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Research Article

Evaluation of three groups of plant growth-promoting extremophilic rhizospheric bacteria on induction of salinity and alkalinity tolerance in GN15 almond (*Prunus amygdalus* L.) rootstocks

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Abstract

The use of indigenous extremophilic bacteria with plant growth-promoting haloalkaliphilic properties will help to cope with biotic and abiotic stresses. The present study focused to investigate the effect of these bacteria to induce salinity resistance in almond rootstocks under soil salinity and alkalinity and compare it with non-stress conditions. The roots of 108 Garnem rootstocks in four different soils (2, 4, 8, and 16 dS m^{-1}) were inoculated with two bacterial strains and sterilized control from three groups of halophilic, alkaliphilic, and haloalkaliphilic isolated from the rhizosphere of almond's cultivation sites. Plant growth-promoting properties, including tri-indole acetic acid production, phosphate mineral dissolution, and exo-polysaccharide production, were measured for alkaliphilic isolates (213.33, 127.55, and 578.11 mg L^{-1}), haloalkaliphilic isolates (77.13, 73.99, and 284.54 mg L^{-1}), and halophilic isolates (15.98, 40.19 and 35.90 mg L^{-1}, respectively). The inoculated plants with halophilic bacteria were found to better grow compared to other inoculated plants. In addition, these plants accumulated more chlorophyll, sugar, and proline. The root fresh and dry weights were maximum by the haloalkaliphilic bacteria inoculation. The alkaliphilic and halophilic bacteria also caused a higher increase in fresh and dry weights of shoots. As well, root and shoot moisture contents were the highest ones in the 16 and 8 dS m^{-1} salinity by haloalkaliphilic inoculation. In general, the application of haloalkaliphilic bacteria was found to have a better effect on root growth and halophilic bacteria in the morphological and physiological properties of almond rootstocks. Compared with 8 dS m^{-1} and other salinity levels, extremophile bacteria had a stronger effect on soil salinity and alkalinity mitigation for almond rootstocks at 16 dS m^{-1} salinity.

Keywords: Exo-polysaccharide, Haloalkaliphilic bacteria, Prolin, Root-to-shoot ratio, Strain, Tri-indole acetic acid

Introduction

Both soil and water salinity and alkalinity are increasingly considered serious threats to agriculture in arid and semi-arid regions like Iran (Ranjbar and Pirasteh-Anosheh, 2015). Of note, Iran is a country with large saline and desert areas, about 12.5% (equal to 204800 km^2) of the lands of which, with saline and alkaline soils, are located in arid and semi-arid regions (Bagheri Rad *et al*., 2007).

Almond (*Prunus amygdalus* L.) is one of the oldest and the most important dried fruits worldwide, the production of which is increasing in recent years (Karimi, 2015). It is estimated that 160,000 hectares of orchards in Iran are dedicated to almond production at the moment (Rahnamoon, 2018). Almond yields are reduced by 25%, 50%, and 100% in electrical conductivity of 2.8, 4.8, and 7 dS m^{-1} respectively (Zarin Kafash, 1992). As well, the almond plant is resistant up to 1.1 g of chlorine per liter in irrigation water (Zarin Kafash, 1992). Garnem (GN15) rootstock, which is a hybrid of peach and almond species, is one of the rootstocks recommended for almond plant due to its salinity and drought-tolerance properties (Ganji Moghaddam and Zamanipour, 2020). Therefore, the selection of salt-tolerant rootstocks in almond is a very suitable strategy used to reduce the effects of salinity, especially in arid regions and saline-alkaline soils. However, only performing this strategy due to the increasing area and amount of salt and alkali soils, is not enough (Eskandari Torbagham, 2017).

Recent studies have shown that soil microorganisms can make plants be more resistant to salinity and

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alkalinity stress. Halophilic and alkaliphilic bacteria in highly saline and alkaline environments can be adapted to the conditions with high salt and pH, and require a certain amount of different types of salt such as sodium chloride and sodium carbonate, for having an optimal growth (Venkateswarlu and Shanker, 2009). Halophilic microorganisms are found in all three domains of archaea, bacteria, and eukaryotes (Zhuang *et al*., 2010). Accordingly, halophilic can create an osmotic balance due to a large amount of salt inside the cell, and the concentration of salt, especially potassium chloride, inside their cells, usually reaches 5 mol. Halophilic proteins, which need this amount of salt for stability and proper functioning, are adapted (Ventosa *et al.*, 2008). Alkaliphilic bacteria maintain their pH around 9.5 in the ambient pH ranged from 9 to 11. These bacteria continue to perform their function with proton transport systems in the cytoplasmic membrane (ATP pump and sodium-with-proton exchange pump) (Horikoshi, 2006). Notably, terrestrial alkaliphilic bacteria are often *bacillus* species (Horikoshi, 2006). Another group of bacteria that can grow under alkaline conditions and in the presence of salt, is known as haloalkaliphilic. Correspondingly, these bacteria were indicated to have adaptation or resistance procedures for their survival and growth under high salinity and pH conditions, which include optimizing the main processes of the cell, enzymatic integration, transportation of essential nutrients both to the outside and inside the cell, and cell membrane function (Singh *et al*., 2010). Some studies have previously shown that another functional potential of these haloalkaliphilic microorganisms is their plant growth-promoting activities (PGPHA), which could lead to better coping with biotic and abiotic stresses (Ganji Moghaddam and Zamanipour, 2020). These microorganisms can be isolated and also propagated from different saline and alkaline environments like marine environments with a low level of salinity to very saline lakes and soil sources like the rhizosphere of plants located in brackish soils (Ganji Moghaddam and Zamanipour, 2020). In the study by Sahay *et al.* (2012), it was shown that among 32 groups of haloalkaliphilic PGPR bacteria, six isolates have phosphate solubilization potential and the SL32 isolate has the highest phosphorus solubility with 981.32 mg L^{-1} phosphate. Moreover, they showed that 50% of the haloalkaliphilic PGPR isolates, which were isolated from Lake Sambar in India, are capable of producing indole-3-acetic acid (IAA). The quantitative comparison of ammonia and tri-indole acetic acid productions in halophilic, alkaliphilic, and haloalkaliphilic bacterial isolates obtained from Khorasan Razavi soils (Eskandari Torbaghan *et al*., 2017) showed that alkaliphilic isolates have the highest ammonia productions (0.055%) among three groups of bacteria, which are 9.5 and 13 times higher than the average of haloalkaliphilic isolates (0.0058%) and halophilic isolates (0.004%), respectively. It was found that most halophilic, alkaliphilic, and haloalkaliphilic bacterial isolates produce IAA with an average of 0.0003, 0.0001, and 0.0021%, respectively. Accordingly, the amount of production in haloalkaliphilic was about 6 and 14.5 times more than those of halophilic and alkaliphilic bacteria, respectively (Eskandari Torbaghan *et al*., 2017). This study showed these 3 groups of bacteria were different in the type and amount of PGP compounds produced with each other (Eskandari Torbaghan *et al*., 2017). Both the isolation and identification of exogenous polysaccharide-producing bacteria in saline soils (Moshabaki *et al*., 2017) showed that the amount of the production of exopolysaccharides by *Citrobacter freundii ATHM38* strain significantly increased ($P < 0.05$) with the increasing salt amount. Therefore, based on these results, this bacterium was selected as the superior strain with the ability to grow in a medium with 25% salt and to produce 168.0 g L^{-1} exopolysaccharide during 24 hours. Changing the amount of sodium and improving the physical condition of the rhizosphere by producing exopolysaccharides (Upadhyay *et al*., 2011), lead to the osmotic regulation of the plant. PGPR isolates by producing exopolysaccharides (which is binding sodium and reduce its availability for plant uptake) (Ashraf *et al*., 2004) and ACC deaminase (which is changing the selectivity of both sodium and potassium for the uptake of the plant) may all decrease the negative effects of salinity stress via reducing sodium adsorption, which consequently increases the ratio of potassium to sodium in plants (Ashraf *et al*., 2004). This study attempted to investigate the effects of halophilic, alkaliphilic, and haloalkaliphilic bacteria isolated from the rhizosphere of almond sites in Khorasan Razavi on the induction of salinity resistance in almond rootstock (GN) under both non-salinity (2 and 4 dS m^{-1}) and salinity (8 and 16 dS m^{-1}) stress conditions of the soil.

