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Original Research

Effect of native growth promoting bacteria and commercial biofertilizers on growth and yield of wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) under salinity stress conditions

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Abstract: Salinity is one of the main obstacles to the production of crops in dry regions of the world. This study focuses on the effects of different strains of plant growth promoting rhizobacteria (PGPR) isolated from native soils on the physiological responses of wheat and barley plants under normal and salt stress conditions. Soil samples were collected from a field in Ilam province, in Iran and bacterial isolates were isolated and screened for salt tolerance, included siderophore and ACC-deaminase production and phosphate solubilizing. Thereafter a two-years greenhouse experiment was conducted as a completely randomized block design with four replications. The applied treatments included bacterial inoculation at five levels (B0: non-inoculation, B1: Siderophore producing + salt-tolerant bacteria, B2: phosphate solubilizing + salt-tolerant bacteria, B3: ACC-deaminase producing + salt-tolerant bacteria, B4: Barvar-2 biological fertilizer, B5: Biofarm-2 biological fertilizer) and salt stress at three levels (S1: 0 dS/m, S2: 4 dS/m, S3: 8 dS/m). Results showed that phosphate solubilizing+ salt-tolerant bacteria resulted in the highest barley grain yield at 4 dS/m salinity level and had no significant difference with ACC-deaminase producing + salt-tolerant bacteria and Barvar-2 biological fertilizer and Biofarm-2 biological fertilizer. The highest proline content in wheat and barley observed in Siderophore producing+ salt-tolerant bacteria at 8 dS/m by 17.48 and 23.42, respectively, followed by phosphate solubilizing+ salt-tolerant bacteria by 16.53 and 19.78. Therefore, the application of isolated growth promoting bacteria can be recommended as an effective biofertilizer in Ilam province.

Key words: Physiological traits; Promotion; Microorganisms; Tolerance.

Introduction

Salt stress is an important growth-limiting factor for most non-halophytic plants. High levels of salt cannot be tolerated by most crops, a fact that severely limits the employment of salt-affected soils for crop production (1). A considerable amount of land in the world is affected by salinity and shows an increasing trend. The United Nations Food and Agriculture Organization (FAO) and United Nations Environment Program (UNEP) reports showing that there are currently 4 million square kilometers of salinized land worldwide with approximately 20% of agricultural and 50% of crop lands in the world being salt-stressed and therefore threatening agricultural productivity (2). To overcome the effects of salinity, scientists are also using several approaches to obtain salt-tolerant plants. These approaches are time-consuming and costly. The mechanisms of salt tolerance are not yet completely clear. Most plants possess several mechanisms to decrease the negative effects of salinity including regulation and compartmentalization of ions, synthesis of compatible solutes, induction of antioxidative enzymes, induction of plant hormones, and changes in photosynthetic pathways (3). Using rhizosphere microorganisms, particularly beneficial bacteria are an alternative strategy that can improve plant performance under stress conditions and, consequently, enhance plant growth through different mechanisms (4). Some plant growth promoting rhizobacteria (PGPR) may cause a direct or indirect stimulation on plant growth and development by providing plants with fixed nitrogen, phytohormones and iron that has been sequestered by bacterial siderophore, and soluble phosphate (5).

Interactions between the soil bacteria, the so-called rhizobacteria, present in soils and the roots of the plant (rhizosphere) and the plants roots have been intensively studied (6, 7, 8). Rhizobacteria are soil bacteria that colonize plant roots, which are able to multiply and occupy all the ecological niches found on the roots at all stages of plant growth (9). Such bacteria may negatively interact with plants, directly by competing for nutrients. Alternatively, the relationship between rhizobacteria and the host plant can be positive. For example, the bacteria may compete with pathogens for survival in the rhizosphere or they may promote mutualistic relationships with plants they were associated, allowing nutrient exchange and stimulating antibiotic production against phytopathogenic agents (10).

