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# Sodium-resistant plant growth-promoting rhizobacteria isolated from a halophyte, *Salsola grandis*, in saline-alkaline soils of Turkey

**Ryota Kataoka [a], Esra Güneri [b], Oguz Can Turgay [b,*], Ahmet Emre Yaprak [c], Bahar Sevilir [b], Isa Başköse [c]**

[a] University of Yamanashi, Faculty of Life & Environmental Sciences, Department of Environmental Sciences, Takeda, Kofu, Yamanashi, Japan

[b] Ankara University, Faculty of Agriculture Department of Soil Science and Plant Nutrition, Ankara, Turkey

[c] Ankara University, Faculty of Science Department of Biology, Ankara, Turkey

## Abstract

Phytoremediation is an expanding field of research in environmental studies due to the benefits of its cost effectiveness and environmental friendliness. The use of this technology in saline and alkaline soils can be a promising approach because soil salinity inhibits crop growth and causes tremendous yield losses in many regions of the world, especially in arid and semi-arid regions. However, little is known about the plants that can be applicable in the phytoremediation of saline soils and role of their rhizobacteria in the phytoremediation processes. In this study, we examined sodium (Na) uptake by the halophyte *Salsola grandis* and screened Na resistant rhizobacteria inhabiting in an extremely saline soil environment. *S. grandis* could uptake Na at the value of 15447 $mg \cdot kg^{-1}$ and transported Na to stem and leaves from roots. We found that 50 out of the 131 strains were Na resistant and 8 out of these 50 strains contributed to the growth of *S. grandis*. Using 16S ribosomal RNA sequencing, we determined these eight strains to be within the genera *Arthrobacter* spp. and *Bacillus* spp. Moreover, four of the eight strains (A22, WP5, B14, AP20) showed traits of being both siderophore and indole-3-acetic acid producers. Therefore, these eight strains appear to be suitable candidates for plant growth-promoting rhizobacteria of *S. grandis*.

## Article Info

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## Introduction

The introduction of irrigation systems has raised agricultural productivity in dry and semi-dry regions. Although only 18% of the cultivated area is irrigated globally, 40% of global food production comes from irrigated agriculture (Siebert et al., 2005). In these areas soil salinization is a major problem and leads to low agricultural productivity. Soil salinity is a worldwide problem affecting about 20% of the world's cultivated land and nearly half of all irrigated land (Zhu, 2001). According to the FAO/UNESCO soil map of the world, approximately 1.5 million hectares of land in Turkey are suffering from salinity and sodicity problems (Kendirli et al., 2005).

Phytoremediation has attracted rising attention as a suitable technology that can be applied not only in polluted but also in saline soil remediation because of its low economic cost and environmentally friendly

* Corresponding author.
Ankara University, Faculty of Agriculture Department of Soil Science and Plant Nutrition, Diskapi, Ankara, 06110 Turkey
Tel.: +90 312 596 1231 E-mail address: O.Can.Turgay@agri.ankara.edu.tr
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process (Qadir et al., 2002, 2003). Some reports suggest that the harvested plant, if not plowed back to the same soil, can contribute significantly to the removal of salts such as sodium (Na) from salt-affected soils (Ravindrana et al., 2007; Qadir et al., 2007). The genus *Salsola* (Chenopodiaceae) comprises about 120 species; they are widespread herbaceous or shrubby halophytic plants, especially in the brackish grounds of the moderate and subtropical regions of Europe, Asia, Africa and North America (Tundis et al., 2008). *Salsola soda*, typically grows in coastal regions and saline conditions, was used as a companion plant because of its high accumulation of Na (Colla et al., 2006; Zuccarini, 2008). In 1999, *Salsola grandis*, a species closely related to *S. soda*, was reported as a new species of genus *Salsola* (Freitag et al., 1999). This plant is an annual halophytic plant growing in open habitats with sparse vegetation where local semi-desert climate creates an extreme saline soil habitat (eg. pH over 9 and Na over 3500 $mg \cdot kg^{-1}$) that forms on highly clayey salt-containing marly substrate, in Nallıhan Region, Ankara Province, Turkey. To the best of our knowledge, there is no information on how this plant manages abiotic stress factors and survives in such a harsh environment and also no previous report about its Na uptake from soil.

