put in Erlenmeyer flasks containing 100 ml of NB culture medium which was shaken at 120 rpm for 72 h (26-30 °C). Pure bacterial suspensions had an adjusted population (5×108 CFU / ml) and were isolated from the culture medium by centrifugation at 15000 rpm for 1 min at 4 °C.

Surface sterilization of the seeds, which were collected from a wild population in Fars province, was performed according to a relevant protocol. First, the seeds were soaked in 96% ethyl alcohol for 10 seconds and, after washing by sterile distilled water, they were soaked for a second time in sodium hypochlorite (0.5 %) for 2 minutes. Then, the seeds were thoroughly washed with sterile distilled water and, finally, 25 seeds were placed in each sterile petri dish (8 cm diameter) containing 25 ml of water agar medium. Then, 5 µl of the bacterial suspension was dropped on each seed. The petri dishes were placed in an incubator under 28 °C and a photoperiod of 12 h, with light/darkness each spanning 12 h. The relative humidity averaged at 70%.

Germinated seeds were counted every 24 hours. Counting continued until the cumulative number of germinated seeds reached a stable state (up to 21 days). Seeds with visible radicles of about 2 mm were considered as germinated. Seedling length was also measured and the germination percentage (Gmax), germination rate (GR), germination uniformity, (GU) and time to 5, 10, 50, 90 and 95% of germination (D5-D95) were calculated using the Germin program.

Effect of bacterial treatment and growth media on the licorice seedling growth criteria

In order to evaluate the combined effects of rhizospheric and endophytic bacteria, which were isolated from licorice plants (Table 1), and to measure the effects of different organic, growth media on the growth of licorice seedlings, a factorial experiment was conducted based on a completely randomized design with three replications in the Research Greenhouse of Department of Horticultural Science, University of Tehran in 2019.

First, the dormancy of licorice seeds was broken by scarification with 75% sulfuric acid for 20 minutes, which was aimed at removing the hard seed coat (Ghadiri et al., 2000). Then, the scarified seeds were thoroughly washed several times with sterile distilled water and were planted in sterilized 70 cell seedling trays which were filled with sterilized perlite and sand (1: 1 ratio). To maintain proper humidity, the seeds were irrigated daily with sterile distilled water during the growth period.

Four weeks after seed sowing, the four-leaf stage seedlings (with similar sizes) were transferred to the growth media treatments according to the following: M1 (sand + perlite (1:1)), M2 (1000 g soil + 100 g cow manure), M3 (1000 g soil + 40 g mycorrhizal fungi (a combination of three species of *Rhizophagus irregularis*, *Glomus mosseae* and *Glomus etunicatum*)) and M4 (1000 g soil + 100 g cow manure + 40 g mycorrhizal fungi).

After transplanting, each pot was inoculated with 10 µl of bacterial suspension with an adjusted population (5×10^8 CFU /ml) at 8 levels as follows: B1 (no bacteria), B2 (S72), B3 (S28), B4 (E101), B5 (E56), B6 (B1+B2), B7 (B3+B4), B8 (B1+B2+B3+B4). After inoculation, the pots were weighed daily and irrigated with distilled water to the extent of 80% field capacity. Two months after transplanting, the seedlings were taken out of the pots and were washed with water. Then, morphological traits were measured, including seedling height, root depth, root diameter, total root length (with the Digimizer software), stem diameter, number of nodes, as well as fresh and dry weights of shoots and roots.

Statistical analysis

Statistical analyses were performed using SAS software version 9.4. The data were reported as means ± SD (standard deviation) for 3 replications. The results were subjected to analysis of variance (ANOVA) according to Fisher's protected LSD test ($p\leq0.05$) using the Stat Graphics Plus version 4.0.

Results

Germination quality criteria

Licorice seed germination was initially very low due to its hard seed coat. According to variance analysis, rhizospheric and endophytic bacteria significantly affected seed germination characteristics (Table 2). The results of control seeds showed that seed germination percentage, seed germination uniformity and time to 95% of germination were 28%, 16.6 and 17.98 days, respectively (Table 3).

Based on the results, the use of endophytic bacteria improved germination, compared to rhizospheric bacteria, so that the highest germination percentage (30.66%) was obtained by E56 bacteria and then E101 bacteria (29.33%). However, they did not show a significant difference with the control (28%) (Table 3). As shown in Table 3, the mixture of endophytic

bacteria (E101 + E56) had the highest germination rate (0.32 per day) and did not show a significant difference with the control (0.27 per day) and E101 bacteria (0.27 per day). In general, the results showed that endophytic bacteria were more effective than rhizospheric bacteria in improving the germination rate and germination percentage.

Table 2. Analysis of variance (ANOVA) regarding the application of indigenous rhizospheric and endophytic bacteria on seed germination characteristics of licorice (G. *glabra* L.)

Sources of variance (S.O.V)	df	Mean square								
		Gmax	GR	GU	D05	D10	D50	D90	D95	SL
Isolates	7	62.47*	0.013*	25.87*	0.51*	0.93*	9.27*	22.6*	22.74*	0.94*
Error	14	5.5	0.001	0.43	0.009	0.019	0.6	1.089	1.004	0.15
CV (%)		9.5	16.72	5.04	13.02	6.51	13.19	7.49	7.01	6.64

G max: Germination percentage GR: Germination rate GU: Germination uniformity D05: Day to 5% germination D10: Day to 10% germination D50: Day to 50% germination D90: Day to 90% germination D95: Day to 95% germination SL: Seedling length
*significantly different at 5% level

Table 3. The effect of indigenous rhizospheric and endophytic licorice (G. *glabra* L.) PGP on its seed germination

Bacteria	GMAX(%)	GR (day^{-1})	GU	D05	D10	D50	D90	D95	SL(cm)
S72	24±2.82	0.17±0.006	13.2±0.49	0.63±0.06	0.93±0.04	7.46±0.35	14.13±0.69	14.4±0.36	4.77±0.36
S28	25.33±0.94	0.12±0.022	10.4±0.58	1.65±0.07	2.3±0.14	9.42±0.42	12.7±1.34	13.35±1.22	5.65±0.26
E101	29.33±1.88	0.27±0.036	12.65±0.69	0.64±0.031	0.95±0.07	4±0.40	13.6±0.42	13.97±0.44	6.47±0.16
E56	30.66±2.35	0.17±0.015	16.47±0.28	0.21±0.028	0.42±0.031	5.92±0.531	16.9±0.4	17.28±0.42	5.9±0.46
S72+S28	22.66±1.88	0.19±0.012	8.65±0.81	0.56±0.11	0.78±0.13	5.33±0.84	9.43±0.74	9.72±0.77	6.18±0.54
E101+E56	18.66±1.88	0.33±0.022	10.5±0.37	0.85±0.14	1.03±0.14	4.75±0.93	11.53±0.12	11.77±0.062	6.04±0.12
MIX	18.66±1.24	0.20±0.06	14.73±0.24	0.57±0.04	0.8±0.14	5.33±0.23	15.53±1.39	15.77±1.4	6.47±0.15
CON	28±1.63	0.27±0.02	16.6±0.48	0.68±0.047	1.03±0.12	4.43±0.89	17.63±0.8	17.98±0.87	6.053±0.18
LSD(P<0.05)	4.35	0.65	1.21	0.16	0.25	1.37	1.85	1.80	0.69

S72 (MN698728 *Pantoea agglomerans*), S28 (MN698726 *Serratia rubidaea*), E101 (MN698729 *Pseudomonas azotoformans*), E56 (MN636330 *Pseudomonas frederiksbergensis*), Control (Non-inoculated seeds)
G max: Germination percentage R50:Germination rate GU: Germination uniformity D05: Day to 5% germination D10: Day to 10% germination D50: Day to 50% germination D90: Day to 90% germination D95: Day to 95% germination SL: Seedling length

According to Table 3, the control seeds (non-inoculation with bacteria) had the highest value of germination uniformity. Given that a higher value of germination uniformity indicates synchronization in the process of seed germination, the mixture of rhizospheric bacteria (S72 + S28) had the most positive effect on increasing the synchronization and uniformity of the germination process of licorice seeds, which showed a significant difference with the control. In general, the use of most bacterial treatments, compared to the control, had an effective contribution to the improvement of germination uniformity.