Bacteria capable of mobilizing phosphates, able to produce siderophore and ACC-deaminase have been frequently isolated from soil using agar culture medium (11). These kinds of rhizosphere bacteria are capable of enhancing plant growth through increasing phosphorous and iron availability for the plants and production

of stress tolerance. In accordance, they are classified as plant growth-promoting rhizobacteria (PGPR). It is of interest that phosphate mobilizing activity of selected strains is frequently accompanied by the capacity to synthesize plant hormones (12). The present study objective is to investigate the response of wheat and barley plants to normal, medium and high NaCl treatment conditions (0, 4 and 8 dS/m) in the presence of plant growth promoting bacteria which isolated from a wheat field soil and two commercial biofertilizers.

Materials and Methods

Sampling and Isolation of Bacteria

A composite soil sample was collected from field sites under wheat cultivation near Ilam, Ilam province, Iran. In September 2017, a total of 25 soil samples were taken from field plots. Bacteria were isolated from soil samples by adding 1 g of soil to 10 m1 of sterile 0.05% Tween 80 solution, vortexing for the 30s, sonicating on ice for 15 min in a Branson model 3210 sonication bath, and vortexing for an additional 30s. Sonicated soil samples were serially diluted in 0.05% Tween 80 and spread plated onto 0.1 X tryptic soy agar (TSA; Difco, Becton Dickinson, Sparks, Md). Plates were incubated at 28°C for 2 days, and individual colonies from each sample were randomly selected to screen for PGP traits.

Screening for PGP Traits

Totally 20 individual and morphologically different single colonies were prepared and exposed to screening tests. The colonies were evaluated for salt tolerance using nutrient agar culture medium containing various concentrations of NaCl and $MgCl_2$ salts. After inoculating the plates having different salinities, isolates were classified as most sensitive to most tolerant isolate based on colonies quality and size. Finally, the tolerant and most tolerant isolates were selected for screening other PGP traits. Totally 10 salt-tolerant isolates were selected and were evaluated in terms of phosphate solubilizing ability (Sperber, 19858), ACC-deaminase production (13) and siderophore production (14) and finally three isolates were selected as Siderophore producing+ salt-tolerant bacteria, phosphate solubilizing+ salt-tolerant bacteria and ACC-deaminase producing + salt-tolerant bacteria and were utilized for greenhouse experiments.

Greenhouse experiments

Ilam 105 wheat genotype and Khorram barley genotype were employed for greenhouse pot experiment. Seeds were surface-sterilized as described by (15). Bacterial suspensions in sterile distilled water ($4*10^9$ cfu ml^{-1}) were used for seed inoculation but control seeds were treated only with sterile distilled water. Two commercial biofertilizers were purchased and utilized in the greenhouse experiment in order to compare the efficiency of native bacteria and the commercial ones for increasing growth and yield of wheat and barley plants. The inoculated seeds (20–30 seeds) were incubated at room temperature overnight and transferred onto sterile filter papers (Whatman No. 42) in Petri dishes and after germination, transferred into pots. After 4-month from germination and transferring into the pots, salt stress was imposed through a mixture solution of NaCl and $MgCl_2$. Initially 4 and 8 dS/m solutions were prepared and the corresponding pots for each salt stress level were irrigated with saline water. Pots exposed to salt stress were irrigated 8-times during the growth. During the growth period, pots moisture was maintained at 70-80 percent of field capacity in order to avoid any moisture stress. Plants were harvested about 5 months after germination. At harvest traits including plant height, grain yield, total proline, carbohydrates content, and SPAD value were measured (16).

Statistical analysis

A two-years greenhouse experiment was conducted as a completely randomized block design with four replications in the years 2017 and 2018. The applied treatments include bacterial inoculation at five levels (B0: non-inoculation, B1: Siderophore producing+ salt-tolerant bacteria, B2: phosphate solubilizing+ salt-tolerant bacteria, B3: ACC-deaminase producing + salt-tolerant bacteria, B4: Barvar-2 biological fertilizer, B5: Biofarm-2 biological fertilizer) and salt stress at three levels (S1: 0 dS/m, S2: 4 dS/m, S3: 8 dS/m). Data were analyzed using SPSS V. 20 and mean comparisons were conducted using Duncan multiple range test at 5 percent probability level.