Materials and methods

Collect rhizospheric soil: In order to isolate the native halophilic, alkaliphilic, and haloalkaliphilic bacteria, the soil sampling process was performed in four regions of the rhizosphere consisting of different almond groves in Khorasan Razavi province from the depth of the almond rhizosphere (30-50 cm). The geographical characteristics of the sampling site were recorded using GPS (Table 1). The obtained samples were then transferred to the laboratory in sterile containers in less than 48 hours at 4 °C.

Isolation and purification: The isolation and purification of 54 isolates obtained from halophilic, alkaliphilic, and haloalkaliphilic groups from the soil samples were performed using their specific culture medium (Table 2). Thereafter, these purified isolates were preserved for the long-term using the liquid nitrogen method (Horikoshi, 1999).

PGPR characteristics measurements in strains: After the isolation, to select the best strains, some of their plant growth-promoting activities, including the production of tri-indole acetic acid (Glickmann and

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Table 1. Geographical data of rhizosphere of almond groves sampled from Khorasan Razavi province (Iran)

No.	Location of sampling	Geographic coordinate of sampling					Height (m)	Number of samples
		Second	Minute		Degree	Aspect		
1	Soleimania village - Sabzevar Quchan road	37	10	15	36	N■	1046	1
		34	10	46	57	E△		
2	Klavash village-sheshtamad of Sabzevar	74	38	02	36	N	1045	1
		24	33	46	57	E		
3	Kizur village- sheshtamad of Sabzevar	85	28	59	35	N	1185	1
		49	03	45	57	E		
4	Chehelpo village - Kuhsorkh areaa - Kashmar	89	01	38	35	N	1783	1
		73	12	31	58	E		

East direction E△, North direction N■

Table 2. Specific culture media of halophilic, alkaliphilic, and haloalkaliphilic bacterial isolates

Compounds	Amount (g L^{-1})		
	Halophile■	Alkalophile	Haloalkalophile
Glucose	1	10	-
Poly Peptone	-	5	-
Yeast extract	10	5	10
Di potassium hydrogen phosphate	-	1	-
Magnesium sulphate seven H_2O	9.6	0.2	1
Sodium carbonate	-	10*	18.5▲
Sodium Chloride	81	-	200
Magnesium chloride two H_2O	7	-	-
Calcium chloride	0.36	-	-
Potassium chloride	2	-	2
Sodium hydrogen bicarbonate	0.06	-	-
Sodium bromide	0.026	-	-
Protease Peptone	5	-	-
Casino acid	-	-	7.5
Tri sodium citrate	-	-	3
Manganese (II) chloride	-	-	0.00036
Ferrous sulfate	-	-	0.05
Agar	15	20	20

■pH was adjusted with KOH 1 N on 7.2 before the medium culture sterilization
▲Was sterile from other materials separately and was added to culture medium before isolates cultivation

Dessaux, 1995), production of exopolysaccharides (Ventosa *et al*., 2008), quantitative amount of mineral phosphate solubility (Tricalcium Phosphate) by Sperber medium, were determined under laboratory conditions.

Plant materials: Garnem (GN15) almond rootstocks (Called GN in this text) obtained from Royan Nahal Mahalat farm (http://www.royannahal.ir/) were used in this experiment.

Testing the PGPR characterization of strains under potting conditions: In order to investigate some growth characteristics of GN almond rootstock under non-stress and soil salinity and alkalinity stress conditions, two superior strains, which were identified and isolated from each bacteria group (halophilic, alkaliphilic, and haloalkaliphilic) with the highest production potential related to plant growth-promoting characteristics (including IAA, PSB, and EPS) along with control, were tested on almond GN (Garfi x Nemared) rootstock (salt-tolerant rootstock) under open-air and potted conditions. Factorial experiments are performed based on a completely randomized design with the following three factors: (1) different soil EC levels, including S2=2 dS m^{-1}, S4=4 dS m^{-1}, S8=8 dS m^{-1}, and S16=16 dS m^{-1}; (2) bacterial type (halophilic, alkaliphilic, and haloalkaliphilic); and (3) strain type (selected from any type of bacteria group) and sterilized control (inoculum with the type of bacteria consumed in their own culture medium, which was sterilized), were performed in this study in three replications.

Soil preparation and rootstocks bacteria inoculation to the GN15 rootstocks: In order to prepare the substrate culture medium for rootstocks, we considered two main characteristics, according to the characteristics of bacterial isolates, including (1) the amount of electrical conductivity (EC) and (2) the sodium adsorption ratio (SAR) of the soil. Four types of saline and alkaline soils with 2, 4, 8, and 16 dS m^{-1} electrical conductivities and SARs of 9.69, 14.99, 14.21, and 19.72, respectively, were prepared from the composition and mixing of three groups of different natural soils (Table 3). The isolates were then inoculated into the roots of 108 almond GN rootstocks through a fresh bacterial culture medium with 10^7 to 10^8 cells per ml. Thereafter, each one of the inoculated rootstocks was planted in a pot with dimensions of 26 × 17 × 23 cm, which contained 8 kg of the four mixed soil

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Table 3. Characteristics of three groups of different natural soils used to prepare the culture substrate for rootstocks

No	Parameter	Unit	River Sand	Shir Hesar zona	Sahab soil
1	Sand	%	99.4	70.7	70.4
2	Silt	%	0.6	16.9	24.6
3	Clay	%	0	12.4	5
4	Soil Texture	-	Sand	Sandy Loam	Sandy Loam
5	pH	-	8.55	7.9	8.1
6	EC	dS m^{-1}	0.540	60.32	1.18
7	SP	%	31.48	44.41	34.32
8	N_t	%	0.01915	0.02335	0.03
9	P_{avl}	mg L^{-1}	0.164	2.65	7.86
10	K_{avl}	mg L^{-1}	2.7	11.25	15.53
11	OC	%	0.2225	0.2709	0.3483
12	Lime	%	10.75	49.75	13
13	Gypsum	%	5.085	5.848	0.2063
14	Na	meq L^{-1}	0	526.99	0
15	Ca+Mg	meq L^{-1}	20.8	76	40
16	SAR	-	0	85.47	0
17	$SO_4^=$	meq L^{-1}	6.75	754.11	14.75
18	Cl^-	meq L^{-1}	1.2	386.8	5.2

Table 4. Characteristics of four mixed soils were used as a culture substrate for rootstocks

No	Parameter	Unit	2 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	16 dS m^{-1}
1	Sand	%	72.8	64.8	58.8	62
2	Silt	%	18	22	26	21
3	Clay	%	9.2	13.2	15.2	17
4	Soil Texture	-	Sandy loam	Sandy loam	Sandy loam	Sandy loam
5	pH	-	7.35	7.17	6.98	6.72
6	EC	dS m^{-1}	2.18	4.12	8.00	16.42
7	SP	%	24.31	23.21	23.70	24.37
8	N_t	%	0.0247	0.0159	0.08945	0.0324
9	P_{avl}	mg L^{-1}	0	0	0	0
10	K_{avl}	mg L^{-1}	6.97	6.97	6.97	5.47
11	OC	%	0.287	0.185	0. 380	0.375
12	Lime	%	9.5	13.5	19	52.25
13	Gypsum	%	0.4006	0.7775	1.531	3.167
14	Na	meq L^{-1}	71	124.0	139.0	200
15	Ca+Mg	meq L^{-1}	107.37	136.9	191.36	205.7
16	SAR	-	9.69	14.99	14.21	19.72
17	$SO_4^=$	meq L^{-1}	27.25	51.5	100.0	205.25
18	Cl^-	meq L^{-1}	8.0	34.0	82.4	271

(Table 4).

Measurement of morphological traits: By passing four months from the transfer of almond tissue culture rootstocks to the mixed soils (Table 4) and bacterial inoculation, some growth characteristics of rootstocks, including plant height, stem diameter (calculated using a caliper with 0.01 accuracy), leaf area, number of lateral branches, number of leaves, leaf losses, and chlorophyll index (SPAD) were measured and the plants were then harvested from the soil's surface. Afterward, the fresh weights of shoots and roots were separately measured and after placing in the oven for 48 hours at 72 °C, their dry weights and moisture contents were calculated.

Measurement of biochemical traits: Proline (Bates *et al*., 1973), soluble, insoluble and total sugar amount (Dubois *et al*., 1956) were also determined in both dry and ground samples.

