Turkish Journal of Botany

Volume 48 | Number 2 Article 3

3-19-2024

Isolation and assessment of halophilic rhizobacteria plant growth-promoting traits for alleviating salt stress in wheat

Alev SEZEN
aosezen@gmail.com

Ömer Faruk ALGUR
ofalgur@yahoo.com

Ferruh AŞÇI
f_asci@aku.edu.tr

Arzu ÜNAL
arzuunal@gmail.com

Recommended Citation

SEZEN, Alev; ALGUR, Ömer Faruk; AŞÇI, Ferruh; and ÜNAL, Arzu (2024) "Isolation and assessment of halophilic rhizobacteria plant growth-promoting traits for alleviating salt stress in wheat," *Turkish Journal of Botany*: Vol. 48: No. 2, Article 3. https://doi.org/10.55730/1300-008X.2797
Available at: https://journals.tubitak.gov.tr/botany/vol48/iss2/3

TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2024) 48: 79-90

doi:10.55730/1300-008X.2797

Isolation and assessment of halophilic rhizobacteria plant growth-promoting traits for alleviating salt stress in wheat

Alev SEZEN[1], Ömer Faruk ALGUR[1], Ferruh AŞÇI[2*], Arzu ÜNAL[3]
[1]Atatürk University, Department of Biology, Erzurum, Turkiye
[2]Afyon Kocatepe University, Department of Molecular Biology and Genetics Afyonkarahisar, Turkiye
[3]Iğdır University, Faculty of Agriculture, Department of Biotechnology Iğdır, Turkiye

Received: 26.09.2023 • **Accepted/Published Online:** 12.03.2024 • **Final Version:** 19.03.2024

Abstract: In this study, 22 halophilic bacteria were isolated from plants collected together with rhizosphere soil from habitats with high salt content in and around Erzurum. Various plant growth-promoting (PGP) properties of these isolates (nitrogen fixing, phosphate solubilizing, ACC deaminase, and IAA and siderephore production) were determined. Bacteria positive for PGP properties and various combinations with these bacteria were subjected to pot experiments in saline medium (greenhouse conditions) and their effects on growth parameters (root and stem length, fresh and dry weight, protein, and chlorophyll and carotenoid content) of wheat plants were determined. As a result of the research, the isolates were evaluated as successful isolates 5%–41% yield increase in root length, 3.82%–54.97% in stem length, 7.14%–60.71% in wet weight, 5%–32.5% in dry weight, 13.78%–29.68% in dissolved protein content, 83.40%–120.22% in total chlorophyll content, and 45.79%–120.22% in total carotenoid content. 16S rRNA analysis showed that these 22 halophilic bacterial strains belonged to 11 different bacterial species (*Bacillus pumilus*, *Bacillus endophyticus*, *Paenobacillus lautus*, *Planococcus citreus*, *Staphylococcus sciuri*, *Staphylococcus saprophyticus*, *Leucobacter iarius*, *Sphingomonas echinoides*, *Bacillus simplex*, *Bacillus cereus*, *Pantoea aggloremans*). All isolates obtained in this study are new and original isolates. Inoculations with the isolates found in our study were much more effective and provided significant yield increases on wheat plants under saline irrigation compared to previous studies.
These results conclude that the obtained isolates promote plant growth under saline conditions and have biofertilizer potential for wheat plants under saline conditions.

Key words: Plant growth-promoting bacteria, salt stress, halophilic/halotolerant bacteria, wheat

1. Introduction

Since beginning of the 21st century, increasing soil and water salinity, environmental pollution, and scarcity of water resources have received more attention globally. Soil salinity is one of the most important destructive environmental stresses leading to reduced crop yield and quality in cultivated areas (Yamaguchi and Blumwald, 2005; Shahbaz and Ashraf, 2013). Salinity is a key factor that inhibits crop yield and production and plays a vital role in plant growth and production. Salinity-affected soils have been a major limiting factor for important crops worldwide (Shannon and Grieve, 1999; Bacilio et al., 2004). The growing human population and the decrease in available land for agriculture pose a threat to sustainable agriculture (Shahbaz and Ashraf, 2013). One of the most important measures to be taken to prevent these problems is the establishment of the necessary agricultural strategies (Glick, 2014). By 2050, a significant increase (estimated at 50%) in grain yields of important crops such as rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.) is required to meet the food needs of the projected population (Godfray et al., 2010). Losses in wheat-cultivated areas are mainly due to drought and high soil salinity, and the area of land affected by salinity is increasing day by day. Adaptations and mitigation strategies are widely needed to cope with a wide range of such impacts. However, such strategies are long, inconclusive, and costly, and there is a need to develop simple and low-cost biological methods for salt stress management that can be used in the short term. Research under these conditions requires an appropriate biotechnological effort, not only to increase crop yields but also to improve soil health through the interaction of soil microorganisms and plant roots (Lugtenberg et al., 2002). Soils under salt stress are known to suppress plant growth (Paul, 2012). It is well known that soil biota

* Correspondence: f_asci@aku.edu.tr

has beneficial effects on salt-exposed plants. Isolating microorganisms present in the rhizosphere of saline plants can be an effective way to achieve this goal, and recently, plant growth-promoting rhizobacteria (PGPR) have been used for this purpose (Dimkpa et al., 2009). Unless their intrinsic characteristics such as tolerance to saline conditions, genetic diversity, synthesis of compatible solutes, production of plant growth-promoting hormones, biocontrol potential, and interactions with cultivated plants are exploited, halotolerant PGPRs could play an important role in improving wheat crop productivity in this context. Previous studies show that the use of PGPRs is a promising alternative to alleviate salinity-induced plant stress (Yao et al., 2010). Therefore, the role of microorganisms in the management of biotic and abiotic stresses is becoming very important. This study will be helpful to explore the potential of native salt tolerant strains of PGPR and further their utilization as biofertilizers for wheat crop to minimize yield losses due to salt stress. In the future, this could lead to developing an effective bioformulation for such problematic soils.

2. Materials and methods

2.1. Soil sampling and plants isolation of halotolerant bacteria

The area where plant and rhizospheric soil samples are collected is a rock salt area on the mountain called Sağır Tepe, considering that the chances of obtaining halotolerant, halophilic, or moderate halophilic bacteria would be high. The collected plants belonged to Boraginaceae, Asteraceae, Poaceae, Lamiaceae, Plantaginaceae, Fabaceae, Equisetaceae, and Ranunculaceae, and our effective isolates were isolated from these plant rhizospheres. Since these plants were not in the flowering period, they could only be identified at the family level. This mountain is located within the boundaries of Esendurak neighborhood (formerly named Sağır Village), 15 km north of Tortum District in Erzurum. It is 1950 m high. The samples were collected from the rhizosphere of various wild halophile plants at a depth of 0–5 cm. They were placed in sterile airtight bottles, labeled with the date of collection, and transported to the laboratory for further investigation. Soil samples taken from the rizospheric region of previously planted plants and brought to the laboratory after the plant roots were thoroughly mixed and homogenized, series dilutions of 10^{-1} to 10^{-6} were prepared from each. From each of these samples, 15 g/L bacto peptone, 5 g/L trptone, 2.5 g/L dextrose, 15 g/L agar, and NaCl and 1 L pure water (pure water device: Nüve NS 112) were used for the isolation of bacteria from the previously prepared agar medium containing 1 M NaCl. After incubation at room temperature for 2–4 days, the bacteria were inoculated in three different molarity media modified with 1.75 M, 2 M, and 2.5 M NaCl. At the end of 2–4 days, isolates growing on these media were identified. Each of the purified isolates was given a code number and stored in glycerin medium at –80 °C.