Results

Plant height

Results of analysis of variance showed that main effect of planting year, salinity level, application of biological fertilizers and the interaction of salinity and biological fertilizers have all significantly ($P < 0.01$) affected wheat and barley plants height (Table 1). Mean comparison results showed that plant height in the second year was higher than the first year. Wheat plant height in the second year of the experiment was 10% higher than the measured values in the first year (Table 2). Mean comparison results for the interaction of salinity levels and biological fertilizers on wheat and barley plants height presented in (Figs 1 and 2), respectively. Plants in control treatment plots were higher compared to those plants in plots exposed to salinity levels. The salinity level of 8 dS/m resulted in the lowest plant height of both wheat and barley. Among the studied bacterial treatments, the

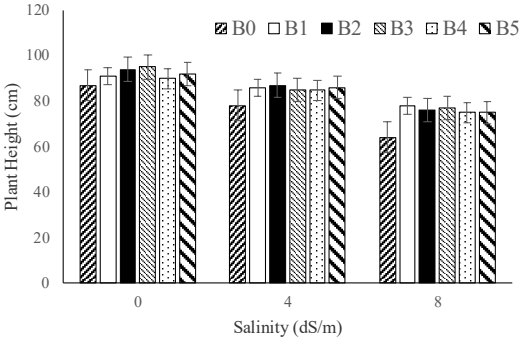

Figure 1. Interaction effect of salinity levels and inoculation with native bacterial treatments and commercial biofertilizers on wheat plant height. B0: non-inoculation, B1: Siderophore producing + salt-tolerant bacteria, B2: phosphate solubilizing + salt-tolerant bacteria, B3: ACC-deaminase producing + salt-tolerant bacteria, B4: Barvar-2 biological fertilizer, B5: Biofarm-2 biological fertilizer.

Table 1. Analysis of variance of the effect of salinity stress, bacterial inoculation and cropping year and their interactions on growth, yield and physiological traits of wheat and barley plants.

SOV	df	Mean Squares									
		Height		Grain Yield		Proline		Total Carbohydrates		SPAD	
		Wheat	Barley	Wheat	Barley	Wheat	Barley	Wheat	Barley	Wheat	Barley
Replication	3	219.0^{ns}	937^{ns}	1003428^{ns}	1576230^{ns}	7.7^{ns}	7.7^{ns}	117.9^{ns}	503^{ns}	107^{ns}	117^{ns}
Factor A	1	0.00^{ns}	676^{ns}	86987*	1739796^{ns}	386.7*	676*	4.1^{ns}	676^{ns}	348^{ns}	18760^{ns}
Error	3	30.0	0.0	0.0	190969	0.0	0.0	134	0.0	0.0	164.5
Factor B	2	3641.3**	2833*	41350170**	486427808**	61.7**	627.9*	5.8**	11.07$_{*}$	322**	34.8*
AB	2	0.0^{ns}	1.08*	19663*	1913^{ns}	0.68**	1.08**	5.1**	1.08^{ns}	12.4**	139.8**
Factor C	5	306.1*	261.6^{ns}	724112*	787091**	8.97**	87.4**	15.4**	17.6**	380.2**	180**
AC	5	0.0^{ns}	0.26^{ns}	11245^{ns}	39511**	1.48**	0.28^{ns}	0.84**	0.26^{ns}	17.2**	9.6**
BC	10	27.4**	19.4**	203597**	534353**	11.92**	47.9**	0.73**	13.5**	41.5**	52.6**
ABC	10	0.0^{ns}	0.25^{ns}	19822^{ns}	43131^{ns}	0.44^{ns}	0.25^{ns}	2.39^{ns}	0.25^{ns}	7.6^{ns}	5.6*
Error	102	0.0	3.6	0.0	4217	0.02	0.0	0.20	0.41	0.2	0.2
CV	-	0.01	2.2	9.7	2.5	1.35	7.6	4.63	5.54	5.78	1.19

*, ** and ns: significant at 5%, significant at 1% and non-significant, respectively.