Statistical analysis: Data analyses were performed using MSTAT-C software and to compare the means, the least significant difference test (LSD) was used at the probability level of 5%.

Results

Evaluation of IAA, PSB and EPS production in the isolates: By comparing tri-indole acetic acid productions in the halophilic isolates, it was shown that only 11 isolates, namely *H12*, *H10*, *H5*, *H22*, *H19*, *H15*, *H17*, *H26*, *H11*, *H18*, and *H9*, produced tri-indole acetic acid between 395 and 7.190 mg L^{-1} (Table 5). The highest amounts of IAA production in alkaliphilic isolates with 1815, 1554, 1355, 1202, and 801.7 mg L^{-1}, were observed in *A16*, *A11*, *A12*, *A7*, and *A14*, respectively, showing several significant differences ($P<0.05$) (Table 5). The amount of IAA in the remained alkaliphilic isolates was calculated less than 369.2 mg L^{-1}. As well, all the haloalkaliphilic isolates produced

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Table 5. Mean Comparison of IAA, PSB, and EPS concentrations in halophilic, alkaliphilic, and haloalkaliphilic bacterial isolates

No of Strain	Halophilic			No of Strain	Alkaliphilic			No of Strain	Haloalkaliphilic		
	IAA (mg L^{-1})	PBS (mg L^{-1})	EPS (mg L^{-1})		IAA (mg L^{-1})	PBS (mg L^{-1})	EPS (mg L^{-1})		IAA (mg L^{-1})	PBS (mg L^{-1})	EPS (mg L^{-1})
H_1	0.000 ^{j}	98.79 ^{b}	3.640 ^{n}	A_1	158.6 ^{k}	140.3 ^{f}	101.8 ^{ab}	HA_1	413.9 ^{a}	0.0000 ^{h}	90.25 ^{ab}
H_2	0.000 ^{j}	104.8 ^{a}	20.45 ^{h}	A_2	138.2 ^{l}	255.6 ^{b}	135.4 ^{ab}	HA_2	381.4 ^{b}	13.89 ^{gh}	78.46 ^{bc}
H_3	0.000 ^{j}	42.12 ^{gh}	50.32 ^{a}	A_3	235.5 ^{i}	34.73 ^{k}	93.73 ^{ab}	HA_3	384.8 ^{b}	0.0000 ^{h}	67.97 ^{cd}
H_4	0.000 ^{j}	40.91 ^{gh}	20.93 ^{g}	A_4	128.1 ^{l}	19.45 ^{l}	50.70 ^{ab}	HA_4	248.1 ^{e}	144.4 ^{b}	71.71 ^{cd}
H_5	125.0 ^{c}	26.06 ^{j}	7.867 ^{l}	A_5	111.2 ^{m}	226.4 ^{c}	114.7 ^{ab}	HA_5	288.9 ^{c}	105.6 ^{d}	64.54 ^{cd}
H_6	0.000 ^{j}	21.21 ^{klmn}	31.99 ^{e}	A_6	96.96 ^{m}	272.2 ^{a}	128.8 ^{ab}	HA_6	231.4 ^{f}	63.89 ^{e}	60.03 ^{d}
H_7	0.000 ^{j}	3.030 ^{p}	44.79 ^{c}	A_7	1202 ^{d}	251.4 ^{b}	488.6 ^{ab}	HA_7	220.6 ^{fg}	180.6 ^{a}	102.8 ^{a}
H_8	0.000 ^{j}	45.15 ^{fg}	5.183 ^{m}	A_8	105.1 ^{m}	0.0000 ^{m}	0.0000 ^{b}	HA_8	280.6 ^{cd}	122.2 ^{c}	94.02 ^{ab}
H_9	7.190 ^{i}	83.03 ^{c}	34.15 ^{d}	A_9	369.2 ^{f}	198.6 ^{d}	189.7 ^{ab}	HA_9	269.8 ^{d}	122.2 ^{c}	102.6 ^{a}
H_{10}	133.5 ^{b}	77.88 ^{d}	22.95 ^{f}	A_{10}	187.2 ^{j}	230.6 ^{c}	143.3 ^{ab}	HA_{10}	197.3 ^{h}	22.22 ^{g}	23.00 ^{e}
H_{11}	14.97 ^{h}	56.67 ^{e}	0.0000 ^{r}	A_{11}	1554 ^{b}	141.7 ^{ef}	572.8 ^{ab}	HA_{11}	213.1 ^{gh}	38.89 ^{f}	92.88 ^{ab}
H_{12}	395.0 ^{a}	25.76 ^{jk}	0.5861 ^{qr}	A_{12}	1355 ^{c}	51.39 ^{j}	469.5 ^{ab}	-	-	-	-
H_{13}	0.000 ^{j}	20.91 ^{lmn}	0.0000 ^{r}	A_{13}	282.0 ^{h}	44.45 ^{jk}	112.0 ^{ab}	-	-	-	-
H_{14}	0.000 ^{j}	0.0000 ^{p}	0.0000 ^{r}	A_{14}	801.7 ^{e}	109.7 ^{g}	311.7 ^{ab}	-	-	-	-
H_{15}	44.25 ^{f}	18.79 ^{mno}	4.319 ^{n}	A_{15}	290.2 ^{h}	73.61 ^{i}	127.4 ^{ab}	-	-	-	-
H_{16}	0.000 ^{j}	21.21 ^{klmn}	11.60 ^{j}	A_{16}	1815 ^{a}	155.6 ^{e}	660.8 ^{a}	-	-	-	-
H_{17}	25.03 ^{g}	87.58 ^{c}	2.684 ^{o}	A_{17}	0.0000 ^{n}	0.0000 ^{m}	0.0000 ^{b}	-	-	-	-
H_{18}	13.86 ^{h}	45.15 ^{fg}	1.586 ^{p}	A_{18}	338.1 ^{g}	90.28 ^{h}	149.9 ^{ab}	-	-	-	-
H_{19}	68.10 ^{e}	17.88 ^{no}	0.9255 ^{pq}	-	-	-	-	-	-	-	-
H_{20}	0.000 ^{j}	35.15 ^{i}	0.0000 ^{r}	-	-	-	-	-	-	-	-
H_{21}	0.000 ^{j}	49.09 ^{f}	17.86 ^{i}	-	-	-	-	-	-	-	-
H_{22}	91.50 ^{d}	24.55 ^{jkl}	45.81 ^{b}	-	-	-	-	-	-	-	-
H_{23}	0.000 ^{j}	22.73 ^{jklm}	10.46 ^{k}	-	-	-	-	-	-	-	-
H_{24}	0.000 ^{j}	14.55 ^{o}	34.80 ^{d}	-	-	-	-	-	-	-	-
H_{25}	0.000 ^{j}	38.18 ^{hi}	20.52 ^{h}	-	-	-	-	-	-	-	-
H_{26}	15.23 ^{h}	23.64 ^{jkl}	22.15 ^{g}	-	-	-	-	-	-	-	-
†C	0.000 ^{j}	0.0000 ^{p}	0.0000 ^{r}	†C	0.0000 ^{n}	0.0000 ^{m}	0.0000 ^{b}	†C	0.0000 ^{i}	0.0000 ^{h}	0.0000 ^{f}
††Pv	< 10^{-4}	< 10^{-4}	< 10^{-4}	††Pv	< 10^{-4}	< 10^{-4}	< 10^{-4}	††Pv	< 10^{-4}	< 10^{-4}	< 10^{-4}

† C means control, †† Pv is p-value

(*) Means within a column followed by the same letter are not significantly different at p = 0.05

IAA and the highest values 413.9, 384.8, 381.4, 288.9, 280.6 and 269.8 mg L^{-1}, were obtained from *HA1*, *HA3*, *HA2*, *HA5*, *HA8* and *HA9* isolates, respectively (Table 5). The averages of IAA production with 587.11, 284.53, and 35.90 mg L^{-1} were determined in the alkaliphilic, haloalkaliphilic, and halophilic isolates, respectively. Correspondingly, these were about sixteen and eight times higher in the alkaliphilic and haloalkaliphilic isolates than the halophilic isolates.