2.2. Screening of plant growth promotion abilities

2.2.1. Qualitative and quantitative biological nitrogen fixation (BNF)

Each bacterium growing on medium containing three different NaCl (1 M, 1.75 M, 2 M) concentrations was inoculated by line seeding method on Petri dishes containing solid Burk's N-free medium (Wilson and Knight, 1952; Park et al., 2005). Each bacterium was inoculated in plates, one of which was incubated at 30 °C for 3–5 days. Petri dishes were checked daily. Petrays with bacterial growth from the second day onwards were coded as +++ and those with bacterial growth from the third day onwards were coded as -++ and their nitrogen fixing potential was graded. After 3–4 days at 30 °C in Burk's N-free medium, pure bacteria were inoculated into test tubes containing 5 mL of semisolid JNFb medium prepared according to (Baldani et al., 2014) and their nitrogen fixation properties were tested for the second time.

2.1.2. Phosphate solubilization

Qualitative and quantitative methods were used for the determination of phosphate solubilization potential of the isolates. Firstly, liquid NBRIP-BPB medium was used for qualitative determination. Absorbance values at 600 nm were read and recorded (Mehta and Nautiyal, 2001; Aydogan and Algur, 2014). The experiments were carried out in triplicate, and arithmetic averages of the results were taken. Bacteria that were determined to be effective in dissolving inorganic phosphate were also tested quantitatively separately. In the quantitative determination, the amount of dissolved phosphate in the medium was determined using the Vanadomolibdo phosphoric acid method (Jackson, 1973). Based on the previously prepared standard graph, the phosphate amounts of samples recorded by reading the absorbance values at 600 nm in the UVmini-1240 brand spectrophotometer were calculated as milligram per liter.

2.1.3. Indole-3-acetic acid (IAA) production

Using the method described by Sarwar and Kremer (1995), the bacteria were incubated on a shaker for 3 days. After incubation, the bacterial cultures were centrifuged. One milliliter of the supernatant was reserved and 1 mL was mixed with 3 mL of Salkowski's reagent, and the mixture was kept for 1 h at 30 °C in the dark. Three replicates were made from each sample. In addition, each sample was measured spectrophotometrically at 530 nm after the waiting period (Sarwar and Kremer, 1995; Patten and Glick, 2002). IAA production was calculated in milligram per liter using standard graph values corresponding to the spectrophotometer values.

2.1.4. Siderophores production

This assay was carried out based on the competition for iron between the ferric complexes of an indicator dye, chrome azurol S (CAS), and the siderophores produced by bacteria which apparently have a higher affinity for chelating Fe^{+3} of CAS. Siderophore production by rhizobacterial isolates was detected as described by Schwyn and Neilands (1987) with several modifications. The assay was performed by using CAS agar medium which contains the ternary complex CAS/Fe^{+3}/hexadecyltrimethyl-ammonium bromide as an indicator. Autoclaved CAS agar medium was poured in each Petri dish. Pure bacteria were inoculated into the wells of CAS Blue agar plates and incubated in an oven at 30 °C for 7 days. After 7 days of incubation, the yellow-orange discoloration of the medium and the diameter of the zone formed by the bacteria were measured to determine whether these bacteria produced siderophore.

2.1.5. ACC(1-aminocyclopropane-1carboxylic acid) deaminase activity

The ACC deaminase activity of bacteria was determined by estimating the amount of α ketobutyrate (α-KB) generated by the enzymatic hydrolysis of ACC, following the method described by Honma and Shimomura (1978). Activity of ACC deaminase in isolated PGPB was assayed according to the method described by Shrivastava and Kumar (2013) by measuring the amount of α-KB produced when the enzyme ACC deaminase cleaves ACC, which was determined by comparing the absorbance at 540 nm of a sample to a standard curve of α-kb ranging between 0.05 and 1.0 μmol. To measure specific activity of the cultures, protein estimation was carried out according to Honma and Shimomura (1978). Protein content was estimated by reference to a standard. The activity of ACC deaminase was expressed in nmol α-K /g^{-1} biomass h^{-1}.

2.2. Diagnosis and assessment of effective isolates

As a result of our studies, some classical, biochemical, and molecular methods (MIS and 16S rRNA analysis) were used for the definitive diagnosis and characterization of the bacterial isolates that were determined to be halophilic/halotolerant PGPR and used in pot trials. The fatty acid profiles of the isolates in our study were analyzed one by one by running the MIS device (MIDI, Inc., Newark, USA) as specified in the system manual and the diagnostic results were printed out from the computer (MIS, 2005). The 22 bacterial isolates, selected as the most effective isolates as a result of the experiments, were analyzed for 16S rRNA gene by Refgen company.

2.3. Inoculum preparation

In order to prepare the inoculum to be inoculated into the seeds of cultivated plants, pure bacteria were incubated in tryptic soy broth (TSB) at 30 °C and 180 rpm for 48 h on a shaker. At the end of the incubation, surface-sterilized wheat seeds were thrown into the inoculum cultures, and 4% sugar solution prepared to ensure better adhesion of the inoculum material to the seeds was added (İşler and Coşkan, 2009). The seeds were kept in the culture liquid overnight to allow the seeds to absorb water. In this way, wheat seeds were made ready for pot planting.

2.4. Pot experiments under greenhouse conditions

The pots (153 × 116 mm and capacity to hold 1liter of soil) to be used for sowing the seeds and growing the plants were disinfected with cotton soaked in 95% alcohol and filled with 1/3 of sand, soil, and peat. Three pots were prepared for each bacterium and the pots were labeled with bacterial code numbers and made ready for planting. The research was conducted using a variety of wheat (*Triticum aestivum cv. Kirik*) derived from the Atatürk University Agricultural Faculty Field Crops Department, in the form of a pot experiment with a triple replication test under greenhouse conditions, following the experimental design outlined below. No solution was used for plant nutrition. The experiment was organized as control group containing uninoculated wheat seeds (TSB medium without inoculation + wheat seeds) and groups containing inoculated wheat seeds (TSB medium prepared by inoculating each bacterium separately + wheat seeds). Seeds were sown at a rate of 15 per pot and grown for 16 days under greenhouse conditions. During the 16-day period, initially irrigated with regular water on the first day. Subsequently, saline irrigation was introduced, with tap water containing 50 mM, 100 mM, and 200 mM NaCl applied for 5 days each in three separate periods. At the end of the 16 days, both experimental groups were harvested. At this stage, the plants were removed together with their roots and washed in tap water. Subsequently, root and stem lengths and wet and dry weights were measured. In addition, protein, chlorophyll, and carotenoids were determined in the leaf samples.