Various soil microorganisms were shown to tolerate unwanted elements in soil using various mechanisms including exclusion, active removal, biosorption, precipitation, and extra or intracellular bioaccumulation (Silver, 1996; Whiting et al., 2001; Lasat, 2002; Gadd, 2004). These processes are known to effect the solubility and the bioavailability of toxic elements, such as heavy metals, to plants and therefore interactions between hyperaccumulator plants, metals and plant growth-promoting rhizobacteria (PGPR) have gained increasing attention for the last two decades (Turgay and Bilen, 2012). PGPR are free-living soil bacteria that can either directly or indirectly facilitate rooting and growth of plants. These bacteria serve the functions of N-fixation, production of siderophores, solubilization of minerals like phosphorus and synthesis of phytohormones such as indole-3-acetic acid (IAA). To date, some bacteria such as *Klebsiella* sp. D5A showed the high plant growth promoting activity on the glycophytic crop in saline–alkaline soils (Liu et al., 2014; Liu et al., 2016). Likewise, many studies have been published on beneficial effects of bacterial application on growth of wheat under salt stress (Ashraf et al., 2004; Sadeghi et al., 2012; Upadhyay et al., 2012; Chakraborty et al., 2013; Paul and Lade, 2014).

According to Ruppel et al. (2013), "micro-organisms use similar approaches to cope with oxidative stress and it is likely that microorganisms use the same mechanisms to alleviate salt stress effects in halophytes as in other tolerant plants. However, little is known about the effect of PGPR on the salt accumulation in halophytic plants. The application of PGPR may prove useful when developing strategies to facilitate plant growth in saline soils. Therefore, in the present study, we studied Na-resistant rhizobacteria isolated from the rhizosphere of a halophytic plant, *S. grandis,* and a halotolerant plant, wheat (Triticum aestivum). The aims of this study were to explore Na-resistant indigenous PGPR on *S. grandis*, and determine the effect of these isolates on the salt accumulation in *S. grandis*.

## Material and Methods

### Sampling sites and soil properties

The seeds and five seedlings (2-3 month old) of *Salsola grandis* Freitag, Vural and N. Adıgüzel (Amaranthaceae) were collected from harsh ecological conditions which is including raw gypsum and salt-containing marly substrate located at 28km east-southeast of Nallıhan region (40°05'29.57"N, 031°36'10.26"E) in Ankara Province in November 2015 and in April 2016, respectively. The seeds collected were stored in paper bags at 4°C until use and the seedlings were used to isolate bacteria from rhizosphere soil on the next day after sampling. For the greenhouse experiment, soil was collected from a depth of 0-20 cm in a non-agricultural area (38°20'08.6"N, 033°59'06.5"E) located at the campus of Aksaray University, Aksaray Province, Turkey. This soil was air-dried and stored in room conditions prior to soil analysis and the pot experiment. The soil characteristics are outlined in Table 1.

### Pot experiment

We conducted a pot experiment, setting up *S. grandis* and *Triticum* spp., in triplicate for 82 days on a soil collected from Aksaray district to examine Na uptake of *S. grandis*. *Triticum* spp. (wheat) was used as control to compare the Na uptake with *S. grandis*. Five seedlings (82 days old) of *S. grandis* per pot and three seedlings (82 days old) of wheat per pot were grown at a greenhouse (Ankara University, Ankara, Turkey). The analysis of the Na concentration in *S. grandis* and wheat was performed as follows. Harvested plants were washed carefully using tap water and then distilled water, and then stem-leaves and roots were

separated. After the measurement of the fresh weight, all plant samples were dried at 60°C, and their dry weight was measured. Then, the plants were ground and dry ashing was conducted in a muffle furnace at temperatures of 500 ± 50°C for approximately 20 h. The ash was dissolved in 10 M of nitric acid ($HNO_3$) and the extract solution was analyzed using ICP-OES (Optima 2100 DV; Perkin Elmer, Inc., Shelton, CT, USA) (Kacar and Inal, 2010).

Table 1. Soil properties collected from Aksaray and Nallıhan. The soil from Aksaray was used at both of pot experiment and conical tube one.

| | | Aksaray | Nallıhan |
|---|---|---|---|
| pH ($H_2O$) | | 8.36 | 9.34 |
| EC | (dS/m) | 2.37 | 1.46 |
| Total Nitrogen | (%) | 0.40 | 0.15 |
| Organic Materials | (%) | 5.42 | 0.73 |
| $CaCO_3$ | (%) | 40.3 | 8.45 |
| Phosphate | (mg $kg^{-1}$) | 8.19 | 5.12 |
| Exchangeable Na | (mg $kg^{-1}$) | 746 | 3538 |
| Exchangeable Ca | (mg $kg^{-1}$) | 5501 | 5374 |
| Exchangeable Mg | (mg $kg^{-1}$) | 2282 | 354 |
| Exchangeable K | (mg $kg^{-1}$) | 402 | 290 |

**Isolation of *rhizobacteria* from Salsola grandis and wheat**

Bacteria were isolated from 0.5 g of rhizosphere soils of *S. grandis* and wheat taken from the pot experiment mentioned above and that of naturally growing *S. grandis* taken from Nallıhan. The soil samples were mixed with 5 ml of sterile distilled water with a sufficient vortex and then diluted to $10^{-3}$, $10^{-4}$, and $10^{-5}$. We then placed 50 µl of the resulting soil solutions onto plates of nutrient agar and of potato dextrose agar adjusted to pH 7.0. The plates were then incubated at 25°C for 3 days.