The solubility range of insoluble phosphates in the halophilic isolates was from 104.8 to zero mg L^{-1} between the maximum (*H2*) and minimum (*H14*) isolates (Table 5). The isolates *A6*, *A2*, *A7*, *A10*, *A5*, *A9*, and *A16* with 272.2, 255.6, 251.4, 230.6, 226.4, 198.6, and 155.6 mg L^{-1}, showed the highest abilities to dissolve mineral phosphates among the alkaliphilic isolates group, respectively (Table 5). Among eleven haloalkaliphilic isolates, only nine isolates were found with the ability to dissolving mineral phosphates, the maximum values of which in the *HA7* and *HA4* isolates were 180.6 and 144.4 mg L^{-1}, respectively. In addition, a significant difference was observed between these two isolate ($P < 0.05$). The minimum value with zero mg L^{-1} production was observed in two isolates, named *HA1* and *HA3* (Table 5). Comparison of the average solubilities of mineral phosphates with 127.55, 73.99, and 40.19 mg L^{-1} in the alkaliphilic, haloalkaliphilic, and halophilic isolate groups, were similar to the production of tri-indole acetic acid in the isolates, respectively (Table 5). Accordingly, in the alkaliphilic and haloalkaliphilic groups, this was about three and two times more than the halophilic group, respectively.

The production range of exopolysaccharides of the halophilic isolates was from 50.32 to zero mg L^{-1} (Table 5). The four halophilic isolates, including *H11*, *H13*, *H14*, and *H20* produced no exopolysaccharides. Except for three isolates *A16* (660.8 mg L^{-1}), *A8*, and *A17* (with zero mg L^{-1}) with a significant difference among them ($P < 0.05$); it was shown that no statistically significant differences exist among alkaliphilic isolates (Table 5). All the haloalkaliphilic isolates had the ability to produce exopolysaccharides (Table 5). The highest production rates were observed in the *HA7* isolates (102.8 mg L^{-1}) and *HA9* (102.6 mg L^{-1}) with no

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Table 6. Interaction effects of soil salinity, bacterial group, and strain type on some growth characteristics of 15GN rootstock

Treatments	Plant height (cm)	Stem diameter (mm)	No. of leaf plant^{-1}	No. of lateral branch plant^{-1}	Leaf loss (%)	Leaf area (cm^2)
S2× H0	87.67 ^{bcd}	6.527 ^{fghijk}	66.33 ^{e}	3.333 ^{efg}	4.333 ^{jklm}	34.60 ^{abcde}
S2× H10	91.33 ^{b}	6.327 ^{ghijk}	73.67 ^{ab}	4.667 ^{abcd}	3.667 ^{lmno}	34.02 ^{abcde}
S2× H22	87.33 ^{cd}	5.743 ^{ijklm}	60.67 ^{gh}	3.667 ^{def}	2.000 ^{opq}	38.29 ^{a}
S2× A0	84.33 ^{de}	6.047 ^{hijkl}	67.33 ^{de}	5.000 ^{abc}	2.667 ^{mnop}	34.96 ^{abcd}
S2× A7	95.67 ^{a}	6.630 ^{fghij}	61.67 ^{gh}	4.333 ^{bcde}	4.000 ^{klmn}	53.92 ^{abc}
S2× A11	83.33 ^{e}	6.867 ^{efgh}	71.67 ^{bc}	4.333 ^{bcde}	5.667 ^{ijk}	34.67 ^{abcde}
S2×HA0	87.67 ^{bcd}	6.277^{ghijk}	67.33 ^{de}	3.667 ^{def}	4.000 ^{klmn}	31.98 ^{abcde}
S2×HA7	85.67 ^{de}	7.370 ^{defg}	66.00 ^{e}	4.000 ^{cdef}	6.000 ^{ij}	28.46 ^{defg}
S2×HA9	91.17 ^{bc}	6.840 ^{efghi}	56.67 ^{j}	4.667 ^{abcd}	5.667 ^{ijk}	36.59 ^{ab}
S4× H0	68.50 ^{g}	7.627 ^{cdef}	73.33 ^{ab}	5.333 ^{ab}	3.667 ^{lmno}	24.17 ^{fgh}
S4× H10	75.33 ^{f}	8.590 ^{abc}	57.67 ^{ij}	3.667 ^{def}	5.667 ^{ijk}	29.20 ^{cdefg}
S4× H22	67.00 ^{g}	8.213 ^{abcd}	61.67 ^{gh}	4.333 ^{bcde}	2.333 ^{nop}	31.25 ^{abcdef}
S4× A0	62.33 ^{i}	7.783 ^{bcde}	57.67 ^{ij}	3.667 ^{def}	2.667 ^{mnop}	35.58 ^{abcd}
S4× A7	73.00 ^{f}	8.783 ^{ab}	65.00 ^{ef}	5.000 ^{abc}	3.333 ^{lmno}	30.90 ^{bcdef}
S4× A11	62.83 ^{hi}	7.603 ^{cdef}	48.33 ^{l}	4.667 ^{abcd}	4.000 ^{klmn}	31.88 ^{abcde}
S4×HA0	73.00 ^{f}	8.040 ^{bcd}	53.00 ^{k}	4.667 ^{abcd}	4.667 ^{jkl}	30.41 ^{bcdef}
S4×HA7	74.33 ^{f}	9.180 ^{a}	56.00 ^{j}	5.667 ^{a}	5.667 ^{ijk}	34.07 ^{abcde}
S4×HA9	66.67 ^{gh}	8.567 ^{abc}	60.33 ^{ghi}	4.333 ^{bcde}	7.000 ^{i}	27.72 ^{efg}
S8× H0	51.67 ^{kl}	4.933 ^{lmnop}	18.00 ^{p}	3.667 ^{def}	22.00 ^{f}	0.5800 ^{m}
S8× H10	50.67 ^{l}	6.860 ^{efgh}	36.33 ^{m}	5.000 ^{abc}	17.33 ^{gh}	4.641 ^{lm}
S8× H22	57.00 ^{j}	6.403 ^{ghijk}	24.33 ^{o}	3.667 ^{def}	19.00 ^{g}	14.23 ^{hij}
S8× A0	55.67 ^{j}	5.660 ^{jklmn}	12.00 ^{q}	4.000 ^{cdef}	29.33 ^{d}	2.830 ^{m}
S8× A7	51.33 ^{i}	6.860 ^{efgh}	35.00 ^{m}	4.000 ^{cdef}	16.00 ^{h}	22.26 ^{ghi}
S8× A11	55.00 ^{jk}	6.900 ^{efgh}	5.000 ^{s}	4.333 ^{bcde}	44.33 ^{a}	0.8867 ^{m}
S8×HA0	55.00 ^{jk}	6.533 ^{fghijk}	8.333 ^{r}	3.667 ^{def}	43.67 ^{a}	1.289 ^{m}
S8×HA7	56.00 ^{j}	6.677 ^{efghij}	13.33 ^{q}	3.667 ^{def}	40.00 ^{b}	2.448 ^{m}
S8×HA9	50.67 ^{l}	5.470 ^{klmno}	0.000 ^{t}	2.000 ^{h}	34.33 ^{c}	0.000 ^{m}
S16× H0	25.33 ^{pq}	4.503 op	56.33 ^{j}	4.000 ^{cdef}	1.000 ^{pqr}	13.52 ^{jk}
S16× H10	34.67 ^{n}	4.943 ^{lmnop}	63.00 ^{fg}	5.333 ^{ab}	3.333 ^{lmno}	13.14 ^{jk}
S16× H22	39.33 ^{m}	6.117 ^{hijk}	29.33 ^{n}	4.333 ^{bcde}	26.67 ^{e}	1.673 ^{m}
S16× A0	22.67 ^{q}	4.630 ^{mnop}	60.33 ^{ghi}	3.000 ^{fgh}	0.3333 ^{qr}	12.01 ^{jk}
S16× A7	29.83 ^{o}	4.212 ^{p}	66.00 ^{e}	2.333 ^{gh}	0.3333 ^{qr}	10.15 ^{kl}
S16× A11	26.83 ^{op}	4.307 ^{p}	70.00 ^{cd}	4.667 ^{abcd}	0.000 ^{r}	15.16 ^{ijk}
S16×HA0	28.00 ^{op}	4.983 ^{lmnop}	76.00 ^{a}	5.333 ^{ab}	0.3333 ^{qr}	18.12 ^{hij}
S16×HA7	29.17 ^{op}	4.467 ^{op}	74.33 ^{ab}	4.000 ^{cdef}	0.3333 ^{qr}	10.19 ^{kl}
S16×HA9	27.00 ^{op}	4.547 ^{nop}	60.00 ^{hi}	3.667 ^{def}	1.333 ^{pqr}	14.98 ^{jk}
Pvalue	0.0000	0.0296	0.0000	0.0004	0.0000	0.0000

(*) Means within a column followed by the same letter are not significantly different at p = 0.01
(S2= 2 dS m^{-1}, S4=4 dS m^{-1}, S8=8 dS m^{-1}, S16=16 dS m^{-1}, H= Halophilic, A-Alkaliphilic, HA=Haloalkaliphilic)

statistically significant difference, and the lowest value was also observed in *HA10* (23.0 mg L^{-1}). The average production capacities of exopolysaccharide in the three groups of bacteria were determined as 213.93, 77.13, and 15.98 mg L^{-1} for the alkaliphilic, haloalkaliphilic and halophilic groups, respectively. Correspondingly, this showed higher production capacity as thirteen and five times in the alkaliphilic and haloalkaliphilic groups, compared to the halophilic group, respectively (Table 5).