Table 2. Interaction effect of salinity levels and inoculation with native bacterial treatments and commercial biofertilizers on wheat and barley plants proline and total carbohydrates content.

Treatments	Proline		Total Carbohydrates	
	Wheat	Barley	Wheat	Barley
S1B0	8.94^{e}	13.12^{d}	8.85^{c}	9.64^{d}
S1B1	8.98^{e}	13.67^{d}	10.09^{b}	12.83^{b}
S1B2	7.38^{e}	10.64^{e}	10.19^{b}	12.41^{b}
S1B3	7.78^{e}	9.78^{e}	11.28^{a}	12.12^{b}
S1B4	8.39^{e}	12.65^{d}	8.44^{d}	10.90^{c}
S1B5	5.36^{f}	5.54^{f}	8.23^{d}	8.50^{d}
S2B0	9.88^{d}	14.64^{d}	8.62^{d}	12.56^{b}
S2B1	10.58^{d}	12.58^{d}	8.36^{d}	9.49^{d}
S2B2	12.28^{c}	17.22^{c}	8.32^{d}	11.15^{c}
S2B3	10.75^{d}	13.00^{e}	11.5^{a}	12.71^{b}
S2B4	12.59^{c}	17.02^{c}	10.5^{b}	11.94^{b}
S2B5	10.95^{d}	14.45^{d}	10.5^{b}	11.61^{c}
S3B0	11.86^{c}	15.86^{cd}	9.82^{c}	10.77^{c}
S3B1	17.48^{a}	23.42^{a}	9.67^{c}	11.55^{b}
S3B2	16.53^{b}	19.78^{b}	10.93^{b}	12.18^{b}
S3B3	13.53^{c}	17.29^{c}	11.24^{a}	13.98^{a}
S3B4	13.72^{c}	17.90^{c}	9.86^{c}	12.43^{b}
S3B5	11.50^{c}	14.50^{d}	9.49^{c}	11.25^{b}

B0: non-inoculation, B1: Siderophore producing + salt-tolerant bacteria, B2: phosphate solubilizing + salt-tolerant bacteria, B3: ACC-deaminase producing + salt-tolerant bacteria, B4: Barvar-2 biological fertilizer, B5: Biofarm-2 biological fertilizer.

highest plant obtained for ACC-deaminase producing + salt-tolerant bacteria by 95 cm (wheat) and 94 cm (barely) which had no significant difference with phosphate solubilizing + salt-tolerant bacteria. In all salinity levels, no-inoculation treatment had the lowest plant height for wheat and barley. At 4 dS/m salinity level, under phosphate solubilizing + salt-tolerant bacteria the highest plant height was obtained for the wheat plant while for barley, the highest plant height was recorded under Siderophore producing + salt-tolerant bacteria had (Figs 1 and 2). Enhancement of wheat plant height has been reported by application of PGPR bacteria (17, 18). Physiological activity and production of plant hormones by bacteria after their introduction into the soil are likely to be important for interaction with the plants. Inoculated bacteria accelerated the growth of lateral roots in the plants, while the effect was repealed in auxin mutants, suggesting the involvement of IAA in the growth-promoting bacterial effect (19). Results are in consistent with (12) and (20) findings.