The results revealed that the time to 5, 10 and 50% of germination in rhizospheric bacteria (S28) was higher than in other treatments. This significant difference showed that the longer seed germination time was affected by this treatment (Table 3). However, the control seeds took more time to reach 90 and 95% of germination. The control treatment also differed significantly from other bacterial treatments, except in the case of E56 bacteria. It should be noted that in this regard, a mixture of rhizospheric bacteria (S72 + S28) was associated with the shortest time to reach 90 and 95% germination, which indicates the beneficial role of these bacteria in reducing

the time to 90 and 95% of germination. It showed a significant difference with other treatments (Table 3).

Based on the results, the maximum seedling length was obtained by using E101 bacteria and a mixture of all bacteria, followed by a mixture of rhizospheric bacteria, although there was no significant difference with the control. For a better comparison of seed treatments, the germination process of licorice seeds is shown in the conditions of non-inoculation with bacteria (control) and inoculation with rhizospheric and endophytic bacteria of licorice plants (Fig. 1).

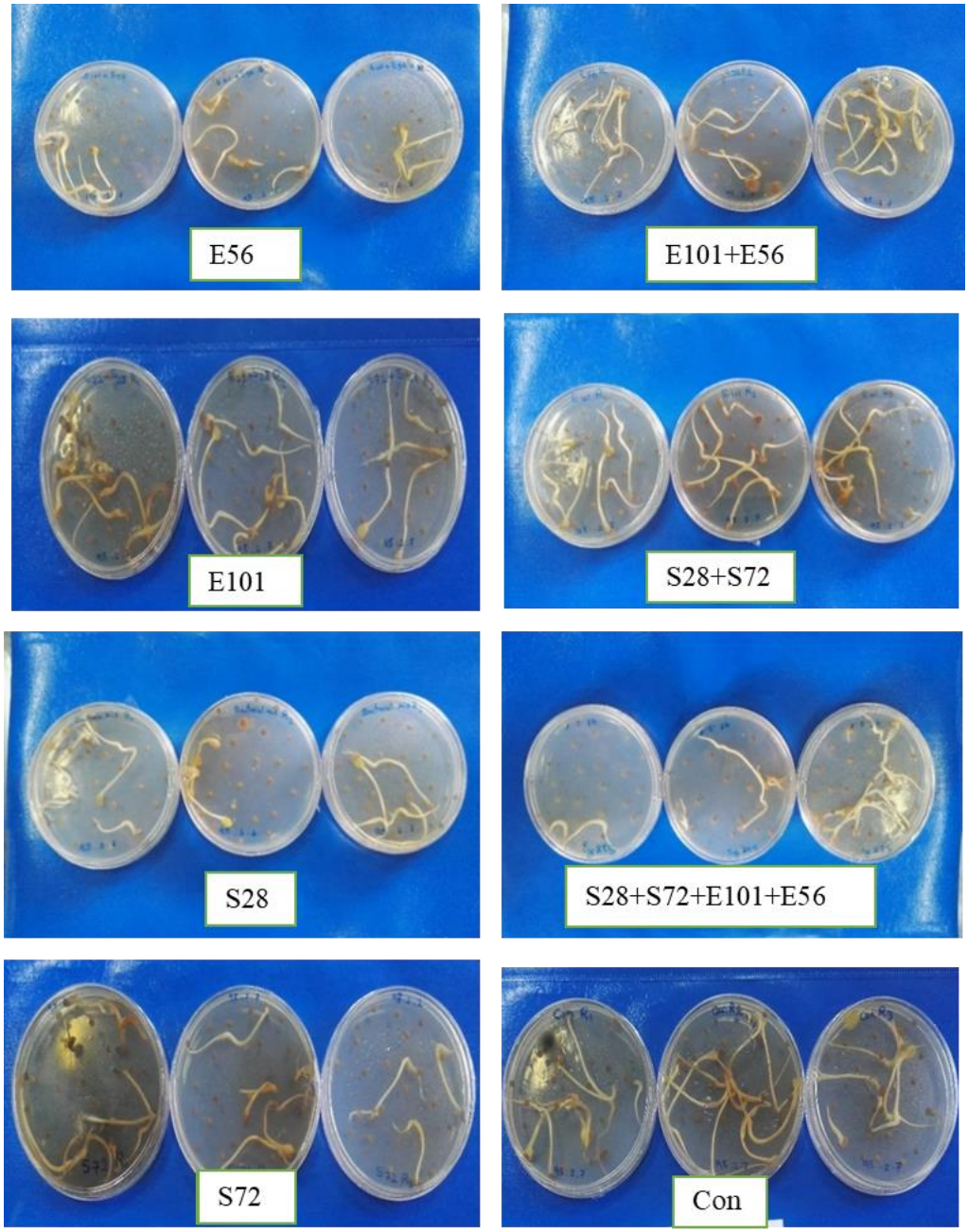

Fig. 1. The effect of indigenous rhizospheric and endophytic licorice (G. *glabra* L.) PGP on its seed germination

Seedling growth criteria

The results indicated that the simple and interaction effects of bacterial treatments and growth media were significant on seedling height, stem diameter, number of nodes, root diameter, root depth, total root length, shoot and root fresh and dry weights (Table 4).

Seedling height

The results showed that the highest seedling

height (33.25 cm) was obtained by applying M2B8 (soil + cow manure and inoculation by a mixture of bacteria) with an increase of 44.46% and 13.67%, compared to M1B1 and M2B1. Nonetheless, there was no significant differences between most other treatments and M2B8 (Fig. 2).

Based on the results, it can be concluded that using M2 (cow manure + soil) and M4 (cow manure + soil + mycorrhiza fungi), in combination with bacteria, played an effective role in increasing plant height, compared to the control and also M3 and M1.