2.4.1. Root and stem lengths of plants

Root and height lengths of the plants removed from each pot were measured with a ruler and calculated by taking the average.

2.4.2. Wet and dry weights of plants

An equal number of wheat plants were removed from each pot, weighed on a precision balance (Denver GERMANY, TP - 214), and the wet weight per plant was calculated by taking the average. Dry weight was determined by placing the plants, whose wet weight was measured, in aluminum foil and allowing them to dry in an oven at 70 °C for 48 h. When the last two weighings of a plant were equal, it was removed from the oven and weighed on a precision balance. The average of the two weights was calculated, and the dry weight per plant was determined (Kaçar, 1972).

2.4.3. Determination of soluble protein in plant leaf tissue

Protein determination was carried out in leaf samples of wheat plant using the method proposed by Bradford (1976). Absorbance values at 595 nm were measured, and protein content was determined spectrophotometrically. Utilizing a standard graph, the results were calculated as protein per gram of tissue for each sample, with absorbance values estimated by measuring the optical density at 595 nm (OD_{595}) using a spectrophotometer (Shimadzu, JAPAN, UVmini-1240).

2.4.4. Determination of chlorophyll and carotenoids in plant leaf tissue

After harvesting from the wheat plant, 0.5 grams of grain was mixed. The examined samples were placed in a mortar, and the extract was crushed with 5 mL of preprepared cold 80% acetone (80 mL of acetone was supplemented with pure water and prepared in 100 mL). The molecule was centrifuged at 3500 × *g* (6000) rpm for 15 min at the centrifugal tube. After centrifugation, the supernatant was completed with 10 mL of acetone. Subsequently, the absorptions were measured on the spectrophometer, at 645 and 663 nm separately. The zero adjustment of the spectrophotometer was done with 80% acetone. For the determination of carotenoids, the same samples were also read at 450 nm (Strain and Svec 1966).

2.5. Statistical analysis of the results

The experiments were conducted in triplicate and the values obtained were analyzed with LSD multiple comparison test using SPSS 12.0 statistical program (Yıldız and Bircan, 1991).

Twenty-two bacterial isolates, selected as the most effective isolate as a result of the experiments, have been sent to the Refgen company for analysis of the 16S rRNA gene. The National Center for Biotechnology Information GenBank accession numbers for the sequences of halotolerant bacterial strains are from MN086818 through MN086870.

3. Results

3.1. Characteristics of the selected halotolerant PGP

Isolates obtained from various plant rhizosphere soil samples collected from areas with high salt content showed growth in media containing 1.75 M, 2 M, and 2.5 M salt concentrations (Table 1). The plant growth promoting properties of the potentially effective isolates growing at high salt concentrations were also tested. For this purpose, the growth of these 22 isolates was observed on the 2nd, 3rd, and 4th day in nitrogen-free medium at a temperature of 30 °C, and the results are presented (Table 1). Some of the isolates (ASD9, ASD14, ASD22, ASD25, ASD31, ASD54, ASD55, ASD58, ASD59) exhibited optimal growth at 30 °C (Figure 1). To confirm the nitrogen fixation of these isolates, their growth on semisolid medium was also observed (Figure 2).

When the inorganic phosphate solubilizing potential

Table 1. Growth potential of the isolates on medium containing various concentrations of NaCl (1.75 M, 2 M, and 2.5 M NaCl), on nitrogen-free medium and findings obtained from PGP properties.

Isolate no.	1.75M NaCl	2M NaCl	2.5M NaCl	Growth in NF medium (30 °C)	Inorganic P (600 nm)	Inorganic P (mg/L)	IAA production (mg/L)	ACC production (α-KB/ g^{-1} biomass h^{-1})	Siderophore production (cm)
ASD1	+	+	-	-++	0.729	60	59.8	1224.490	1.2
ASD8	+	+	+	+++	0.411	97.06	78.8	2342.86	1.2
ASD9	+	+	+	+++	0.263	70.06	48	437.788	1.1
ASD14	+	+	-	+++	0.9	113.8	8	1136.930	0.9
ASD18	+	+	-	-++	0.104	103.03	20.1	5997.310	0.9
ASD19	+	+	-	+++	0.403	136.8	50.6	154.668	1
ASD22	+	+	+	+++	0.337	104.13	78.8	229.053	0.6
ASD23	+	+	+	+++	0.132	140.6	64.2	454.439	0.9
ASD25	+	-	-	+++	0.123	116.7	111.7	2788.22	1.2
ASD31	+	+	+	-++	0.6	99.5	50	1201.01	0.8
ASD36	+	+	+	+++	0.529	98.06	33.7	109.495	1.1
ASD38	+	+	+	+++	0.755	104.7	7.2	147.696	1
ASD44	+	+	+	+++	0.851	99.6	42.7	93.292	1.1
ASD48	+	+	+	+++	0.588	100.2	103.8	159.691	1.0
ASD54	+	+	-	+++	0.1	57.2	60.7	114.999	0.5
ASD55	+	+	-	±++	0.947	82	61	696.668	0.7
ASD58	+	+	-	+++	0.14	70.5	79.7	110.47	0.7
ASD59	+	+	+	+++	1.2	59.26	51.8	126.16	1
ASD64	+	+	+	-++	0.74	65	117	750.394	1
ASD65	+	-	-	+++	0.93	91.8	60	990.704	1.0
ASD68	+	+	-	±++	0.437	103.8	55.7	118.2	0.7
ASD69	+	+	-	±++	0.235	97.33	33.8	591.84	1

Figure 1. Growth of some isolates in nitrogen-free (NF) medium.

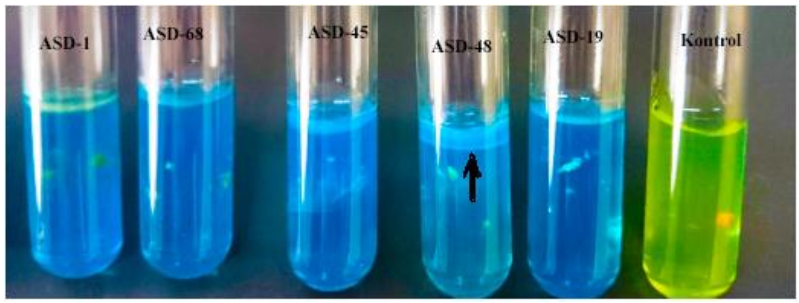

Figure 2. The blue color of some isolates on semisolid NF medium and the layer formed near the surface.

of the isolates were analyzed by comparing the qualitative and quantitative results (Table 1), the isolates with the best phosphate solubilizing properties were ASD8, ASD14, ASD18, ASD19, ASD22, ASD23, ASD25, ASD31, ASD36, ASD38, ASD48, ASD68, and ASD69, respectively. The most effective isolates in terms of IAA production potential are ASD8, ASD22, ASD25, ASD48, ASD48, ASD58, and ASD64, respectively. The color changes of some isolates corresponded to their potential to produce IAA. 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity of the isolates was determined (Table 1). ASD1, ASD8, ASD14, ASD18, ASD18, ASD25, ASD31, ASD55, ASD64, and ASD65 were the most effective isolates in terms of ACC deaminase potential. The ability of the isolates to produce siderophore and the orange colored zones they formed in the medium. ASD1, ASD8, ASD25, ASD9, ASD36, and ASD44 isolates produced significant levels of siderophores and formed yellow-orange zones in the medium.