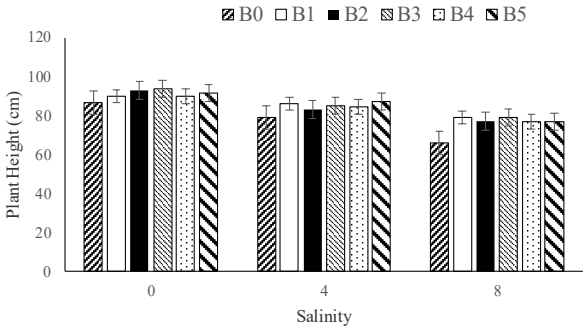

Figure 2. Interaction effect of salinity levels and inoculation with native bacterial treatments and commercial biofertilizers on barley plant height. B0: non-inoculation, B1: Siderophore producing + salt-tolerant bacteria, B2: phosphate solubilizing + salt-tolerant bacteria, B3: ACC-deaminase producing + salt-tolerant bacteria, B4: Barvar-2 biological fertilizer, B5: Biofarm-2 biological fertilizer.

Grain yield

According to results of the analysis of variance, inoculation of soils with native bacteria and commercial biofertilizers and different salinity levels in both years of experiment significantly ($P<0.01$) affected wheat and barley grain yield (Table 1). The mean comparison showed that different bacteria treatments had a different impact on wheat plant grain yield. In all bacterial inoculation treatments (native and/or commercial) 8 dS/m salinity level resulted in the lowest grain yield and 0 dS/m had the highest grain yield. Inoculation of siderophore producing + salt-tolerant bacteria resulted in highest wheat grain yield (4543 kg/ha) but showed no significant difference with phosphate solubilizing+ salt-tolerant bacteria (4386 kg/ha). Under inoculation

of ACC-deaminase producing + salt-tolerant bacteria at 8 dS/m plants showed the highest grain yield however there was no significant difference with plants under Siderophore producing+ salt-tolerant bacteria and phosphate solubilizing + salt-tolerant bacteria. No-inoculation treatment resulted in the lowest grain yield at all three salinity levels (Fig. 3). Mean comparison results of the effects of studied treatments on barley yield showed a similar trend as wheat in which increasing salinity level resulted in lower barley grain yield and 8 dS/m had the lowest grain yield in all bacterial inoculation treatments. Among bacterial inoculation treatments, Siderophore producing + salt-tolerant bacteria resulted in the highest barley grain yield by 3867 kg/ha with no significant difference with phosphate solubilizing + salt-tolerant bacteria with grain yield of 3788 kg/ha at 9 dS/m salinity level (Fig. 4). Phosphate solubilizing + salt-tolerant bacteria resulted in the highest barley grain yield at 4 dS/m salinity level and had no significant difference with ACC-deaminase producing + salt-tolerant bacteria and Barvar-2 biological fertilizer and Biofarm-2 biological fertilizer (Fig. 4). Inoculation of winter wheat with plant growth promoting bacteria has shown that significantly enhanced grain yield under a growth chamber study (21). (22) demonstrated that (S. *proteamaculans*) significantly increased plant height, grain yield, 100-grain weight, and straw yield of wheat under less than 15 dS/m salinity.

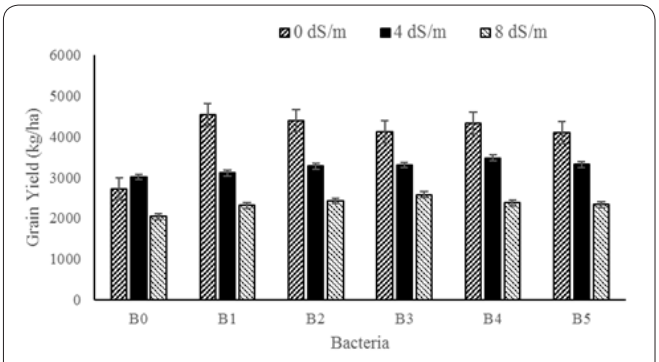

Figure 3. Interaction effect of salinity levels and inoculation with native bacterial treatments and commercial biofertilizers on wheat plant grain yield. B0: non-inoculation, B1: Siderophore producing + salt-tolerant bacteria, B2: phosphate solubilizing + salt-tolerant bacteria, B3: ACC-deaminase producing + salt-tolerant bacteria, B4: Barvar-2 biological fertilizer, B5: Biofarm-2 biological fertilizer.