Table 4. Analysis of variance (ANOVA) regarding the different levels of bacteria and growth media on the morphological characteristics of licorice (G. *glabra* L.) seedlings

Sources of variance (S.O.V)	df	Mean square									
		SH	SD	NN	RDI	RDE	TRL	HFW	RFW	HDW	RDW
Growing media	3	393.34*	0.18*	31.18*	8.85*	212.73*	322336.46*	2.013*	0.048*	0.24*	0.079*
Isolate	7	40.22*	0.036*	17.9*	1.56*	49.31*	16932.09*	0.0224*	0.33*	0.015*	0.027*
Growing media* Isolate	21	50.79*	0.038*	8.69*	0.81*	32.43*	18428.16*	0.515*	0.12*	0.044*	0.021*
Error	64	10.77	0.0097	1.81	0.15	1.98	2421.87	0.017	0.008	0.0039	0.0026
CV(%)		13.76	9.74	8.65	11.02	7.03	28.81	13.35	12.36	20.96	24.41

SH: Seedling height, SD: Stem diameter, NN: Number of nodes, RDI: Root diameter, RDE: Root depth, TRL: Total root length, HFW: Shoot fresh weight, RFW: Root fresh weight, HDW: Shoot dry weight, RDW: Root dry weight.
*significantly different at 5% level

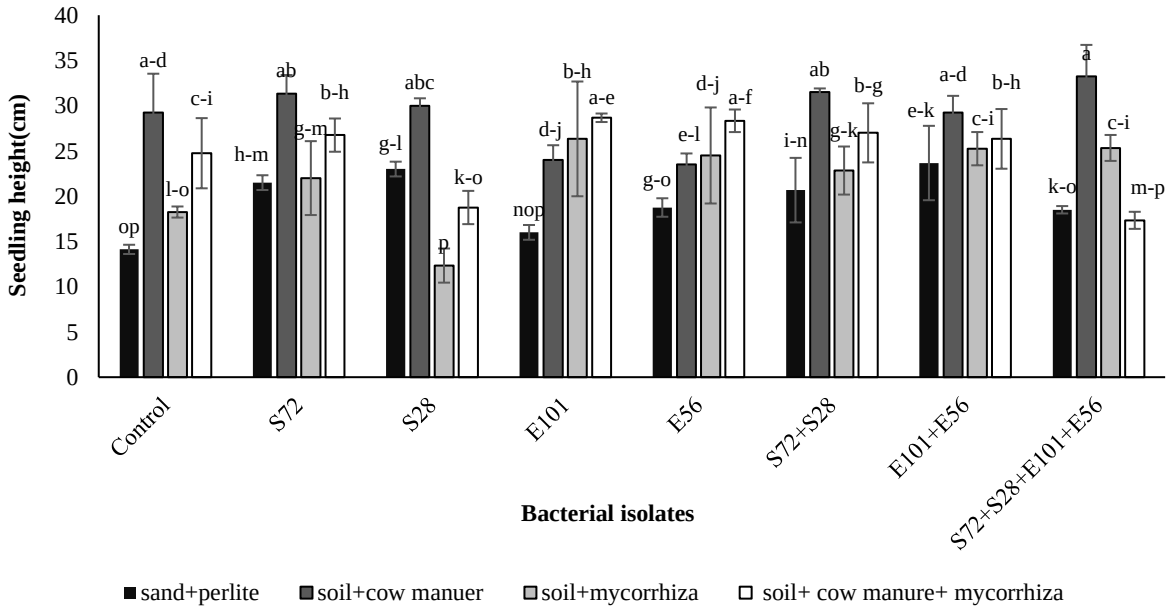

Figure 2. Interaction effect of different levels of bacteria and growth media on height of licorice seedlings

Stem diameter

As can be seen in Table 5, the mixture of cow manure, soil and mycorrhizal fungi (M4), that were inoculated by E56, resulted in the highest stem diameter (1.31 mm) with an increase of up to 36.64%, compared to the control (M1B1). However, this treatment had no significant difference with the effects of M4B4, M2B3, M3B8, M2B5, M2B8.

Number of nodes

Among the different culture media, M1B2 and M2B8 resulted in the highest number of nodes (20 nodes) which showed a 35% increase in the number of nodes, compared to the control. Nonetheless, these two treatments did not differ

significantly with the effects of M4B2. The lowest number of nodes was observed in response to M3B3 (soil + mycorrhiza + S28) (Table 5).

Root diameter

According to Table 5, the highest value of root diameter was observed in response to M4B5, M2B5 and M4B4 treatments. These treatments increased the percentage of root diameter up to 61.42, 59.06 and 56.20%, respectively, compared to the control. The application of sand + perlite without bacterial inoculation (M1B1) resulted in the lowest root diameter (2 mm).

Root depth

The deepest roots were observed in response to M4B4 (inoculation of mixture of cow manure + soil + mycorrhizal with E101), M1B5 (inoculation of sand + perlite with E56), M1B4 (inoculation of sand + perlite with E101) and M1B7 (inoculation of sand + perlite with E101 + E56), which resulted in an increase of 24.77, 24.43, 1.24 and 21.99%, compared to the control, respectively (Table 5).

As mentioned above, the maximum depth of the roots was observed in seedlings that were inoculated with endophytic bacteria, either alone or in combination with both M4 and M1 media. It could be proposed that, due to a lack of soil and nutrients in the M1 medium, the roots grew more extensively and were able to absorb more nutrients from the soil. Similar to root diameter, in the case of this factor, the effect of endophytic bacteria was obviously efficient in comparison with the effect of rhizospheric bacteria.

Total root length

The results showed that the highest total root length, measured by the Digimizer software, was 597.14 cm in response to the growth media of sand + perlite, inoculated with S28 (M1B3), which increased significantly up to 85.41%, compared to the control. The lowest total root length was obtained by the application of soil + mycorrhiza + cow manure, without bacterial inoculation (M4B1) (Fig. 3 and 6).

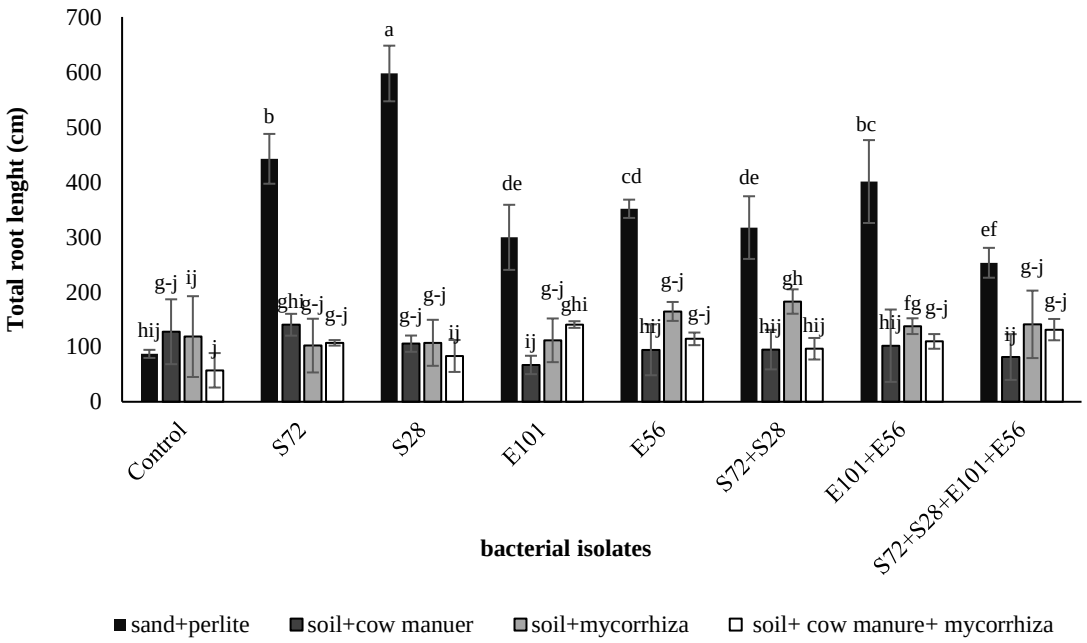

Figure 3. Interaction effect of different levels of bacteria and growth media on total root length of licorice seedlings

Shoot fresh weight

The application of M4 medium, inoculated with S72 bacteria and a mixture of bacteria (B8), resulted in the highest shoot fresh weight (with an increase of 58.2 and 57.75 %, compared to the control, respectively). Meanwhile, they did not have significant differences with the effect of M4B4. Also, the lowest shoot fresh weight was observed in response to the growth media of soil + mycorrhiza which was inoculated by S27 (M3B2) and/or S28 (M3B3) (Table 5).