3.2. Identification and characterization of effective isolates

Gram, endospore, and catalase tests were performed on 22 isolates used in pot experiments. According to these tests, ASD1, ASD8, ASD9, ASD18, ASD19, ASD22, ASD25, ASD31, ASD55, ASD59, ASD68, and ASD69 isolates had positive gram, endospore and catalase tests. Gram and catalase tests of ASD14, ASD23, ASD36, ASD38, ASD44, ASD48, and ASD58 isolates were positive, while endospore tests were negative. Only catalase tests of ASD54, ASD58, and ASD64 isolates were positive. 16S rRNA results of isolates ASD1, ASD8, ASD25, ASD31, ASD55, ASD68, and ASD69; *Bacillus pumilus*, isolate ASD36; *Staphylococcus sciuri*, isolates ASD38 and ASD44; *Staphylococcus saprophyticus*, isolates ASD19, ASD59; *Bacillus cereus*, isolates ASD9 and ASD22; *Bacillus endophyticus*, isolate ASD18; *Bacillus licheniformis*, isolates ASD14 and ASD23 *Cellulosimicrobium cellulans*, isolate ASD48; *Leucobacter iarius*, isolate ASD54; *Sphingomonas echinoides*, isolate ASD65; *Bacillus atrophaeus*, isolate ASD58; *Stenotrophomonas maltophilia*, isolate ASD64; *Pantoea agglomerans*.

3.3. Effect of inoculation on growth traits of wheat in pot trials

A pot experiment was conducted by inoculating the isolates to wheat seeds. According to the results of the experiment, ASD55 isolate showed the best effect on plant growth (Figure 3).

Figure 3. Images of wheat plants inoculated with the control group and ASD55 isolate on the 15th day.

3.3.1. Effect of saline irrigation on growth traits of wheat

3.3.1.a. Root and stem length of wheat

The analysis of the values obtained from root and stem length measurements is summarized in Table 2. In the light of these data, the positive contributions of ASD8, ASD55, ASD69, and ASD55 isolates to the increase in root length compared to the control group without bacterial inoculation were statistically significant ($p < 0.05$). Only isolate ASD1 (10.53 ↓ %) had a negative effect on root length compared to the control group and caused a decrease in root length. On the other hand, the other isolates had a positive effect on root length by showing a significant increase compared to the control group (Figure 4).

The effect of ASD9, ASD55, ASD65, ASD22, ASD48, and ASD54 isolates on stem length was statistically significant ($p < 0.05$). None of the isolates had a negative effect on stem length. Inoculation with different isolates had a positive effect on root and stem length of wheat plants compared to the control group and generally increased (Table 2).

3.3.1.b. Wet and dry weight

The analysis of the findings obtained from wet and dry weight measurements is summarized in Table 2. As seen in this table, ASD69 and ASD58 isolates led to statistically significant ($p < 0.05$) increases in wet weight, while ASD19, ASD36, ASD69, ASD58, ASD54, ASD59, ASD55, and ASD64 isolates on dry weight compared to the control group without bacterial inoculation.

ASD1 (3.57↓%), ASD25 (10.71 ↓%), and ASD68 (7.14 ↓%) isolates had a negative effect on wet weight and caused a decrease in wet weight per plant. However, all other isolates except these three isolates had a positive effect on wet weight and caused an increase.

It was determined that SD36, ASD69, ASD58, ASD19, ASD54, ASD55, ASD59, and ASD64 isolates led to statistically significant ($p < 0.05$) increases in dry weight compared to the control without bacterial inoculation. In addition, while ASD1 isolate was in the same group with the control, ASD14 (7.5% ↓), ASD25 (15% ↓), and ASD68 (17.5% ↓) isolates caused a decrease in plant dry weight compared to the control. In general, the isolates mostly caused an increase in plant dry weight. Bacterial inoculation had a positive effect on wet and dry weight of wheat plants and caused an increase compared to the control group (Figure 5).

3.3.1.c. Effects of inoculation treatments on dissolved protein content in plants

Statistical analysis of the values obtained from measurements of the dissolved protein content (Figure 6). As shown by the figure, ASD54, ASD69, and ASD-8-9 isolates provided the maximum positive effect on protein content compared to the control group without bacterial inoculation, followed by ASD38 and ASD1 isolates, respectively. Apart from these isolates, ASD8, ASD9, ASD14, ASD18, ASD22, ASD31, ASD36, ASD44, ASD48, ASD55, ASD58, ASD64, ASD65, ASD65, and ASD68 isolates caused a decrease in protein content in wheat plants compared to the control group. The effects of inoculation with different isolates on plant protein content in wheat plants are presented in Table 3.

3.3.1.d. Effects of inoculation with isolates on plant chlorophyll and carotenoid content

The effects of inoculation with different isolates and saline irrigation on chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoid content of wheat plants are presented in Table 3. In the light of the statistical analysis of the results obtained from the chlorophyll a measurements, ASD54 isolate had the highest positive effect compared to the control group and this isolate was followed by ASD18, ASD69, ASD65, and ASD19 isolates. ASD44 and ASD58 isolates had a negative effect on chlorophyll a content in wheat plants compared to the control group (Figure 7). ASD54, ASD18, ASD19, ASD19, ASD69, and

Table 2. Root, stem length; wet and dry weight findings of inoculation with isolates statistical analysis and percentage increase (↑), decrease (↓), and stable (↔) amount.