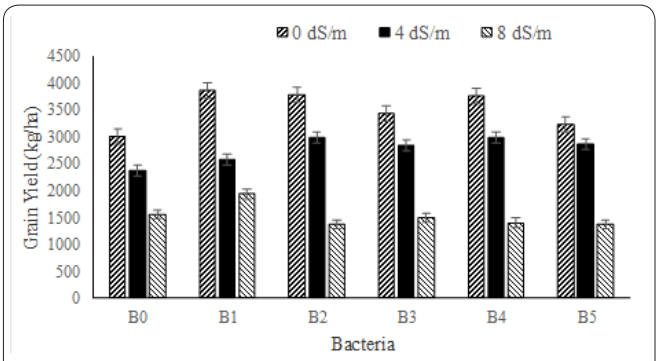

Figure 4. Interaction effect of salinity levels and inoculation with native bacterial treatments and commercial biofertilizers on barley plant grain yield. B0: non-inoculation, B1: Siderophore producing + salt-tolerant bacteria, B2: phosphate solubilizing + salt-tolerant bacteria, B3: ACC-deaminase producing + salt-tolerant bacteria, B4: Barvar-2 biological fertilizer, B5: Biofarm-2 biological fertilizer.

Discussion

Native bacteria and commercial biofertilizers significantly ($P<0.01$) affected wheat and barley plants proline and total carbohydrates content (Table 1). Results showed that salt stress significantly increased proline content in wheat and barley and the highest proline content in wheat and barley observed in the highest salinity level (8 dS/m). Bacterial inoculation significantly affected plants proline and total carbohydrates content. The highest proline content in wheat and barley observed in Siderophore producing + salt-tolerant bacteria at 8 dS/m by 17.48 and 23.42, respectively, followed by phosphate solubilizing + salt-tolerant bacteria by 16.53 and 19.78. At the same salinity level, there was no significant difference between ACC-deaminase producing + salt-tolerant bacteria, Barvar-2 biological fertilizer and Biofarm-2 biological fertilizer for wheat and between ACC-deaminase producing + salt-tolerant bacteria and Barvar-2 biological fertilizer for barley (Table 2). In regard to total carbohydrates content, similar to proline content, total carbohydrates significantly increased by increasing salinity level and reached the highest level at 8 dS/m salinity level. ACC-deaminase producing + salt-tolerant bacteria inoculation at 8 dS/m resulted in the highest carbohydrates content by 11.24 (wheat) and 13.98 (barely) and there was no significant difference between other bacterial treatments in the same salinity level for barley and phosphate solubilizing + salt-tolerant bacteria placed in the second rank for wheat in terms of total carbohydrates content (Table 2). (23) reported that by exposure of the plant to the salinity stress, initially total soluble carbohydrates increased due to the conversion of sucrose to mono-saccharide sugars and (24) reported an increase in proline content by salinity stress in rice. (25) showed that an increase in plant proline content is the plant response to a decrease in water potential in the root medium. In these situations, proline decreases the osmotic potential of root cells and provide requirements for water and nutrient uptake. Synthesis of plant hormones by bacteria stimulates root exudation, thereby providing bacteria with the substrate for their growth (26). Nevertheless, the responses of bacteria of different genotypes to the presence of plants may be distinct (12).