Table 5. Comparison of morphological characteristics of licorice (G. *glabra* L.) seedlings at different levels of bacterial presence and growth media

Growing media	Bacteria	SD (cm)	NN	RDE (cm)	RDI (cm)	RFW (gr)	HFW (gr)
M1	**B1**	0.83±0.077	13±2.4	21.25±0.2	2.006±0.033	0.36±0.02	0.79±0.04
	B2	0.96±0.29	20±0.8	24±1.63	3.20±0.038	0.91±0.008	1.01±0.075
	B3	0.91±0.03	17±0.8	22±0.82	2.85±0.37	0.72±0.69	0.74±0.07
	B4	0.82±0.054	15.66±1.2	28±1.41	2.52±0.4	0.65±0.03	0.5±0.029
	B5	0.81±0.07	14.33±0.5	28.12±0.62	2.40±0.07	0.75±0.03	0.6±0.03
	B6	0.88±0.065	17.33±05	24.5±2.04	2.87±0.1	0.47±0.032	0.94±0.06
	B7	1.04±0.043	17.33±0.9	27±0.81	2.88±0.16	1.06±0.080	0.98±0.06
	B8	0.83±0.1	16±00	20.5±0.4	2.48±0.18	0.49±0.28	0.75±0.32
M2	**B1**	0.9±0.069	15±2.4	14.5±0.4	3.67±0.18	0.67±0.036	1.55±0.24
	B2	1.15±0.11	16±0.8	17.5±0.4	3.73±0.3	0.52±0.04	1.89±0.03
	B3	1.21±0.02	15±0.81	15.66±0.94	4.09±0.22	0.79±0.01	1.53±0.02
	B4	0.96±0.03	14±0.81	19.75±1.83	3.45±0.26	0.58±0.04	0.54±0.05
	B5	1.17±0.05	14.66±0.47	22.33±1.69	4.9±0.24	0.86±0.15	0.77±0.048
	B6	0.98±0.01	15.33±0.94	18.5±0.4	4.03±1.07	0.89±0.14	1.53±0.11
	B7	1.11±0.12	17.66±0.94	22.33±2.05	3.7±0.24	1.015±0.085	1.44±0.004
	B8	1.15±0.14	20±0.81	21±1.63	3.81±0.12	0.91±0.11	1.87±0.17
M3	**B1**	1.05±0.06	11.66±0.47	21±0.81	3.6±0.36	0.51±0.081	0.5±0.11
	B2	0.96±0.11	13.66±0.47	18.5±0.4	3±0.63	0.8±0.036	0.4±0.1
	B3	0.95±0.08	11.33±1.24	16±0.81	3.03±0.34	0.34±0.04	0.33±0.11
	B4	0.96±0.05	13.66±0.047	19.66±0.47	3.45±0.11	0.66±0.087	0.58±0.048
	B5	1.006±0.11	14±1.63	14.66±1.24	4.03±0.06	0.67±0.77	1.01±0.4
	B6	0.99±0.07	14.66±0.47	19±0.81	4.44±0.26	0.76±0.93	0.93±0.89
	B7	1.11±0.11	16.5±0.4	18±0.81	4.36±0.31	1.13±0.1	1.1±0.15
	B8	1.19±0.08	15.33±1.24	15.66±0.23	3.81±0.11	0.7±0.08	1.23±0.12
M4	**B1**	0.87±0.01	14±0.81	16.33±1.24	2.42±0.08	0.24±0.004	0.51±0.02
	B2	0.97±0.08	19±1.63	23±0.81	3.45±0.24	0.56±0.36	1.035±0.23
	B3	0.92±0.03	16±1.63	16.33±1.69	3.75±0.11	0.57±0.028	0.66±0.12
	B4	1.22±0.008	16.33±0.47	28.25±102	4.58±0.47	1.09±0.04	1.71±0.21
	B5	1.31±0.098	17.66±0.94	16±0.81	5.2±0.23	1.22±0.12	1.57±0.09
	B6	1.07±0.099	16±2.16	22.33±1.24	3.65±0.32	0.71±0.12	0.95±0.14
	B7	1.09±0.09	16±00	12.5±1.22	4.39±0.037	0.79±0.043	1.04±0.077
	B8	0.94±0.81	13.33±0.47	16.66±1.24	3.85±0.14	0.55±0.009	0.54±0.021
LSD(0.05%)		0.15	2.22	3.01	0.63	0.145	0.19

SD: Stem diameter, NN: Number of nodes, RDI: Root diameter, RDE: Root depth, HFW: Shoot fresh weight, RFW: Root fresh weight.
M1: Sand: perlite (1:1), M2:1000gr soil + 100gr cow manure, M3: 1000gr soil + 40gr mycorrhiza and M4: 1000gr soil + 100gr cow manure + 40gr mycorrhiza
B1 (no bacteria), B2 (S72), B3 (S28), B4 (E101), B5 (E56), B6 (B1+B2), B7 (B3+B4), B8 (B1+B2+B3+B4)

Root fresh weight

The highest amount of root fresh weight was observed in the growth media of soil + mycorrhiza fungi + cow manure inoculated with E56 bacteria (1.22 gr) with an increase of 70.49%, compared to the control. Nonetheless, there was no significant difference between the effects of M4B5, M3B7 and M4B4 treatments (Table 5).

The lowest root fresh weight was measured in M4B1, M3B3 and M1B1 treatments. Thus, a positive effect of bacterial use on root weight was quite evident, compared to the conditions of non-inoculation (M4B1 and M1B1 treatments). Root weight loss in response to the M3B3 may be due to antagonistic effects between mycorrhizal fungi and *Serratia* bacteria (Table 5).

Shoot dry weight

According to Fig. 4, the highest shoot dry weight was observed in response to the M2B2 (soil + cow manure inoculated with S27) and M4B4 (inoculation of a mixture of cow manure + soil + mycorrhizal fungi with E101) (0.57 and 0.56 gr) which resulted in an increase of 43.85 and 42.85%, compared to the control, respectively. However, these treatments were not significantly different from the effects of M2B7, M4B5, and M2B3. In addition, the inoculation of soil + mycorrhiza with S28 bacteria led to the lowest shoot dry weight.

Root dry weight

The results revealed that the application of soil + mycorrhiza fungi + cow manure (M4) with E56 bacteria increased the root dry weight up to 51.21%, compared to the control, while using soil + mycorrhizal fungi + cow manure as the medium, without bacterial inoculation, which caused the lowest root dry weight (Fig. 5).