Effect of different soil salinity levels, types of bacteria and strains on some morphological and biochemical traits of almond: The analysis of variance indicated that different strains of bacteria and different salinity levels could significantly affect the growth traits, including height, stem diameter, number of leaf and branches, leaf losses, and leaf areas. The plant height was higher in the halophilic inoculated plants under salinity conditions compared to the other plants, and the highest height under three-way interaction effect was obtained in the treatments as S2×*A7*, S2×*H10*, and S2×*HA9* with some significant differences (P <0.05). Under higher salinity conditions, diameter of plant as well as numbers of leaves and branches decreased in all the treatments compared to the 2 dS m^{-1} plants. The highest diameter of plant and number of lateral branches were observed in the S4×*HA7* treatment, with 9.18 mm and 5.66, respectively. The minimum number of leaves and lateral branches were obtained at the salinity of 8 dS m^{-1} in the group of the haloalkaliphilic bacteria. As well, an elevated leaf loss was observed in the plants under S8 conditions compared to the other treatments. Additionally, the inoculation with *HA* enhanced the leaf loss in comparison with both the *A* and *H* treatments

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under S8 conditions. Notably, the maximum leaf loss was found in the S8×*A11* and S8×*HA0* treatments. The largest leaf area was observed in the S2× *H22* plants, and in contrast, the least amount was obtained in the haloalkaliphilic bacteria group when the studied plants were under the S8 condition (Table 6). The status of these mentioned parameters was better in S16 compare to S8 due to the influence of extremophilic bacteria.

The maximum average of chlorophyll was observed in the salinity treatment of 4 dS m^{-1} in the halophilic group (Table 7). In low and high salinity levels (levels 2 and 16 dS m^{-1}), the haloalkaliphilic bacteria, and in moderate salinity levels (4 and 8 dS m^{-1}), the halophilic group showed greater effects on increasing the amount of chlorophyll. The highest chlorophyll was obtained using the S8× *H22* treatment, and in contrast, the least one was obtained using the S8×*HA9* treatment with zero content. It was indicated that salinity could positively affect the proline content of plants, and consequently, proline has significantly ($P < 0.05$) increased under 16 dS m^{-1} conditions compared to the 2 dS m^{-1} (by 10%). The halophilic and haloalkaliphilic inoculation resulted in the increased proline in almond plants compared to the alkaliphilic inoculation. Moreover, under 4 dS m^{-1} salinity, proline has increased in the inoculated halophilic bacteria in comparison with other treatments; however, under 8 dS m^{-1} salinity, proline has increased in the inoculated haloalkaliphilic plant. As well, the soluble, insoluble, and total sugar amounts significantly ($P<0.05$) decreased under high salinity conditions compared to those under the 2 dS m^{-1} conditions (by 79, 92, and 87%, respectively). In addition, inoculated plants with halophilic bacteria showed a higher insoluble and total sugar amounts compared to the other treatments under different salinity conditions. The maximum soluble sugar was recorded in the plant grown under the following treatments: S2×*HA9*, S2×*HA7*, S4×*A0*, S2×*HA0*, S4×*H10*, and S4×*H22*. Moreover, the maximum insoluble and total sugar amounts were accumulated in the S2× *H0* treatment, and the lowest ones were recorded in the S8×*HA7* and S8×*HA0* treatments for insoluble sugar and S8×*HA9* and S8×*HA7* for total sugar (Table 7).

The fresh and dry weights of shoot and root decreased along with salinity increasing (Table 8). Under the influence of bacteria type, the findings showed that the GN rootstocks consisting of halophilic bacteria led to the highest increase in fresh weight of shoots (19.8 g), while the fresh weight of roots was found to be maximum in the haloalkaliphilic group of bacteria with 23.6 g (Data not shown). Moreover, the findings showed that the ratios of fresh weight of shoot to roots were 0.86, 0.87, and 0.74, for the three groups of halophilic, alkaliphilic, and haloalkaliphilic, respectively (Data not shown). Correspondingly, these indicate a greater effect of haloalkaliphilic bacteria on root growth than shoot growth. The highest fresh and dry weights of shoot decreased along with salinity increasing (Table 8). Accordingly, this decrease was more observed in the haloalkaliphilic bacteria, while this trend was found to be inversely affected by the type of bacteria in the fresh and dry weights of roots, so that the maximum fresh and dry weights of the root were observed in the haloalkaliphilic bacteria (Table 8). As well, the highest fresh and dry weights of the plant shoot were observed in the S2×*A7*, S2×*H10*, S2×*H22*, and S2×*A0* treatments with some significant differences, respectively. In addition, these weights of roots decreased along with salinity increasing. The highest amounts of root fresh weight as 39.9, 38.6, 38.6 and 35.9 g were observed in the S2×*HA9*, S4×*A0*, S2×*HA0* and S2×*HA7* treatments with some significant differences observed between the *HA9* and *HA7* isolates (Table 8). The results show that haloalkaliphilic bacteria led to the highest increase in the amount of moisture in both the shoots and roots. The highest percentage of relative moisture of roots was also observed at the highest salinity level (Table 8). Additionally, the S16×*A0*, S16×*A11* and S16×*HA9* treatments with 80.3%, 71.9% and 71.7% had the highest root moisture contents, respectively (Table 8).

Discussion

The exact mechanisms of plant growth stimulation remained largely speculative, some possible explanations in this regard are as follows: (1) production of some hormones like IAA, (2) production solubility of insoluble phosphates (PSB), and (3) exopolysaccharide (Kudoyarova *et al*., 2019). IAA is a phytohormone involved in cell's enlargement, root initiation, and cell's division; therefore, the IAA production activities of halophilic, alkaliphilic, and haloalkaliphilic microorganisms are crucial for plant's growth. Furthermore, IAA-producing microorganisms could improve the root growth as well as root length of plants, which constitute a greater root surface area enabling the plant to get more nutrients from the soil (Kudoyarova *et al*., 2019). Previous studies have discussed the different bacteria with the ability of enhancing fertility of the soil, but the focus is currently shifted on endophytic bacteria that can increase abiotic stress tolerance factors like soil salinity. Rajput *et al*. (2013) in their study stated that the halophilic bacteria *Planococcus rifietoensis*, has IAA producing and phosphate solubilizing activities that could improve the growth and yield of wheat under salinity conditions. Moreover, in the study by Siddikee *et al*. (2010), different strains of bacteria were found to be able to IAA production, nitrogen fixation, phosphate solubilizing, and ammonia production abilities. In general, soluble phosphates and exopolysaccharides directly help in plant growth, as they could act as macronutrients. Various strains belonging to different genera also have the potential of solubilizing insoluble inorganic phosphate compounds (Mendoza-Arroyo *et al*., 2020; Sarikhani *et al*., 2020). It was demonstrated that the production of organic acids results in the acidification of the microbial cell and its surroundings. Previously, the production of organic acids has been

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Table 7. Interaction effects of soil salinity, bacterial group, and strain type on some biochemical parameters of 15GN rootstock