Results of analysis of variance showed that salt stress, microbial fertilizers and the interaction effect of cropping year and microbial fertilizers significantly affected wheat plant SPAD value and salt stress, microbial fertilizers and cropping year and their interactions had significant ($P<0.01$) impact on barley plant SPAD value (Table 1). Mean comparison results of the interaction effect of cropping year and microbial fertilizers showed that second cropping year had the highest SPAD values compared to the first year and among bacterial treatments, ACC-deaminase producing + salt-tolerant isolate in the second year resulted in the highest wheat SPAD value of 55.4 (Fig. 5). The same bacterial isolate at the first cropping year resulted in the highest wheat plant SPAD value (Fig. 5). The interaction of studied treatments on barley plant SPAD value showed that SPAD value in the

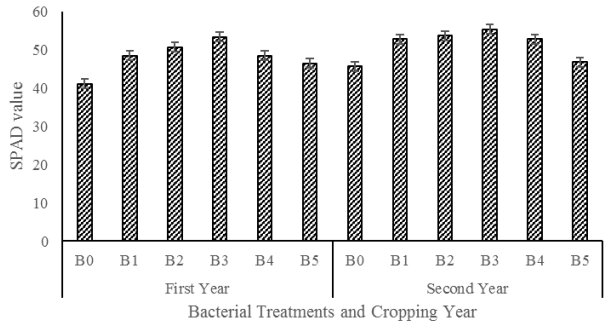

Figure 5. The interaction effect of cropping year and inoculation with native bacterial treatments and commercial biofertilizers on wheat plant SPAD value. B0: non-inoculation, B1: Siderophore producing + salt-tolerant bacteria, B2: phosphate solubilizing + salt-tolerant bacteria, B3: ACC-deaminase producing + salt-tolerant bacteria, B4: Barvar-2 biological fertilizer, B5: Biofarm-2 biological fertilizer.

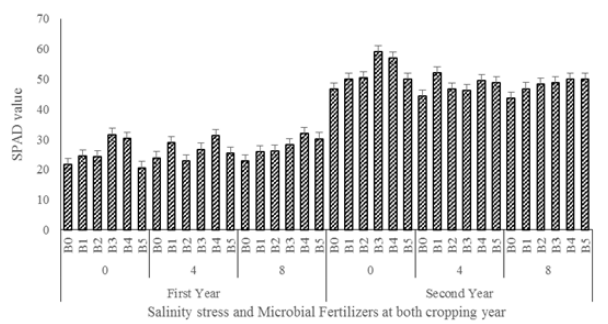

Figure 6. Interaction effect of salinity levels and inoculation with native bacterial treatments and commercial biofertilizers at both cropping years on barley plant SPAD value. B0: non-inoculation, B1: Siderophore producing + salt-tolerant bacteria, B2: phosphate solubilizing + salt-tolerant bacteria, B3: ACC-deaminase producing + salt-tolerant bacteria, B4: Barvar-2 biological fertilizer, B5: Biofarm-2 biological fertilizer.

second year significantly increased and highest SPAD value for barley plant obtained in the second year and 0 dS/m and inoculation with ACC-deaminase producing + salt-tolerant isolate (Fig. 6). The reason for decreasing SPAD value by increasing salinity could be due to the inhibitory effect of salt stress on absorbing and transferring of photosynthetic materials (27). (28) reported that PGPR increased plant chlorophyll content. It seems that these bacteria increase chlorophyll content via increasing nitrogen uptake (29). Gholami et al., 2009 (30) stated that increasing leaf area index and SPAD value through bacterial treatments could be attributed to the production of plant hormones and increasing access to nutrients. Jalili et al (31) and Golamian et al (32) studied on effect of salinity on stevia and camelina respectively.

Results showed that application of native growth promoting bacteria isolated from a field in Ilam province significantly increased wheat and barley plants growth, yield and physiological traits under salinity stress conditions and have the ability to compete with commercial biological fertilizers and were better than commercial ones which could be attributed to their compatibility with soil and climate conditions in the studied area. Therefore, the application of isolated growth promoting bacteria can be recommended as an effective biofertilizer in Ilam province.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Availability of data and material
The datasets supporting the conclusions of this article are included within the article

Competing interests
The authors declare that they have no competing interests.

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