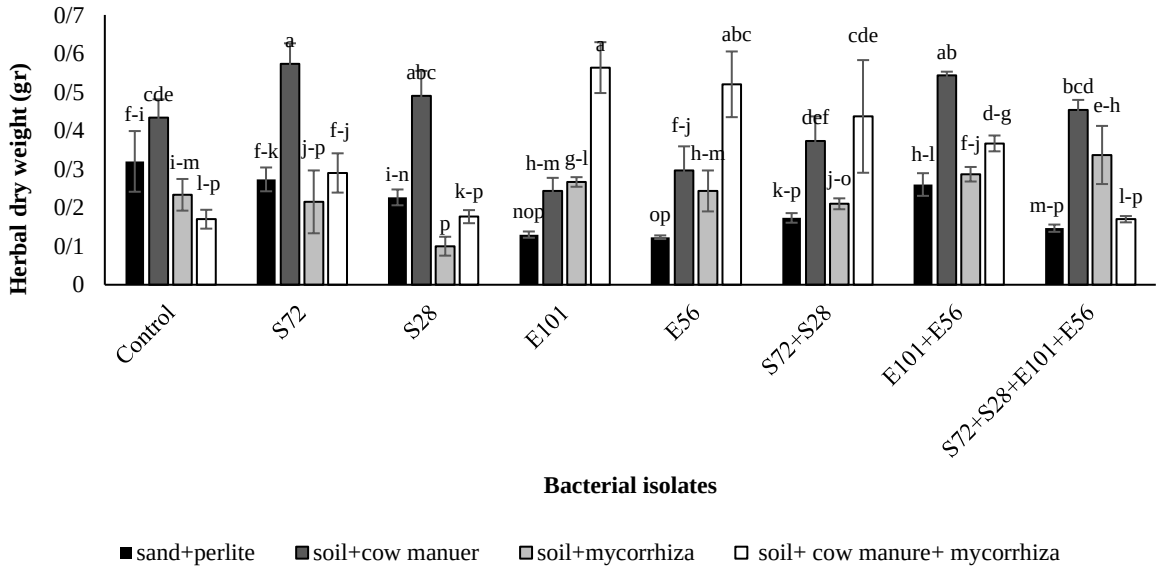

Figure 4. Interaction effect of different levels of bacteria and growth media on shoot dry weight of licorice seedlings

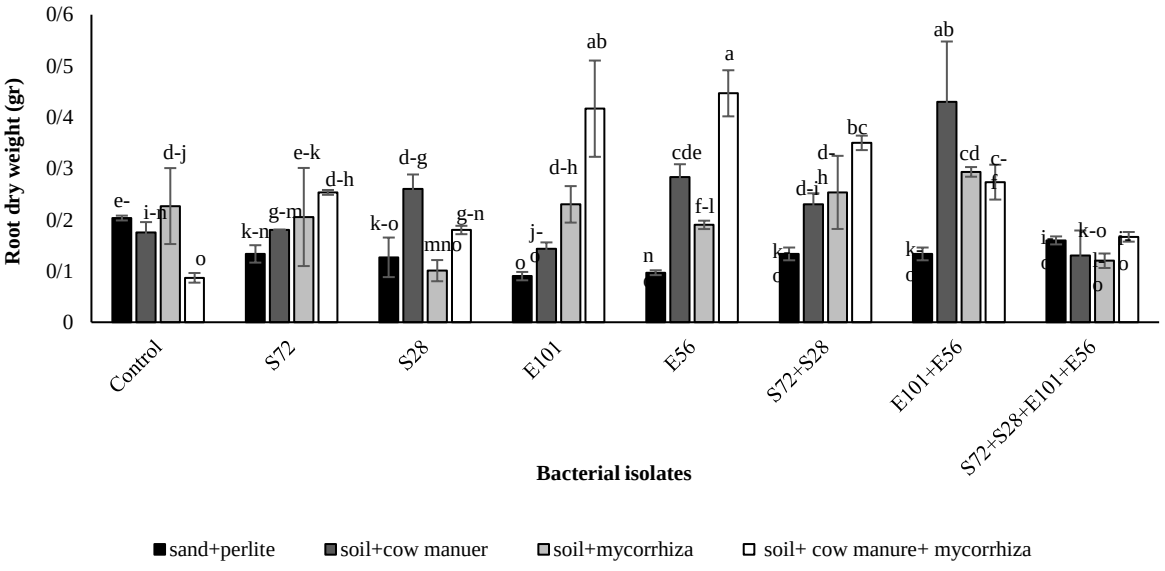

Figure 5. Interaction effect of different levels of bacteria and growth media on root dry weight of licorice seedlings

Figure 6. The highest and the lowest total root length in licorice (G. *glabra* L.) seedlings that were affected by indigenous licorice rhizospheric and endophytic PGP and by growth media (analyzed by the Digimizer software)

Discussion

Soil organic matter deficit, excessive use of chemical fertilizers and increased successive tillage are factors that reduce the amount of soil organic matter and subsequently cause a decrease in soil quality (Dinesh et al., 2010). Improving soil fertility is one of the common strategies in increasing agricultural production. Also, proper fertilizer management has an important effect on improving crop yield and quality. The use of organic fertilizers and biofertilizers effectively contributes to the development of microbial carbon biomass, nitrogen mineralization, soil respiration and enzymatic activity. In this regard, the results of our study showed that the co-application of biological and organic fertilizers in the soil had a positive effect on two-month-old seedlings of licorice. According to the results, it can be stated that the best treatment for the initial growth and establishment of licorice seedling is M4B4. Then, the M4B5 treatment was evaluated as the second best treatment. In other words, the M4 medium (soil + cow manure + mycorrhizal fungi) that was inoculated with two endophytic bacteria, belonging to the genus Pseudomonas (E101 and E56), showed the best performance in the evaluated traits. Soil microorganisms and their activity play an important role in nutrient availability. They also help to improve soil fertility via proper regulations of metabolic activity (Leaungvutiviroj et al., 2010). In this regard, the use of PGPR is a reliable strategy for uniform production, lower risk and lower dependency on chemical fertilizers.

E101 (*Pseudomonas azotoformans*) and E56 (*P. frederiksbergensis*) are endophytic bacteria that were isolated from licorice with milky and cream color colonies in the growth medium, respectively. Isolate E101 showed a significant potential in inorganic phosphate solubilization, IAA production, N fixation, siderophore production and salinity stress. Also, E56 led to inorganic phosphate solubilization, N fixation, siderophore production, and drought resistance (Table 1).

In plant-microorganism symbiosis, the production of plant hormones and bacterial metabolites is the most important factor in stimulating plant growth. Improving the biomass and yield of plants that were inoculated with PGPR is related to their capacity to produce hormones, especially auxins and gibberellins, which increase hairy roots and growth of aerial parts (Riefler et al., 2006). Auxin is an important plant hormone with multiple physiological roles (Tsavkelova et al., 2006; Spaepen and Vanderleyden, 2011). In response to the presence of tryptophan and other small molecules in plant root exudates, some bacteria that coexist with the root have the ability to synthesize and secrete phytohormone indole-3-acetic acid (IAA) (Glick, 2014). These IAAs, together with the endogenous IAA, which are synthesized by plants, affect plant function in various ways (Glick, 2014). These