Isolate no	Root length(cm)	% Root length	Stem length (cm)	% Stem length	Wet weight (g)	% Wet weight	Dry weight (g)	% Dry weight
Control	15.00±0.58^{kl}		19.10±1.10^{jk}		0.28±0.02^{cde}	3.57↓	0.040±0.002^{bcd}	
ASD1	13.42±0.57^{l}	10.53↓	19.83±0.55^{ijk}	3.82↑	0.27±0.05^{de}	39.29↑	0.040±0.0049^{abcd}	↔
ASD8	21.15±0.63^{cd}	41↑	23.75±1.02^{defghı}	31↑	0.39±0.04^{abcde}	39.29↑	0.046±0.0057^{abc}	15↑
ASD9	18.72±0.47^{efgh}	24.8↑	26.70±0.75^{abcd}	39.79↑	0.39±0.034^{abcde}	7.14↑	0.046±0.011^{abc}	15↑
ASD14	16.50±1.17^{iijk}	10↑	21±0.57^{ijk}	9.95↑	0.30±0.058^{bcde}	32.14↑	0.037±0.0058^{cd}	7.5↓
ASD18	15.75±0.51^{kj}	5↑	21.6±0.82^{fghıij}	13.09↑	0.37±0.069^{abcde}	42.86↑	0.045±0.017^{abcd}	12.5↑
ASD19	16.20±0.91^{ijk}	8↑	24.6±0.80^{cdefgh}	28.80↑	0.40±0.058^{abcde}	28.57↑	0.049±0.0017^{abc}	22.5↑
ASD22	18.00±0.96^{ghı}	20	27.16±0.95^{abc}	42.20↑	0.36±0.023^{abcde}	28.57↑	0.042±0.0018^{abcd}	5↑
ASD23	16.05±0.85^{ijk}	7↑	22.65±0.77^{efghıi}	18.59↑	0.36±0.057^{abcde}	10.71↓	0.043±0.002^{abcd}	7.5↑
ASD25	16.06±0.55^{ijk}	7.06↑	20.29±0.71^{iij}	6.23↑	0.25±0.069^{e}	28.57↑	0.034±0.003^{d}	15↓
ASD31	18.50±0.80^{fgh}	23.33↑	24±0.57^{cdefghı}	25.65↑	0.36±0.051^{abcde}	42.86↑	0.045±0.0011^{abcd}	12.5↑
ASD36	17.61±0.22^{hıi}	17.4↑	21.60±1.68^{fghıij}	13.09↑	0.40±0.028^{abcde}	39.29↑	0.054±0.0012^{a}	35↑
ASD38	19.00±0.63^{efgh}	26.66↑	21.14±2.22^{hıij}	10.68↑	0.39±0.011^{abcde}	7.14↑	0.043±0.0058^{abcd}	7.5↑
ASD44	17.29±0.57^{hıij}	14.66↑	21.50±0.86^{ghıij}	12.57↑	0.30±0.034b^{cde}	3.57↓	0.042±0.017^{abcd}	5↑
ASD48	18.90±1.21^{efgh}	26↑	26.80±1.27^{abcd}	40.31↑	0.35±0.014^{abcde}	25↑	0.044±0.0051^{abcd}	10.5↑
ASD54	19.00±0.89^{efgh}	26.2↑	26.50±0.86^{abcde}	38.74↑	0.45±0.021^{ab}	60.71↑	0.052±0.0018^{ab}	30↑
ASD55	20.40±0.66^{cde}	36↑	29.60±1.09^{a}	54.97↑	0.44±0.023^{abc}	57.14↑	0.049±0.011^{abc}	22.5↑
ASD58	19.86±1.02^{defg}	32.4↑	25.50±0.86^{bcdef}	33.51↑	0.43±0.06^{abcd}	53.57↑	0.052±0.023^{ab}	30↑
ASD59	18.79±0.42^{efgh}	25.26↑	24.30±1.55^{cdefghı}	27.23↑	0.41±0.057^{abcde}	46.43↑	0.050±0.0045^{abc}	25↑
ASD64	18.25±0.61^{ghı}	21.66↑	25.93±1.48^{abcde}	35.76↑	0.41±0.057^{abcde}	46.43↑	0.049±0.0057^{abc}	22.5↑
ASD65	20.50±0.97^{cde}	36.66↑	28.65±1.06^{ab}	50↑	0.39±0.023^{abcde}	39.29↑	0.045±0.0023^{abcd}	12.5↑
ASD68	15.75±0.21^{jk}	5↑	16.60±1.68^{k}	13.09↓	0.26±0.034^{e}	7.14↓	0.033±0.0058^{d}	17.5↓
ASD69	20.25±0.57^{cdef}	35↑	24.30±1.55^{cdefghı}	27.23↑	0.43±0.057^{abcd}	53.57↑	0.053±0.017^{ab}	32.5↑

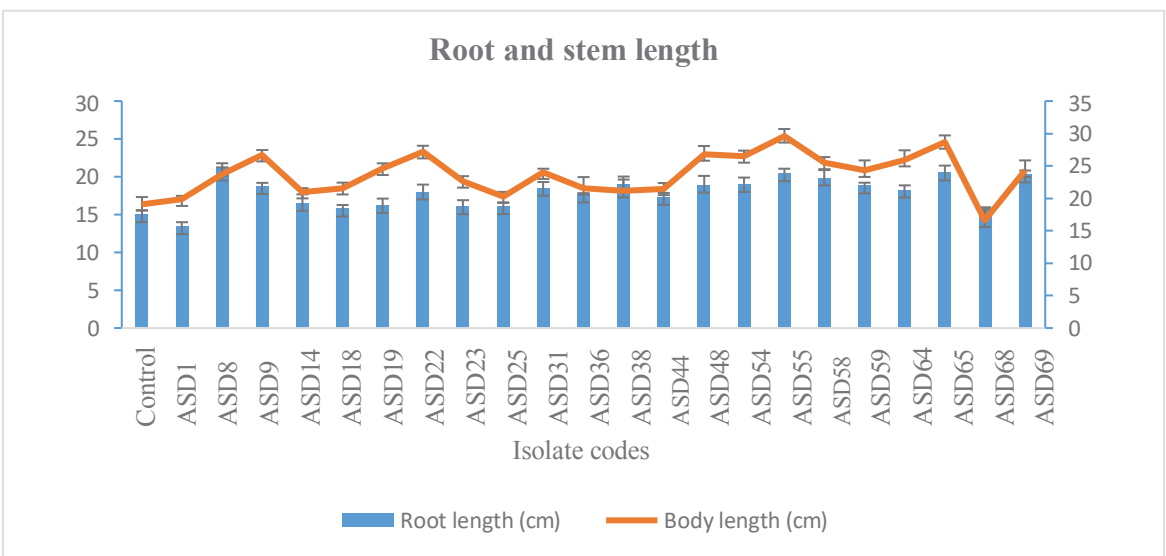

Figure 4. The effects of salt irrigation in different concentrations on the root and stem length of the wheat plant.

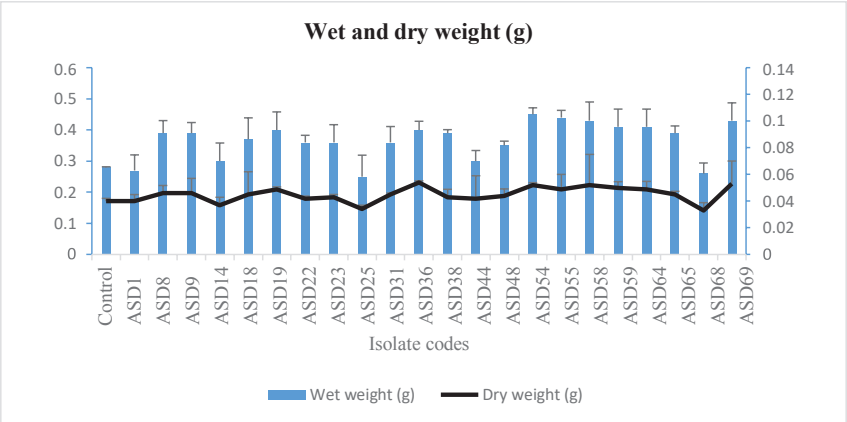

Figure 5. The effects of salt irrigation in different concentrations on wet and dry weight of wheat plants.