Treatments	Chlorophyll content	Proline µmol g DW^{-1}	Soluble sugar %	Insoluble sugar %	Total Sugar %
S2× H0	35.84 ghij	15.74 ijk	2.833 cd	10.03 a	12.87 a
S2× H10	34.72 hijkl	11.95 lm	2.647 cde	7.460 c	10.10 b
S2× H22	37.12 defgh	16.90 ij	2.583 cde	7.623 c	10.21 b
S2× A0	35.88 fghij	17.18 hij	2.223 efg	8.393 b	10.62 b
S2× A7	35.19 hijk	18.90 efghi	2.953 c	5.797 e	8.757 c
S2× A11	34.88 hijkl	27.28 b	3.983 b	6.627 d	10.61 b
S2×HA0	37.24 defgh	8.447 nopq	4.594 a	0.8733 ghijklm	5.467 e
S2×HA7	39.24 cd	20.41 defg	4.667 a	1.177 ghi	5.740 de
S2×HA9	33.37 jkl	26.66 b	4.823 a	1.470 g	6.293 de
S4× H0	41.08 bc	18.31 fghi	3.553b	2.920 f	6.473 d
S4× H10	41.84 ab	34.29 a	4.560 a	1.063 ghijk	5.617 de
S4× H22	39.32 cd	20.35 defgh	4.510 a	1.133 ghij	5.643 de
S4× A0	41.17 bc	12.12 lm	4.633 a	0.9800 ghijkl	5.607 de
S4× A7	39.31 cd	10.36 mnop	3.007 c	0.3707 lmno	4.377 f
S4× A11	29.09 m	11.48 lmn	1.817 fgh	0.4933 jklmno	2.340 ijk
S4×HA0	36.86 defgh	7.370 opq	2.300 ef	1.090 ghij	3.393 gh
S4×HA7	41.16 bc	5.370 qr	1.217 ij	2.407 f	3.617 fg
S4×HA9	38.39 def	14.13 jkl	1.640 hi	0.2967 mno	1.937 jkl
S8× H0	10.27 p	7.187 pq	1.780 gh	0.8633 ghijklm	2.643 hij
S8× H10	13.39 o	10.77 mn	1.870 fgh	0.8200 ghijklmn	2.693 ghij
S8× H22	43.74 a	10.54 mno	1.517 hij	1.243 gh	2.757 ghij
S8× A0	14.26 o	11.44 lmn	0.4800 lm	0.1800 no	0.6600 mn
S8× A7	40.92 bc	10.36 mnop	0.2200 lm	0.3133 lmno	0.5467 mn
S8× A11	9.367 p	2.877 r	0.1933 lm	0.3567 lmno	0.5833 mn
S8×HA0	15.12 o	21.73 cde	0.3067 lm	0.1300 o	0.4400 n
S8×HA7	13.49 o	20.94 def	0.2700 lm	0.0933 o	0.3633 n
S8×HA9	0.000q	12.66 klm	0.0600 m	0.2633 mno	0.3233 n
S16× H0	36.67 efgh	16.27 ij	0.2900 lm	0.2467 mno	0.5400 mn
S16× H10	39.18 cde	21.19 def	0.2167 lm	0.3133 lmno	0.5300 n
S16× H22	20.23 n	21.31 def	0.3900 lm	0.3967 klmno	0.7900 mn
S16× A0	32.49 l	20.53 def	0.3633 lm	0.5833 hijklmno	0.9500 mn
S16× A7	33.09 kl	17.27 ghij	0.4567 lm	0.5033 ijklmno	0.9600 mn
S16× A11	33.98 ijkl	22.82 cd	0.5233 lm	0.6100 hijklmno	1.133 lmn
S16×HA0	35.62 ghij	15.99 ij	0.6500 kl	0.7333 lmno	1.033 lmn
S16×HA7	38.04 defg	22.11 cde	1.123 jk	0.3667 lmno	1.487 klm
S16×HA9	36.19 fghi	24.71 bc	2.387 de	0.5500 ijklmno	2.937 ghi
Pvalue	0.0000	0.0000	0.0000	0.0000	0.0001

(*) Means within a column followed by the same letter are not significantly different at p = 0.01
(S2= 2 dS m^{-1}, S4=4 dS m^{-1}, S8=8 dS m^{-1}, S16=16 dS m^{-1}, H= Halophilic, A-Alkaliphilic, HA=Haloalkaliphilic)

well-documented for different PGPR genera (Macias-Benitez *et al*., 2020). As discussed earlier, the soil contains a wide range of organic substrates, which can be considered as a source of P for plant growth. Accordingly, in order to make this form of P available for plant nutrition, it must be hydrolyzed to inorganic P, at first. The mineralization of most organic phosphorous compounds was done through phosphatase enzymes (Azziz *et al*., 2012). In comparison with other strains, alkaliphilic isolates released a larger amount of another P-solubilizing organic acid, and showed a significant increase in both shoot and root fresh weights when growing under salinity conditions (Table 8). The alkaliphilic isolates were also observed to be able to produce higher EPS. Notably, bacterial EPS production is considered as an important salt-tolerant characteristic. Moreover, exo-polysaccharides can lessen the hostile effect of osmotic stress by augmenting fresh and dry weights, and water content in plants (Shultana *et al*., 2020a). In this regard, different studies have shown the bacterial ability to produce EPS when exposed to the saline condition, compared to non-saline media. Bacterial cells could also be associated with the plant root system to enhance moisture-holding capacity significantly and to defense stem against various stress types. Plant growth-promoting with EPS-producing characteristic chelate different cations like Na^+. As well, it has been found that under salinity stress, bacteria can bind to the Na^+ ion through the secretion of EPS, which consequently reduces sits toxicity in the soil. Therefore, a higher population of EPS-producing bacteria in the rhizosphere zone possibly decrease the concentration of available Na^+ for plant uptake, which consequently alleviates the salt stress effect on plants under a saline

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Table 8. Interaction effects of soil salinity, bacterial group, and strain type on physiological traits of 15GN rootstock

Treatments	Fresh shoot weight (g)	Shoot dry weight (g)	Shoot moisture content (%)	Root fresh weight (g)	Root dry weight (g)	Root moisture content (%)
S2× H0	36.45 cd	16.02 bc	56.04 ghij	31.08 gh	16.48 ef	46.99 ghijk
S2× H10	37.68 ab	16.61 b	55.88 ghij	32.37 efg	18.58 bc	42.55 jk
S2× H22	37.16 bc	16.07 bc	56.75ghij	35.56 bc	19.29 b	45.75 hijk
S2× A0	37.19 bc	16.04 bc	56.81 fghij	34.32 cd	17.87 bcde	47.93 ghijk
S2× A7	38.70 a	17.85 a	53.86 hij	32.26 efg	18.35 bcd	43.10 ijk
S2× A11	35.62 d	16.14 bc	54.66 hij	33.45 de	18.83 bc	43.75 ijk
S2×HA0	35.33 d	15.12 d	57.20 fghij	38.65 a	21.26 a	45.02 hijk
S2×HA7	36.38 cd	15.55 cd	57.21 fghij	35.96 b	19.34 b	45.97 hijk
S2×HA9	30.31 e	13.28 e	56.19 ghij	39.93 a	21.87 a	45.24 hijk
S4× H0	26.17 gh	11.38 f	56.54 ghij	27.30 i	15.01fg	45.03 hijk
S4× H10	25.25 h	10.38 gh	58.83 defgh	32.87 def	17.48 cde	46.80 ghijk
S4× H22	27.64 f	10.89 fg	60.63 defgh	25.78 j	13.97 g	45.71 hijk
S4× A0	26.42 g	11.12 fg	57.87 efghi	38.68 a	22.44 a	41.99 k
S4× A7	29.36 e	12.90 e	56.06 ghij	32.31 efg	17.93 bcde	44.47 ijk
S4× A11	17.61 k	7.357 j	58.25 efghi	24.68 j	12.05 h	51.26 fghi
S4×HA0	23.69 i	9.996 hi	57.79 efghi	31.07 gh	18.70 bc	39.80 k
S4×HA7	23.66 i	9.877 hi	58.22 efghi	30.50 h	16.63 def	45.18 hijk
S4×HA9	22.42 j	9.457 i	57.82 efghi	31.37 fgh	17.72 bcde	43.50 ijk
S8× H0	6.370 o	1.255 q	80.13 a	14.33 m	5.829 lmn	59.21 def
S8× H10	12.97 l	4.934 l	61.83 defgh	18.39 l	8.937 ij	51.10 fghij
S8× H22	12.89 l	4.413 l	65.70 bcde	21.09 k	9.850 i	53.28 fgh
S8× A0	9.567 n	2.577 mn	73.01 ab	13.99 mn	5.307 lmnop	62.15 cde
S8× A7	17.15 k	6.240 k	63.62 cdefg	19.30 l	7.887 jk	58.93 def
S8× A11	8.933 n	3.111 m	65.21 bcdef	12.03l	4.151 nopq	65.46 bcd
S8×HA0	9.590 n	2.747 m	71.06 bc	13.67 mnop	6.094 lm	55.44 efg
S8×HA7	10.99 m	4.573 l	58.02 efghi	13.41 mnopq	5.570 lmno	57.92 def
S8×HA9	6.427 o	1.861 nopq	70.77 bc	12.40 opqr	4.370 mnopq	64.64 bcd
S16× H0	3.933 r	2.373 mno	38.45 k	12.72 nopqr	6.673 kl	47.61 ghijk
S16× H10	5.577 opq	2.337 mnop	57.69 efghi	10.27 st	3.604 pq	66.14 bcd
S16× H22	5.890 op	1.593 opq	72.67 ab	11.58 rs	3.579 pq	70.02 bc
S16× A0	4.387 r	1.940 nopq	55.20 ghij	9.236 t	1.767 r	80.38 a
S16× A7	4.680 qr	2.313 mnop	49.95 ij	9.343 t	2.966 qr	68.51 bc
S16× A11	4.767 pqr	2.420 mn	48.84 j	9.687 t	2.703 qr	71.87 ab
S16×HA0	4.063 r	1.560 pq	60.91 defgh	13.58 mno	3.921 opq	71.61 b
S16×HA7	4.863 pqr	1.597 opq	67.02 bcd	12.22 pqrs	3.718 pq	69.83 bc
S16×HA9	3.992 r	1.173 q	70.42 bc	10.22 t	2.930 qr	71.67 b
Pvalue	0.0000	0.0000	0.0002	0.0000	0.0000	0.0006