ways include proliferation of plant cells, stimulation of cell elongation (Spaepen and Vanderleyden, 2011), plant enzyme catalysis, ACC synthase and ACC formation. In this case, IAA plays a role in stimulating ethylene synthesis (Glick, 2014). Other aspects include the processes of causing cell wall loosening (Glick, 2014) and increasing root secretion, which tend to provide excess nutrients for the growth of rhizospheric bacteria (Elvira-Recuenco & VanVuurde, 2000; Glick, 2014). Since IAA, produced by bacteria, stimulates the root system (Salamone et al., 2005) through over-production of hairy roots, it causes the expansion of lateral roots and the release of saccharides from the cell wall during the root elongation process. As a result of hairy root expansion, a greater access to nutrients occurs for the plant (Widawati and Suliasih, 2018). In this regard, it is reported that inoculation of *Achyranthes aspectra* L. with *Serratia marcescens* AL2-16 caused increases in growth factors such as leaf area, root length, seedling length and aerial parts (dry weight) in this plant (Devi et al., 2016). In another study, the largest aerial biomass was observed in maize plants treated with *Pseudomonas putida* and *S. marcescen* and, in the case of underground parts, the largest biomass was observed in plants inoculated with *S. marcescens* and *Bacillus panthetonicu* (Amogou et al., 2018). Also, Bacillus and Serratia strains resulted in a significant increase in the length of seedlings and corn roots (Koo and Cho, 2009). The properties of two endophytic bacteria (E101 and E56) from this study are in agreement with these previous cases of research.

In this study, although two *Pseudomonas* endophytic bacteria had a significant positive effect on most seedling growth factors in the greenhouse, the results of seed germination in vitro showed that in most of the traits, bacterial treatments performed better than the control group, but in some cases, there was no significant difference when compared with the control. In all bacterial treatments, endophytic bacteria were considered as effective agents in improving the quality of licorice seed germination, compared to rhizospheric bacteria. The ability of PGPB to produce or modify plant hormones, e.g. gibberellins, IAA and ethylene, led to evaluations of their behavior regarding seed germination.

According to the results of previous in vitro experiments, growth-promoting bacteria led to increases in germination and seed growth indexes in plants (Hameeda et al., 2006; Niranjan et al., 2007). Seed bio-priming of medicinal plants such as *Codonopsis pilosula* (Franch.) Nannf. with *Bacillus subtilis* bacterial (Zhao et al., 2016), *Dracocephalum kotschyi Boiss.*, with *Bacillus polymixa* (Bidabadi and Mehralian, 2020) and *Cuminum cyminum* L., with *Pseudomonas* sp. and *Bacillus* sp. (Moradi and Piri, 2018) all positively affected seed germination criteria and seedling growth. During the germination process, respiratory metabolism increases to produce energy and biosynthetic processes (Perata et al., 1997). Enzymes and hormones play an important role in relieving seed dormancy. Hydrolytic enzymes such as α-amylase play an important role in converting stored carbohydrates into soluble sugars (Perata et al., 1997). Therefore, α-amylase activity is required to maintain seed metabolism and seed germination. Thus, the presence of the amylase enzyme in the allerun layer is critical for seed germination, which is regulated by hormones produced during the embryonic stage (Uniyal et al., 2017). The positive effect of PGPR on seed germination was relevant to the ability of bacteria to produce or modify plant hormones, e.g. gibberellins, by reducing ethylene in stressful and non-stressful conditions through the production of ACC diaminase (Safari et al., 2018). This latter enzyme plays a key role in seed germination (Noumavo et al., 2013).

However, Kennedy et al. (2001) and Ghorbanpour and Hatami (2014) reported that some species and strains of bacteria may completely prevent seed germination or reduce seed germination ability. For example, P. aeruginosa bacteria, which were isolated from corn and barley, reportedly inhibited seed germination but, in some cases, had a promoting effect on growth (Tiwari and Singh, 2017). The effect of growth-promoting bacteria on seed germination inhibition may be due to the production of IAA and unknown metabolites caused by bacteria or by stress factors induced by the application of bacteria (Tabatabaei et al., 2016). Therefore, IAA appears to play a dual role based on its concentration (Chauhan et al., 2009). In this regard, a significant correlation was observed between the concentration of auxin produced in bacteria and the degree of inhibition of seed germination in wheat plants when used in association with different bacterial strains (Tabatabaei et al., 2016).

Despite the positive results obtained by the application of growth-promoting bacteria on seed germination in previous cases of research, the results of this study were not so effective on germination, although the positive roles of these bacteria were confirmed in the initial phase of plant establishment herein. It should be noted that the colonization of plant roots by endophytes was evaluated and confirmed. Also, it has to be stated that the possibility of antagonistic effects between rhizospheric bacteria and mycorrhizal

fungi would require further assessments for a better interpretation of the results. This is assumed in light of the fact that, in most traits, the lowest values were observed in M3B3 (soil + mycorrhizal fungi + S28).

Conclusion

Despite the low seed germination percentage in this study, we attempted to provide a new strategy for the cultivation of this plant by eco-friendly methods. In this study, the effect of rhizospheric and endophytic bacteria, isolated from licorice, were tested on seed germination characteristics and initial establishment of the seedlings. Overall, the results of the present study showed that two *Pseudomonas* endophytic bacteria performed better than rhizospheric bacteria in improving seed germination rate, germination percentage and early seedling growth. Also, the inoculation of combined planting substrates (M4) with endophytic bacteria improved most of the growth factors of seedlings, compared to the control. Therefore, it seems that endophytic isolates can be considered as a potential option for incentives to increase the growth of licorice seedlings and to improve the yield of this plant.

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Conflict of interest

No potential conflict of interest was reported by the authors.

References

Aarab S, Ollero FJ, Megías M, Laglaoui, A. 2015. Isolation and screening of bacteria from rhizospheric soils of rice fields in Northwestern Morocco for different plant growth promotion (PGP) activities : An in vitro study. International Journal of Current Microbiology and Applied Science 4, 260–269.

Abudureheman B, Liu H, Zhang D, Guan K. 2014. Identification of physical dormancy and dormancy release patterns in several species (Fabaceae) of the cold desert, north-west China. Seed Science Research 24, 133-145.

Amogou O, Dagbénonbakin G, Agbodjato NA, Noumavo PA, Salami HA, Valère S, Baba-Moussa L. 2018. Influence of isolated PGPR rhizobacteria in Central and Northern Benin on Maize germination and greenhouse growth. American Journal of Plant Sciences 9, 2775–2793. https://doi.org/10.4236/ajps.2018.913201

Ataei Somagh H, Mousavi SM, Omidi H, Mohammadian E, Hemmati M. 2016. Canola seed germination and seedling growth in response to the saline condition and bio-priming. Iranian Journal of Plant Physiology 7, 2149- 2156.

Ayyaz K, Zaheer A, Rasul G, Mirza M S, 2016. Isolation and identification by 16S rRNA sequence analysis of plant growth-promoting *azospirilla* from the rhizosphere of wheat. Brazilian Journal of Microbiology 47, 542–550. https://doi.org/10.1016/j.bjm.2015.11.035

Bidabadi SS, Mehralian M. 2020. Seed bio-priming to improve germination, seedling growth and essential oil yield of *Dracocephalum kotschyi* boiss, an endangered medicinal plant in Iran. Gesunde Pflanzen 72, 17–27. https://doi.org/10.1007/s10343-019-00478-2

Brimecombe MJ, De Leij FAAM, Lynch JM. 2007. Rhizodeposition and microbial populations. In: Pinton R, Varanini Z, Nannipieri P, The rhizosphere biochemistry and organic substances at the soil- plant interface, 2nd ed, CRC Press, New York, pp. 74-98.