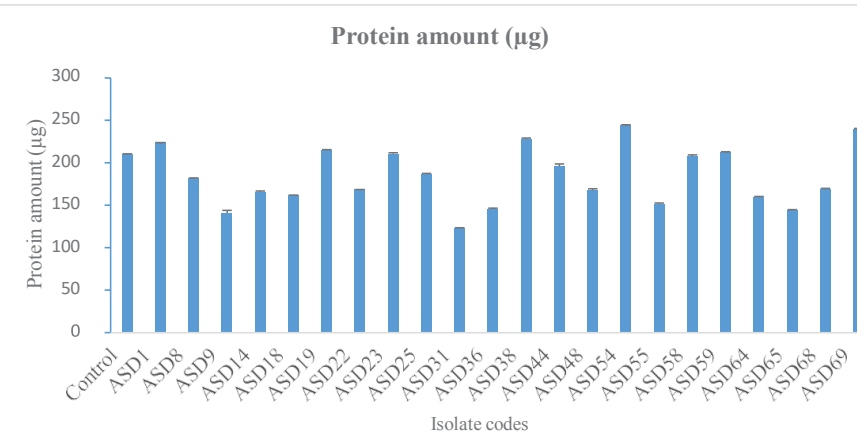

Figure 6. The effects of salt irrigation in different concentrations on protein content in wheat plants.

Table 3. Statistical analyses of protein, chlorophyll (chl.) a, chlorophyll b, total chlorophyll, and carotenoid amounts on wheat plants and their percentage increases (↑) and decreases (↓).

Isolate no.	Protein amount (µg)	mg of chl. a per g of tissue	mg of chl. b per g of tissue	mg of total chl. per g of tissue	% Total chlorophyll content	mg of total carotenoids per g of tissue	% Total carotenoids
Control	210.02±0.58^{de}	8.89±0.27^{i}	3.89±0.04^{h}	12.71±0.35^{ij}		1.49±0.10^{cdef}	
ASD1	223.80±0.11^{bc}	13.81±0.39^{bcdefg}	5.96±0.07^{cdef}	19.65±0.53^{bcdefgh}	54.60↑	2.45±0.14^{abcd}	64.43↑
ASD8	181.44±0.81^{g}	14.04±0.40^{bcdef}	5.64±0.15^{def}	19.53±0.63^{bcdeg}	53.66↑	3.04±0.16^{ab}	104.03↑
ASD9	141.07±3.02^{i}	15.38±1.44^{bcde}	7.02±0.99^{cd}	22.30±1.49^{bcdef}	75.45↑	2.58±0.93^{abcd}	73.15↑
ASD14	165.75±1.18^{h}	15.92±1.55^{bcde}	6.76±0.61^{bcd}	22.56±1.22^{bcde}	77.50↑	2.63±0.59^{abcd}	76.51↑
ASD18	161.38±0.70^{h}	16.98±0.72^{ab}	7.52±0.28^{ab}	24.36±1.06^{ab}	91.66↑	2.73±0.27^{abc}	83.22↑
ASD19	215.18±0.49^{cde}	16.03±1.08^{bcd}	7.18±0.33^{abc}	23.09±1.09^{bcde}	81.67↑	2.83±0.42^{abc}	89.93↑
ASD22	168.39±0.22^{h}	13.40±0.68^{cdefgh}	5.89±0.21^{cdef}	19.18±0.95^{cdefghı}	50.60↑	2.35±0.23^{abcde}	57.72↑
ASD23	210.88±1.07^{de}	12.99±1.31^{defgh}	5.80±0.47^{efgh}	17.25±0.98^{ghi}	35.72↑	2.16±0.49^{abcde}	44.97↑
ASD25	186.72±0.93^{fg}	10.22±1.37^{hı}	4.46±0.90^{gh}	14.63±1.31^{iij}	15.11↑	1.78±0.90^{bcdef}	19.46↑

Table 3. (Continued.)

ASD31	123.28±0.35^{j}	12.43±0.11^{efgh}	5.32±0.02^{efg}	17.54±0.28^{fghı}	38.00↑	1.99±0.04^{abcde}	33.56↑
ASD36	146.13±0.51^{i}	10.38±0.46^{ghı}	4.57±0.14^{gh}	14.86±0.65^{hij}	16.92↑	1.73±0.17^{bcdef}	16.11↑
ASD38	228.14±1.18^{b}	11.10±0.39^{fghı}	4.75±0.11g^{h}	15.76±0.48^{ghij}	23.99↑	1.90±0.14^{bcdef}	27.52↑
ASD44	196.19±2.55^{f}	8.81±0.48^{ı}	3.38±0.21^{h}	12.62±0.63^{ij}	0.07↓	1.56±0.16^{cdef}	4.70↑
ASD48	167.79±1.72^{h}	13.83±0.66^{bcdefg}	6.03±0.16^{cdef}	19.75±0.95^{bcdefg}	55.39↑	2.27±0.25^{abcde}	52.35↑
ASD54	244.78±0.10^{a}	19.83±0.72^{a}	8.33±0.36^{a}	27.99±0.98^{a}	120.22↑	3.28±0.29^{a}	120.13↑
ASD55	151.98±0.77^{ıi}	12.97±0.71^{defgh}	5.70±0.2^{efgh}	18.54±1.01^{defghı}	45.87↑	2.05±0.24^{abcde}	37.58↑
ASD58	207.83±1.63^{e}	8.21±0.63^{ı}	3.84±0.75^{h}	12.16±0.87^{j}	0.87↓	1.36±0.24^{def}	8.72↓
ASD59	211.74±1.40^{de}	12.90±1.01^{defgh}	5.60±0.21^{defg}	18.38±1.08^{efghı}	44.51↑	2.16±0.75^{abcde}	44.97↑
ASD64	159.37±1.06^{hı}	14.40±0.69^{bcdef}	6.22±0.36^{cde}	20.50±0.97^{bcdefg}	61.29↑	2.24±0.26^{abcde}	50.34↑
ASD65	144.25±0.65^{i}	16.37±1.07^{bcd}	7.02±0.14^{bc}	23.48±1.01^{bc}	84.74↑	2.58±0.40^{abcd}	73.15↑
ASD68	169.01±1.03^{h}	13.12±0.54^{cdefgh}	5.52±0.43^{defg}	18.53±0.75^{defghı}	45.79↑	2.01±0.20^{abcde}	34.90↑
ASD69	239.70±0.88^{a}	16.37±0.76^{bcd}	7.06±0.16^{bc}	23.31±1.01^{bcd}	83.40↑	2.54±0.29^{abcd}	70.47↑

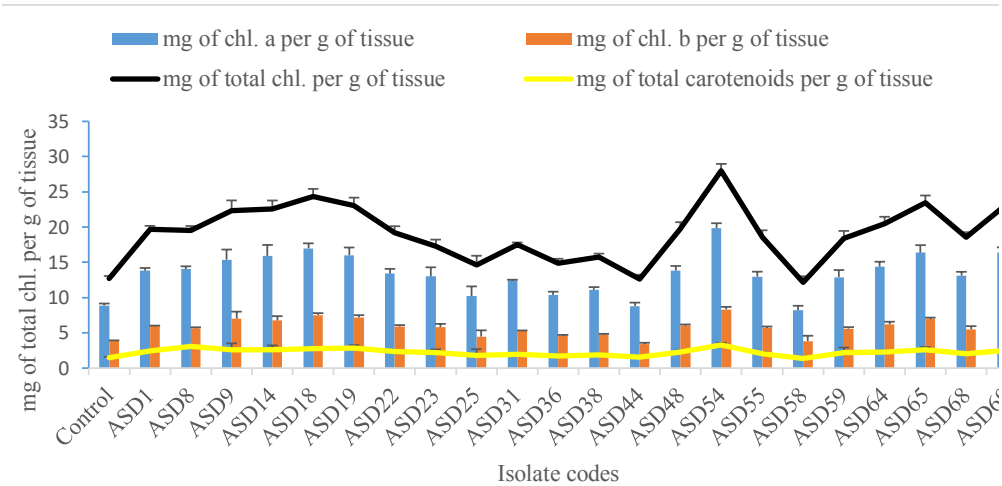

Figure 7. Chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoid content in wheat plants irrigated with NaCl at different concentrations of treatments.