(*) Means within a column followed by the same letter are not significantly different at p = 0.01
(S2= 2 dS m^{-1}, S4=4 dS m^{-1}, S8=8 dS m^{-1}, S16=16 dS m^{-1}, H= Halophilic, A-Alkaliphilic, HA=Haloalkaliphilic)

environment (Shultana *et al*., 2020a; Abd El-Ghany and Attia, 2020).

In our study, the morphological traits such as height, diameter, the number of leaf and branches, leaf areas, fresh and dry weights; as well as the biochemical traits, including chlorophyll, proline, and sugar amount decreased as a result of salinity stress. By investigating the effect of five types of quality irrigation waters on fourteen commercial almond rootstocks, it was displayed that sodium-chloride caused the highest reductions in both survival rate and trunk diameter, followed by sodium-chloride/sulfate and sodium-sulfate. Correspondingly, this indicated that Na^+ and, to a lesser extent, Cl^- were the most toxic ions to almond rootstocks (Shultana *et al*., 2020b; Abd El-Ghany and Attia, 2020). Photosynthetic rate was found to be correlated with trunk diameter and proline leaf ratio (sodium-chloride/control) was significantly correlated with the exclusion of Na^+ and Cl^-, which consequently affected the survival rate directly (Shultana *et al*., 2020b; Abd El-Ghany and Attia, 2020). In a study, the effect of salinity stress on morphological characteristics of some selected almond genotypes grafted on GF677 rootstock was investigated and then reported (Momenpour *et al.*, 2015). By applying salinity stress and increasing its concentration, growth indices, including branch height, branch diameter, total leaf number, green leaves, leaf density on the main branch, leaf area and its ratio, fresh and dry weights of leaves, fresh and dry weights of shoots, fresh and dry weights of roots, all decreased in total studied genotypes (Momenpour *et al.*, 2015). The highest stem diameter, number of lateral branches, and chlorophyll index were obtained at the salinity of 4 dS m^{-1}, showing a positive

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effect of salinity at low to moderate levels, despite the availability of nitrogen, phosphorus, and potassium. Accordingly, this was consistent with the results obtained by other researchers (Ganji Moghaddam and Zamanipour, 2020). In the current study, the lowest stem diameter was obtained at the salinity of 16 dS m^{-1}, and those of chlorophyll index and lateral branch were obtained at the salinity of 8 dS m^{-1}. Momenpour and Imani (2019) observed the lowest branch of six almond genotypes in the 6 dS m^{-1} treatment. In this regard, the lowest number of leaves and the highest losses were also observed at the salinity of 8 dS m^{-1} (Table 6). Frequently, under water stress conditions, the plant reduces its photosynthetic surface by reducing both the number and shrinking of leaves, and following the reduction in leaf area, the plant's photosynthetic capacity decreases. Consequently, this event causes more leaf losses and reduces the photosynthetic surface (Alinejadian Bidabadi *et al*., 2018). The highest amount of proline was obtained at a salinity of 16 dS m^{-1} (Table 7). Along with salinity levels increasing, the amount of proline accumulation also increased in the green texture. The increased proline production due to the decreased electrolyte output finally increased the relative amounts of leaf water and selective K^+ uptake as well as stress tolerance (Grover *et al*., 2011). Zrig *et al*. (2016) in their study mentioned that Garnem offered a higher degree of protection against salinity due to their ability in limiting the loss of photosynthetic activity by maintaining stomatal conductance as well as protecting chlorophyll and cytosolic assimilatory enzymes from toxic ions (Na^+ and Cl^-). Additionally, Mazzetto grafted onto Garnem rootstocks reported higher gs rates, carotenoids/ chlorophyll and anthocyanins/chlorophyll ratios, and a better nutritional status (higher K^+ and Ca^{2+} as well as a lower Na^+) compared to GF677 (Zrig *et al*., 2016). Another previous study also showed that GN15 accumulated less Na^+ and Cl^- compared to GF677 and bitter almond (Zrig *et al*., 2011). It seemed that GN15 was more able to tolerate the excess of toxic ions using anthocyanins, which are abundant in their red leaves and free polyamines for more efficiently responding to stress than the other almond rootstocks (Zrig *et al*., 2011).

Along with salinity increasing, plant height decreased and alkaliphilic bacteria showed less amount of increase in plant height at different salinity levels compared to the other two groups studied in this research. The highest stem diameter and number of lateral branches were obtained with no significant difference in bacterial type at the salinity level of the 4 dS m^{-1}. Thereafter, the addition of growth-accelerating bacteria (PGPR), as biofertilizers, to 10% of the soil microbial population seemed to increase the enzymatic activity of the soil, which consequently provided the conditions for the absorption of more nutrients. More assimilation were provided during the process of photosynthesis, in order to build cell walls during cell turgescence in plants and to increase plant height (Hassan *et al*., 2014). Due to the higher mean of tri-indole acetic acid in the alkaliphilic isolates (587.11 mg L^{-1}) compared to the other two groups of bacteria (Table 5), the increase in height of the alkaline isolates was justified. The use of extremophiles bacteria, which were adapted to soil salinity and alkalinity, was found to be effective under high salinity conditions such as 16 and 8 dS m^{-1}, because it increased the number of leaves at the salinity of 16 dS m^{-1}. The haloalkaliphilic PGPR isolates isolated from the soil of Gujarat in India, including *Bacillus pumilus* and *Pseudomonas pseudoalcaligenes* (Jha and Subramanian, 2014), were indicated to be able to alleviate the effects of salinity stress on paddy soils in rice (*Orayza sativa*). These bacteria led to a 16% increase in germination, 8% greater viability, 27% increase in dry weight, and 31% increase in plant height. Moreover, rice inoculated with PGPR increased the concentration of nitrogen by 26%, phosphorus by 16%, and potassium by 31% in the plant, while at the same time, the concentration of sodium decreased by 71% and calcium by 36% under the salinity conditions (Jha and Subramanian, 2014). The use of microorganisms for saline soil restoration may be regarded as an environmentally sustainable, safer, and more efficient method, as the halophilic microorganisms have the potential of eliminating salt from saline soils (Arora *et al*., 2013).