Chauhan JS, Tomar YK, Indrakumar Singh N, Seema A, Debarati A. 2009. Effect of growth hormones on seed germination and seedling growth of black gram and horse gram. Journal of American Science 5, 79-84.

Chouitah O, Meddah B, Aoues A, Sonnet P. 2011. Chemical composition and antimicrobial activities of the essential oil from *Glycyrrhiza glabra* leaves. Journal of Essential Oil-Bearing Plants 14, 284–288.

Das S, Nurunnabi TR, Parveen R, Mou AN. 2019. Isolation and characterization of Indole Acetic Acid producing bacteria from rhizosphere soil and their effect on seed germination. International Journal of Current Microbiology and Applied Sciences 8, 1237–1245.

Deepa CK, Dastager SG, Pandey A. 2010. Isolation and characterization of plant growth promoting bacteria from non-rhizospheric soil and their effect on cowpea (*Vigna unguiculata* (L.) Walp.) seedling growth. World Journal of Microbiology and Biotechnology 26, 1233–1240. https://doi.org/10.1007/s11274-009-0293-

Devi KA, Pandey P, Sharma GD. 2016. Plant Growth-Promoting Endophyte Serratia marcescens AL2-16. enhances the growth of *Achyranthes aspera* L., a medicinal plant. Hayati Journal of Biosciences 23, 173-180. https://doi.org/10.1016/j.hjb.2016.12.006

Dinesh R, Srinivasan V, Hamza S, Manjusha A. 2010. Short-term incorporation of organic manures and biofertilizers influences biochemical and microbial characteristics of soils under an annual crop [*Turmeric (Curcuma longa* L.)]. Bioresource Technology 101, 4697–4702. https://doi.org/10.1016/j.biortech.2010.01.108

Dutta S, Sarkar A, Dutta S. 2019. Characterization of *Pseudomonas aeruginosa* MCC 3198 and its potential for growth promotion of seedlings of the medicinal plant *Celosia cristata* L. International Journal of Current Microbiology and Applied Sciences 8, 985–997. https://doi.org/10.20546/ijcmas.2019.804.114

Elvira-Recuenco M, VanVuurde JWL. 2000. Natural incidence of endophytic bacteria in pea cultivars under

field conditions. Canadian Journal of Microbiology 46, 1036–1041.

Fuggersberger-Heinz R, Franz G. 1984. Formation of glycyrrhizinic acid in *Glycyrrhiza glabra* var. typica. Planta Medica 50, 409-413.

Ghadiri H, Torshiz B. 2000. Effects of scarification and temperature on germination of licorice (*Glycyrrhiza glabra* L.) seeds. Journal of Agricultural Science and Technology 2, 257-262.

Ghanbari A, Rahimian Mashhadi H, Nasiri Mahalati M, Kafi M, Rastgou M. 2006 Ecophysiological aspects of liquorice (*Glycyrrhiza glabra* L.) germination under different temperatures. Iranian Journal of Field Crops Research 3, 263 - 275.

Ghorbanpour M, Hatami M. 2014. Biopriming of Salvia officinalis seed with growth promoting rhizobacteria affects invigoration and germination indices. Journal of Biological and Environmental Science 8, 29–36.

Glick BR. 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiological Research 169, 30–39. https://doi.org/10.1016/j.micres.2013.09.009

Gupta V, Kak A, Singh BB 1997. Studies of germination and seedling vigor in licorice (*Glycirrhiza glabra*). Journal of Medicinal and Aromatic Plant Sciences 2, 412–413.

Hameeda B, Rupela OP, Gopal Reddy, Satyavani K. 2006. Application of plant growth promoting bacteria associated with composts and macrofauna for growth promotion of Pear millet (Pennisetum glaucum L.). Biology and Fertility of Soils 43, 221– 227.

Hosseinzadeh H, Nassiri Asl N. 2007. Review of antiviral effects of Glycyrrhiza glabra L. and its active component, glycyrrhizin. JournalJournal of Medicinal Plants 6, 1-12.

Islam S, Akanda AM, Prova A, Islam MT, Hossain MM. 2016. Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. Frontiers in Microbiology 6, 1–12. https://doi.org/10.3389/fmicb.2015.01360

Jin Q, Duan L, Li J, Dong X, Tian X, Wang B, Li Z. 2006. Scarification damages by sulphuric acid and their effects on vigor, germination and emergence of *Glycyrrhiza uralensis* Fisch seeds. Seed Science and Technology 34, 227-231.

Kennedy AC, Johnson BN, Stubbs TL. 2001. Host range of a deleterious rhizobacterium for biological control of downy brome. Weed Science Society of America 4, 792-797.

Kesaulya H, Baharuddin, Zakaria, B, Syaiful, SA. 2015. Isolation and physiological characterization of PGPR from potato plant rhizosphere in medium land of Buru Island. Procedia Food Science 3, 190–199. https://doi.org/10.1016/j.profoo.2015.01.021

Khalid A, Arshad M, Zahir ZA. 2004. Screening plant growth promoting rhizobacteria for improving growth and yield of Wheat. Journal of Applied Microbiology 96, 473-480. https://doi.org/10.1046/j.1365-2672.2003.02161.x

Khosravi M, Shahraki D, Ghobadinia M, Saeidi K. 2018. The effect of seed bio-priming treatments on morphological indices of Moldavian Balm (*Dracocephalum moldavica* L.) under water deficit stress. Environmental Stresses in Crop Sciences 11, 353-363.

Kokate CK, Purohit AP, Gokhale SB. 2004. Pharmacognosy. 26th ed. Pune: Nirali prakashan, pp. 212-216.

Koo SY, Cho KS. 2009. Isolation and characterization of a plant growth-promoting rhizobacterium, Serratia sp. SY5. Journal of Microbiology and Biotechnology 19, 1431–1438. https://doi.org/10.4014/jmb.0904.04014

Leaungvutiviroj C, Piriyaprin S, Limtong P, Sasaki K. 2010. Relationships between soil microorganisms and nutrient contents of *Vetiveria zizanioides* (L.) Nash and *Vetiveria nemoralis* (A.) Camus in some problem soils from Thailand. Applied Soil Ecology 46, 95–102. https://doi.org/10.1016/j.apsoil.2010.06.007

Lee X, Azevedo MD, Armstrong DJ, Banowetz GM, Reimmann C. 2013. The *Pseudomonasaeruginosa* antimetabolite L-2-amino-4-methoxy-trans-3-butenoic acid inhibits growth of *Erwinia amylovora* and acts as a seed germination-arrest factor. Environmental Microbiology Reports 5, 83–89.

Majeed A, Kaleem Abbasi M, Hameed S, Imran A, Rahim N. 2015. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Frontiers in Microbiology 6, 1–10. https://doi.org/10.3389/fmicb.2015.00198

McPhail KL, Armstrong DJ, Azevedo MD, Banowetz GM, Mills DI. 2010. 4-Formylaminooxyvinylglycine, an herbicidal germination arrest factor from *Pseudomonas* rhizosphere bacteria. Journal of Natural Product 73, 1853-1857. http:// dx.doi.org/10.1021/np1004856

Moradi A, Piri R. 2018. Enhancement of salinity stress tolerance in Cumin (*Cuminum cyminum* L .) as affected by plant growth promoting rhizobactria during germination stage. Journal of Plant Process and Function Iranin Society of Plant Physiology 6, 47-55.