ASD65 isolates had a positive effect on the increase in chlorophyll b content. ASD44 and ASD58 isolates had a negative effect on chlorophyll b content compared to the control group. These isolates and combinations, which had negative effects on chlorophyll a and chlorophyll b content, also had negative effects on total chlorophyll content. ASD54, ASD18, ASD65, and ASD69 isolates had a positive effect on total chlorophyll content. According to the effects of the isolates on the total carotenoid content in wheat plants, ASD54, ASD8, ASD18, and ASD19 isolates showed positive effects. According to the percentage increase and decrease rates of inoculation with isolates on total chlorophyll content in wheat plants, ASD54, ASD18, ASD65, and ASD69 isolates showed maximum positive effect compared to the control group and provided a significant increase in total chlorophyll content. Isolates ASD44 and ASD58 caused a decrease in total chlorophyll content compared to the control group. According to the percentage increase and decrease ratios of inoculation with isolates on total chlorophyll content in wheat plants, ASD54, ASD8, ASD19 and ASD18 isolates had a positive effect on total carotenoid content compared to the control group. Only ASD58 isolate had a negative effect and caused a decrease in total carotenoid content.

4. Discussion

In this study, we aimed to isolate new halophilic PGPRs based on salt stress-PGPR applications, which are the focus of current PGPR studies, and to determine the usability of the new isolates for wheat cultivation in saline soils. For this purpose, various levels of salt-tolerant bacteria were isolated from different habitats containing high levels of salt. These bacteria were then tested for various PGP properties (nitrogen fixation, solubilizing insoluble

phosphate, IAA production, ACC deaminase production, and siderophore production) and according to their activities, the isolates (22) to be used in pot experiments were decided. Finally, these isolates were taken into pot experiments to be irrigated with water containing various ratios of salt and the results were discussed in the light of the literature. According to Kushner (1978, 1993), our 22 bacterial isolates can be classified as intermediate halophiles. Our isolates ASD8, ASD9, ASD22, ASD23, ASD31, ASD36, ASD38, ASD44, ASD48, ASD59, and ASD64 also grew on media containing 2.5 M NaCl and can be classified as extreme halophiles according to Margesin and Schinner (2001). It was determined that 80.66% of these halophilic isolates were gram-positive and 13.63% were gram-negative. All of our total halophilic isolates were found to exhibit significant positive traits including nitrogen fixation, IAA production, siderephore production, phosphate solubilizer, and ACC deaminase production. These PGP properties of our isolates were compared with the phosphate solvent and siderephore producing, ACC deaminase and IAA producing properties of various PGPR bacteria in the literature. When the literature was examined, it was found that ASD23, ASD19, ASD14, and ASD25 isolates had phosphate solubilizing properties; ASD8, ASD9, ASD14, ASD19, ASD23, ASD25, and ASD45 isolates had siderophore production properties; ASD18, ASD25, ASD14, ASD8, and ASD64 isolates in terms of ACC deaminase production; ASD25 and ASD64 isolates in terms of IAA production have much higher potential than the isolates determined in the literature.

Among our isolates whose PGP properties were determined, the ones that were good in terms of 4–5 parameters measured were selected instead of the ones that were good in terms of a single parameter and these isolates were used in pot experiments. These parameters are the most frequently used in both physiological research and PGPR research (Egamberdieva and Kucharova 2009; Ramadoss et al., 2013; Singh et al., 2016).

Our pot experiment results are summarized (Tabless 2 and Table 3). According to these results, when each of our results were compared with the control group; ASD8, ASD65, and ASD69 isolates were found to be the most successful isolates in plant root length and they provided a yield increase of 5%–41%. In plant stem length, ASD9, ASD22, ASD48, ASD54, ASD55, ASD55, and ASD65 isolates were found to be the most successful isolates and in yield increases between 3.82% and 54.97% were achieved. On wet weight, ASD54 and ASD55 isolates were found to be the most successful isolates and yield increases between 7.14% and 60.71% were achieved. ASD1, ASD25, and ASD68 isolates caused decreases in yield on stem length. ASD19, ASD36, ASD54, ASD54, ASD55, ASD58, ASD59, ASD64, and ASD69 isolates were found to be the most successful isolates on dry weight and they provided a yield increase of 5%–32.5%. ASD1, ASD14, ASD25, and ASD68 isolates caused decreases in yield on stem length. ASD1, ASD38, ASD54, ASD69, ASD54, and ASD69 isolates were found to be the most successful isolates on dissolved protein content and yield increases of 13.78%–29.68% were achieved. All isolates except from ASD1, ASD38, ASD54 and ASD69 isolates caused decreases in yield on protein amount compared to the control. ASD18, ASD54, ASD65 and ASD69 isolates were found to be the most successful isolates on total chlorophyll content and they provided yield increases between 83.40% and 120.22%. ASD44, ASD58 isolates decreases in yield on total chlorophyll content. ASD8, ASD18, ASD19, ASD19, and ASD54 isolates were found to be the most successful isolates on total carotenoid amount and they provided yield increases between 45.79% and 120.22%. Our ASD58 isolate caused decreases in yield on total carotenoid amount. In all these results, we should not overlook that all of our isolates were significantly effective both in terms of PGP characteristics and in pot experiments. According to the results of our pot experiment, our bacteria, which were generally found to be successful and 16S rRNA analyzed, were species that were previously used in PGPR studies and found to be more or less successful. *Sphingomonas echinoides* and *Leucobacter iarius*, which were found to be successful in our research, are the species whose PGPR success was determined for the first time in this study. Most of the inoculants and combinations we used in our study, for example; ASD55 and ASD65 isolates inoculants were much more successful than the inoculants used in previous studies. Inoculations made with both the isolates in our study and our combinations were much more effective and provided significant yield increases on wheat plants irrigated with saline irrigation compared to previous studies. In this context, we can say that the isolates and combinations we obtained stimulate plant growth under saline conditions and have biofertilizer potential for wheat plants under saline conditions.