The minimum number of leaves, their area, and chlorophyll index were obtained in the group of haloalkaliphilic. To be adapted to the increase in external osmotic pressure, extremophile bacteria can accumulate ions (especially sodium). One of the signs of sodium accumulation in plants is leaf loss (Ganji Moghaddam and Zamanipour, 2020). Mathivanan *et al*. (2014) in their study showed that the inoculation of *Rhizobium*, *Pseudomonas*, and *Bacillus* into peanuts increased leaf area as a photosynthetic area by 1.3 times. Furthermore, alkaliphilic with higher production rates of PGPA increased leaf length, width, and leaf area in GN almond rootstocks. Due to the higher IAA average production (284.5 mg L^{-1}) compared to the PBS (73.9 mg L^{-1}) and EPS (77.1 mg L^{-1}) in the haloalkaliphilic bacteria group, this group of bacteria consequently reduced GN rootstocks' length, width, and finally leaf area compared to even halophilic bacteria, which had a lower average of all growth-promoting properties (IAA, PBS and EPS with 35.90, 40.19, and 15.98 mg $L^{-1,}$ respectively). The production of growth regulators by bacteria with the ability to enhance plant growth-promoting properties can be considered one of the most justified adaptations proposed in order to explain the effect of these bacteria on increasing the chlorophyll's content (Ashraf and Foolad, 2007). It was reported that the presence of bacteria caused an 18% increase in total chlorophyll compared to control in basil (*Ocimum basilicum*) (Mohammadi Babazeidi *et al*., 2018). As well, the treatment of bare rootstocks of almond with a strain of *Agrobacterium rhizogenes* was reported to increase the early growth of trees (Strobel

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and Nachmias). These results showed that the group consisting of halophilic bacteria caused the highest increase in fresh weight of shoots (19.8 g), insoluble sugar (2.8%), and total sugar amount (5.0%), while the fresh weight of roots was the maximum in the haloalkaliphilic group of bacteria with 23.6 g (Data not shown). In the study by Zahir *et al.* (2000), it was found that the abilities of rhizosphere bacteria such as *Pseudomonas putida*, *Bacillus subtilis*, and *Enterobacter cloacae* in producing auxin (IAA) vary according to the type of bacteria and the conditions of the culture medium. In the present study, the haloalkaliphilic bacteria produced more indole auxin compounds compared to the other two groups of bacteria; therefore, this group caused a greater increase in root growth. Accordingly, these results also showed that the ratios of fresh weight of shoot to roots for the three groups containing halophilic, alkaliphilic, and haloalkaliphilic were 0.86, 0.87, and 0.74, respectively, which indicate a greater effect of haloalkaliphilic bacteria on root growth compared to shoot growth. The highest percentage of humidity in the shoot was also observed in the group of haloalkaliphilic. Biofertilizers containing *Azospirillum*, *Pseudomonas*, and *Azotobacter* bacteria in cooperation with plant roots increased the level of moisture absorption and with extensive root network through water and salt uptake, they increased both leaf area and the relative amount of water in the plant (Sprent and Sprent, 1990). Inoculation with growth enhancers of Rhizobium and *Azotobacter* also increased the water use efficiency and the relative amount of water in leaves that were under field conditions in Boroujerd, Iran (Khosravi *et al*., 2012).

The treatment of apple seedlings and rootstocks with strains of bacteria isolated from some plants' roots, including wild grape (*Vitis* spp.), *Potentilla*, *Prunus*, *Fragaria*, *Rosa*, *Pyrus*, *Rubus*, *Malus*, *Crataegus* and *Spiraea* spp., resulted in some significant growth increases up to 65% and 179% in seedlings and rootstocks, respectively (Khosravi *et al*., 2012). In this regard, the rootstocks treated with the PGPR showed up to 102% more active lateral root nodes. Accordingly, the most active PGPR strains were found as *fluorescent Psedomonas* spp. and enteric bacteria (Caesar and Burr, 1978). It seems that one of the possible reasons for the increase in plant height in GN almond rootstocks in the halophilic group may be less amount of IAA produced, which consequently reduced the inhibition of ethylene in this group compared to the haloalkaliphilic and alkaliphilic groups. The highest stem diameter and the number of lateral branches were also obtained with no significant difference in bacterial type at the salinity level of the 4 dS m^{-1} (Table 6). Sometimes, increasing salinity to a level under special conditions due to the type of salinity and its ionic composition, does not only bring a negative effect on some plant growth indices, but also has a nutritional effect (Ganji Moghaddam and Zamanipour, 2020). The minimum number of leaves and lateral branches were obtained at the salinity of 8 dS m^{-1} in the group of haloalkaliphilic bacteria. Salinity stress has been reported to have the ability to increase ethylene production in plants, resulting in leaf and petal abscission, organ aging, and premature plant death. Therefore, reducing stress due to ethylene levels, can eventually reduce some of the effects of stress on plants. In fact, one of the high photosynthetic losses of the plant was found as about 40% through root secretion, so it is obvious that more ACC, as a precursor to ethylene production under stress, can be released from the plant roots. Afterward, by the enzyme ACC, bacterial deaminase is hydrolyzed to ammonium and alpha ketobutyrate, meaning that more ACC secretions from plant roots cause distances from the ethylene synthesis pathway in the plant and also reduce the amount of ACC converted to ethylene by ACC oxidase (Siddikee *et al*., 2011). As a result, leaf abscission would be reduced. However, higher production of IAA in the haloalkaliphilic bacteria and possibly its uptake from roots according to available sources (Sahay *et al*., 2012; Siddikee *et al*., 2011), are responsible for increasing plant ethylene levels and more leaf losses in the haloalkaliphilic group. The leaf area as well as the fresh and dry weights of the shoot decreased along with increasing salinity (Table 8). As well, this decrease was more in the haloalkaliphilic bacteria, while this trend was observed to be inversely affected by the type of bacteria in the fresh and dry weights of roots, so that the maximum fresh and dry weights of the root was observed in the haloalkaliphilic bacteria group. By examining the characteristics of the three groups of halophilic, alkaliphilic, and haloalkaliphilic bacteria in vitro, it was shown that the haloalkaliphilic isolates were superior to the other two groups in terms of the production of growth-promoting properties (Eskandari Torbaghan *et al*., 2017). Additionally, these halophilic, alkaliphilic, and haloalkaliphilic isolates increased the yield of wheat by about 30.3%, 43.6%, and 44.2%, respectively, compared to the control (Eskandari Torbaghan *et al*., 2017). Evaluation of salinity resistance of wild almond species, including *Amygdalus scoparia*, *A. Arabica*, *A. elaegnifolia*, and *A. haussknechtii* under salinity stress resulted from the irrigation with sodium chloride solution at different concentrations, including control, 25, 50, 75, and 100 mM, showed that the amounts of vegetative factors, plant pigments, and plant biomass decreased; and proline accumulation increased by increasing salt concentration. Increasing salinity stress consequently decreased the uptakes of copper, zinc, iron, manganese, and potassium, which also increased the uptakes of magnesium, sodium, chlorine, nitrogen, phosphorus, and calcium (Jahanbazy *et al*., 2014). Additionally, the maximum amount of chlorophyll was observed in the salinity treatment of the 4 dS m^{-1} in the halophilic group. At both low and high salinity levels (levels 2 and 16 dS m^{-1}, respectively), haloalkaliphilic bacteria, and in moderate salinity (4 and 8 dS m^{-1}), the halophilic group had greater effects on increasing the amount of

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chlorophyll. Of note, salt could affect plant photosynthetic compounds such as enzymes, chlorophyll, and carotenoids (Sultana *et al*., 1999). Izanloo *et al*. (2008) in their study stated that increasing chlorophyll content and root growth can be considered mechanisms for increasing plant resistance to abiotic stresses.

Conclusion

The use of the selected isolates of extremophile native bacteria in this study, with their plant growth-enhancing properties, showed the ability to increase the rootstock resistance of GN almonds under soil salinity and alkalinity stress compared to the none stress condition (2 and 4 dS m^{-1}). For example, the percentage of leaf losses and leaf area at 8 dS m^{-1} was more than at the 16 dS m^{-1} salinity, which showed an increase in bacterial efficiency with increasing salinity. Due to the higher production of tri-indole acetic acid compared to other growth-promoting properties in the haloalkaliphilic bacteria, it can increase root growth, root-to-shoot ratio, and plant moisture content, and consequently increase water use efficiency and relative water content in the plant facing osmotic stress. As well, halophilic bacteria (especially *H10* strain) showed the greatest effect on increasing proline, chlorophyll, and carbohydrate contents. Therefore, the inoculation of these extremophilic bacteria that produce colonies on the roots under higher salinity and alkalinity of the soil (more than 8 dS m^{-1}), is recommended for growing horticultural crops in saline-alkaline soils.

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