Mousavi M, Omidi H. 2019. Seed priming with bio-priming improves stand establishment, seed germination and salinity tolerance in canola cultivar (Hayola 401). Plant Physiology 9, 2807–2817. https://doi.org/10.22034/IJPP.2019.667138

Niranjan SR, Shetty NP, Shetty HS. 2007. Seed bio-priming with Pseudomonas fluorescens isolates enhances growth of pearl millet plants and induces resistance against downy mildew. Journal of Integral Pest Management 50, 41-48. http://dx.doi.org/10. 1080/ 09670870310001626365.

Noumavo P, Kochoni E, Didagb YO, Adjanohoun A, Allagbe M, Sikirou R, Gachomo EW, Kotchoni SO, Baba-

Moussa L. 2013. Effect of different plant growth promoting rhizobacteria on maize seed germination and seedling development. American Journal of Plant Sciences 32, 629–640.

Omar HR, Komarova I, El-Ghonemi M, Fathy A, Rashad R, Abdelmalak HD, Yerramadha MR, Ali Y, Helal E, Camporesi EM. 2012. Licorice abuse: Time to send a warning message. Therapeutic Advances Endocrinology and Metabolism 3, 125-138.

Ozturk M, Altay V, Hakeem KR, Akcicek E. 2017. Licorice from botany to phytochemistry. Springer Press. p. 139.

Pastorino G, Cornara L, Rodrigues F, Oliveira MBPP. 2018. Liquorice (*Glycyrrhiza glabra*): A phytochemical and pharmacological review, (July), 2323–2339. https://doi.org/10.1002/ptr.6178

Perata P, Guglielminetti L, Alpi A. 1997. Mobilization of endosperm reserves in cereal seeds under anoxia. Annual Botany 79, 49-56. http://dx.doi.org/10.1093/oxfordjournals.aob.a010306

Rafieiolhossaini M, Tadayon M R, Mazhari M. 2014, The effect of dormancy breaking treatments on seed germination of Licorice medicinal plant (*Glycyrrhiza glabra* L.). Journal of Crops Improvement 16, 809-817.

Riefler M, Ondrej N, Miroslav S, Thomas S. 2006. Arabidopsis cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development, and cytokinin metabolism. The Plant Cell 18, 40-54. https://doi.org/10.1105/tpc.105.037796

Rizzato G, Scalabrin E, Radaelli M, Capodaglio G, Piccolo O. 2017. A new exploration of licorice metabolome. Food Chemistry 221, 959–968.

Safari D, Jamali F, Nooryazdan HR, Bayat F. 2018. Evaluation of ACC deaminase producing *Pseudomonas fluorescens* strains for their effects on seed germination and early growth of wheat under salt stress. Australian Journal of Crop Science 12, 413–421. https://doi.org/10.21475/ajcs.18.12.03.pne801

Salamone IEGD, Hynes RK, Nelso, LM. 2005. Role of cytokinins in plant growth promotion by rhizosphere bacteria. In: Z.A. Siddiqui (Ed.), PGPR: Biocontrol and Biofertilization, Springer, The Netherlands, pp. 173–195.

Sinha SK, Prasad SK, Islam MA, Gurav SS, Patil RB, AlFaris NA, Aldayel TS, AlKehayez NM, Wabaidur SM , Shakya A. 2020. Identification of bioactive compounds from *Glycyrrhiza glabra* as possible inhibitor of SARS-CoV-2 spike glycoprotein and non-structural protein-15: a pharmacoinformatics study. Journal of Biomolecular Structure and Dynamics, DOI: 10.1080/07391102.2020.1779132

Scagliola M, Pii Y, Mimmo T, Cesco S, Ricciuti P, Crecchio C. 2016. Plant physiology and biochemistry characterization of plant growth promoting traits of bacterial isolates from the rhizosphere of barley (*Hordeum vulgare* L .) and tomato (*Solanum lycopersicon* L .) grown under Fe sufficiency and deficiency. Plant Physiology and Biochemistry 107, 187–196. https://doi.org/10.1016/j.plaphy.2016.06.002

Sharma S, Thokchom RA. 2014. Review on endangered medicinal plants of India and their conservation. Journal of Crop and Weed 10, 205-218.

Sharma PC, Yelne MB, Dennis TJ. 2005. Database on medicinal plants used in Ayurveda. 2nd. Central Council for Research in Ayurveda and Siddha., New Delhi, pp. 561- 566

Spaepen S, Vanderleyden J. 2011. Auxin and plant-microbe interactions. Cold Spring Harbor Laboratory Press, 1, 3(4): a001438.

Srivastava M, Purshottam DK, Srivastava A, Misra P. 2013. In vitro conservation of *Glycyrrhiza glabra* by slow growth culture. International Journal of Biotechnology and Research 3, 49- 58.

Tabatabaei S, Ehsanzadeh P, Etesami H, Alikhani HA, Glick BR. 2016. Indole-3-acetic acid (IAA) producing pseudomonas isolates inhibit seed germination and α-amylase activity in durum wheat (*Triticum turgidum* L.). Spanish Journal of Agricultural Research 14, 1–10. https://doi.org/10.5424/sjar/2016141-8859

Tiwari P, Singh JS. 2017. A plant growth promoting rhizospheric *Pseudomonas aeruginosa* strain inhibits seed germination in *Triticum aestivum* (L) and *Zea mays* (L). Microbiology Research 8, 73–79. https://doi.org/10.4081/mr.2017.7233

Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI. 2006. Microbial producers of plant growth stimulators and their practical use: A Review. Applied Biochemistry and Microbiology 42, 117–126.

Uniyal N, Chauhan A, Sharma K, Mitra D. 2017. Plant growth promotion by bacterial inoculum with different conditions and estimation of enzyme activity from germinating seeds and bacterial isolates. Current Trends in Biotechnology and Pharmacy 12, 53–64.

Widawati S, Suliasih 2018. The effect of plant growth promoting rhizobacteria (PGPR) on germination and seedling growth of *Sorghum bicolor* L. Moench. IOP Conference Series: Earth and Environmental Science 166, 94–99. https://doi.org/10.1088/1755-1315/166/1/012022

Yu JY, Ha JY, Kim KM, Jung YS, Jung JC, Oh S. 2015. Anti-inflammatory activities of licorice extract and its active compounds, glycyrrhizic acid, liquiritin and liquiritigenin, in BV2 cells and mice liver. Molecules 20, 13041–13054. https://doi.org/10.3390/molecules200713041

Zhang Q, Ye M. 2009. Chemical analysis of the Chinese herbal medicine Gan-Cao (licorice). Journal of Chromatography A 1216, 1954–1969. https://doi.org/10.1016/j.chroma.2008.07.072

Zhao K, Li J, Zhang X, Chen Q, Liu M, Ao X, Penttinen P. 2018. Actinobacteria associated with *Glycyrrhiza inflata* Bat. are diverse and have plant growth promoting and antimicrobial activity. Scientific Reports

8,1–13. https://doi.org/10.1038/s41598-018-32097-8

Zhao Q, Wu YN, Fan Q, Han QQ, Paré PW, Xu R, Zhang JL. 2016. Improved growth and metabolite accumulation in *Codonopsis pilosula* (Franch.) Nannf. by inoculation of Bacillus amyloliquefaciens GB03. Journal of Agricultural and Food Chemistry 64, 8103–8108. https://doi.org/10.1021/acs.jafc.6b03390