In our study, our isolates coded ASD8, ASD9, ASD19, ASD31, ASD38, ASD48, ASD54, ASD55, ASD58, ASD59, ASD64, ASD65, and ASD69 demonstrated success in saline conditions. Further investigations into biofertilizer production should involve conducting field trials to identify suitable inoculants and carrier materials that perform well in field conditions, potentially contributing to new projects. In this context, other isolates with good PGP properties and binary and triple combinations should be evaluated in further studies. *Sphingomonas echinoides* and *Leucobacter iarius*, which were found to be successful in this study and diagnosed through 16S rRNA analysis, were previously unknown species according to our literature research. This finding is a first and adds originality to our research.

5. Conclusion

This study was conducted to explore the beneficial effects of isolated halotolerant PGPR based on salt stress applications on the growth of wheat plant under greenhouse conditions. Under saline conditions, halo-tolerant PGPR, play a vital role in the amelioration of physiological abnormalities induced by salts in plants. Halotolerant PGPR are involved in inducing the salt tolerance in various plants to help them survive under saline conditions and followed by improvement in their morphological parameters. Halotolerant PGPR are natural microflora that enhances plant growth and crops productivity but all of these are not explored yet. In the future, halotolerant PGPR can be utilized as biofertilizers to mitigate salt stress and enhance crop production in an economically sustainable manner.

Conflict of interest

The authors declare no conflicts of interest.

References

Aydogan MN, Algur OF (2014). *Solubilization of Mazidagi* rock phosphate by locally isolated Aeromonas hydrophyla MFB-25. Journal of Pure and Applied Microbiology 8:413-420.

Bacilio M, Rodriguez H, Moreno M, Hernandez Juan-Pablo Bashan Y (2004). Mitigation of salt stress in wheat seedlings by a gfp-tagged *Azospirillum lipoferum*. Biology and Fertility of Soils 40 (3):188-193.

Baldani JI Reis VM, Videira SS, Boddey LH, Baldani VLD (2014). The art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semi-solid media: a practical guide for microbiologists. Plant and Soil 384 (1-2): 413-431.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72 (1-2): 248-254.

Cao Y, Liao L, Xu X-w, Oren A, Wang C et al. (2008). Characterization of alcohol dehydrogenase from the haloalkaliphilic archaeon *Natronomonas pharaonis*. Extremophiles 12:471-476.

Dimkpa C, Weinand T, Ash F (2009). Plant-rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell and Environment 32: 1682–1694.

Egamberdieva D, Kucharova Z (2009). Selection for root colonising bacteria stimulating wheat growth in saline soils. Biology and Fertility of Soils 45 (6): 563-571.

Egamberdieva D (2009). Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. Acta Physiologiae Plantarum 31 (4): 861-864.

Glick BR (2014). Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiological Research 169: 30–39.

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D et al. (2010). Food security: The challenge of feeding 9 billion people. Science 327: 812-818.

İşler E, Coşkan A (2009). Farklı bakteri (*Bradyrhizobium Japonicum*) aşılama yöntemlerinin soyada azot fiksasyonu ve tane verimine etkisi. Ankara Üniversitesi Ziraat Fakültesi Tarım Bilimleri Dergisi 15 (4): 324-331 (in Turkish).

Honma M, Shimomura T (1978). Metabolism of 1-aminocyclopropane-1-carboxylic acid. Agricultural and Biological Chemistry, 42 (10), 1825-1831.

Jackson ML (1973). Soil chemical analysis, advanced course: publ. by the author, Dept. Soil Science, Univ. Wisc., Madison, Wisconsin, 183-204.

Kaçar B (1972). Toprak ve Bitki Analizleri, Ankara Üniversitesi. Basımevi, Ankara (in Turkish).

Kushner DJ (1978). Life in High salt and solute concentrations: halophilic bacteria. Microbial Life in Extreme Environments. London, UK: Academic Press, pp. 317-368.

Kushner DJ (1993). Growth and nutrition of halophilic bacteria, p. 87-103. In: Vreeland RH and Hochstein LI (editors), The Biology of Halophilic Bacteria. Boca Raton, FL, USA: CRC Press.

Lugtenberg B, Chin-A-Woeng T, Bloemberg G (2002). Microbeplant interactions: principles and mechanisms. Antonie Van Leeuwenhoek 81: 373–383.

Margesin R, Schinner F (2001). Bioremediation (natural attenuation and biostimulation) of diesel-oil-contaminated soil in an alpine glacier skiing area. Applied and environmental microbiology 67 (7): 3127-3133.

Mehta S, Nautiyal CS (2001). An efficient method for qualitative screening of phosphate-solubilizing bacteria. Current Microbiology 43 (1): 51-56.

MIS (2005). MIS Operating Manual. 125 Sandy Dr. Newark, DE.

Park M, Kim C, Yang J, Lee H, Shin W et al. (2005). Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. Microbiological Research 160 (2): 127-133.

Patten CL, Glick BR (2002). Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Applied and Environmental Microbiology 68 (8): 3795-3801.

Paul D (2012). Osmotic stress adaptations in rhizobacteria. Journal of Basic Microbiology pp. 52:1-10.

Ramadoss D, Lakkineni VK, Bose P, Ali S, Annapurna K (2013). Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. Springer Plus 2 (6): 1-7.

Sarwar M, Kremer RJ (1995). Determination of bacterially derived auxins using a microplate method. Letters in Applied Microbiology 20 (5): 282-285.

Schwyn B, Neilands JB (1987). Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry 160 (1): 47-56.

Shahbaz M, Ashraf M (2013). Improving salinity tolerance in cereals. Critical Reviews in Plant Sciences 32: 237–249.

Shannon MC, Grieve CM (1999). Tolerance of vegetable crops to salinity. Science Horticulturae 78: 5-38.

Shrivastava UP, Kumar A (2013). Characterization and optimization of 1-Aminocyclopropane-1-Carboxylate Deaminase (ACCD) activity in different rhizospheric PGPR along with *Microbacterium* sp. strain ECI-12A. International Journal of Applied Sciences and Biotechnology, 1 (1), 11-15.

Singh RP, Jha PN (2016). Alleviation of salinity-induced damage on wheat plant by an ACC deaminase-producing halophilic bacterium *Serratia* sp. SL-12 isolated from a salt lake. Symbiosis 69 (2): 101-111.

Singh RP, Jha PN (2016). Mitigation of salt stress in wheat plant (*Triticum aestivum*) by ACC deaminase bacterium *Enterobacter* sp. SBP-6 isolated from *Sorghum bicolor.* Acta Physiologiae Plantarum 38 (5):1-12.

Strain HH, Svec WA (1966). Extraction, separation, estimation and isolation of the chlorophylls. The chlorophylls,1, 22-66.

Wilson PW, Knight SC (1952). Experiments in bacterial physiology. Burgess Publishing Company, Minneapolis, MN.

Yamaguchi T, Blumwald E (2005). Developing salt-tolerant crop plants: challenges and opportunities. Trends in Plant Science 10 (12): 615– 620.

Yao L, Wu Z, Zheng Y, Kaleem, I and Li C. (2010). Growth promotion and protection against salt stress by *Pseudomonas putida* Rs-198 on cotton. European Journal of Soil Biology 46 (1), 49-54.

Yıldız N and Bircan H (1991). Araştırma ve deneme metotları. Atatürk Üniversitesi, 697 (in Turkish).