**Screening of Na-resistant bacteria**

Potato dextrose broth was adjusted to pH 7.0 using 1M potassium hydroxide (KOH) and the media with Na (750 mM) and without Na (0 mM) were prepared we then divided 5 ml of the media into 15-ml centrifugation tubes. Na concentration was set referring to Hotchkiss (1923). Individual bacteria were inoculated to each tube and incubated at 25°C for two days. After incubation, the optimal density for bacterial growth was measured at 600 nm. The strains which grew to more than half the number of those growing in the control (0 mM Na) tubes were selected as Na-resistant bacteria.

**Screening of PGPR**

In order to handle high number of strains in experimental condition, a sterile conical tube was set to test 50 strains with 4 replications at the same time in terms of microbes-Na uptake association. Saline soil collected from Aksaray was sieved (<2 mm) and autoclaved at 121 $^0$C for 120 min, and then 20 g of the sieved soil was put into a 50-ml conical centrifuge tube treated with 0.5 ml mineral nutrient solution (N, 100 mg $kg^{-1}$; P, 100 mg $kg^{-1}$; K, 125 mg $kg^{-1}$). The bacterial suspension was adjusted to an optimal density of 1.0 at 600 nm. It was then inoculated with 1 ml bacterial suspension into each tube. The seeds were soaked in 0.5% of sodium hypochlorite (NaOCl) for 2 min for brief surface-sterilization and then rinsed using distilled water. Seeds were then individually transplanted into the tube and we prepared four replications for each bacterial isolate. The seedlings were grown in the plant growth chamber (15°C / 25°C night/day temperature, 55% relative humidity and 12 hours photo period) and harvested at 35 days of the plant development. The yield of shoots and roots was determined based on fresh and dry weight. Moreover, the Na concentration in the harvested plant was also measured using ICP-OES. The data obtained were statistically analyzed using Student's *t*-test to compare the plant growth between seedlings with and without each bacterial species ($P < 0.05$).

**Biochemical assay of PGPR properties**

**Determination of IAA**

The cultures were grown for 4 days at 25°C in IAA production media (30 g of glucose, 2g of beef extract, 3g of $CaCO_3$, 1mM (final concentration) of tryptophan, pH 7, 1 L of distilled water) and centrifuged at 10000*g* for 10 min. The IAA was determined in 300 µl of supernatant using 1.2 ml of Salkowski's reagent at 535 nm (Acuña et al., 2011).

### Determination of phosphate solubilization

The media developed by Pikovskaya (1948) was used for quantitative estimation of tri-calcium phosphate solubilization by the isolates. Strains selected as PGPR were inoculated to the media and incubated at 25°C for 7days. As evaluation of P solubilization, we used following index; - No clear zone, ± detectable of the clear zone but very weak activity, + detectable of clear zone.

### Siderophore production

Chrome azurol S (CAS) medium was prepared using a slightly modified method of Schwyn and Neilands (1987) and Pérez-Miranda et al. (2007). The medium for 100 ml of overlay was prepared as follows: CAS 6.04 mg, hexadecyltrimetyl ammonium bromide (HDTMA) 7.3 mg, Piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES) 3.04 g, and 1 ml of 1 mM $FeCl_3 \cdot 6H_2O$. Siderophore detection was achieved after 10 mL overlays of this medium were applied over the agar plates containing cultivated microorganisms to be tested for siderophore production. The CAS-blue agar changed to light orange or yellow if bacteria produce the siderophore. – No color change, + change the color, ++ color change detected in all over the medium.

### 16S rRNA analysis of selected bacteria

To identify the isolated microorganisms, the nucleotide sequences of their 16S rDNA were investigated using molecular techniques. DNA was extracted using 100 µl of 2 × CTAB buffer [2.0% (w/v) CTAB; 1.4 M NaCl; 100 mM Tris-HCl (pH 8.0); 20 mM EDTA (pH 8.0)]. After vortexing, the sample solution was incubated for 30 min at 60°C. An equal volume of chloroform was added to the sample solution and mixed to emulsify the mixture. The mixture was centrifuged at 15300*g* for 20 min at room temperature, and the supernatant was transferred to a new tube. A 2.5 times volume of ethanol was added and the mixture was incubated at room temperature for 20 min, then centrifuged at 15300*g* for 20 min at 4°C. The resulting DNA pellet was rinsed with 70% ethanol and dried. Each dried DNA pellet was dissolved in 50 µl sterilized distilled water. The 16S rRNA gene (700 bp) of each isolate was amplified by a T100™ Thermal Cycler (Bio-rad, CA, USA), with 1 cycle of 95°C for 5 min, 30 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 1 min and a final extension at 72°C for 7 min. The 40-µl polymerase chain reaction (PCR) mixtures contained 1.5 µl DNA extract, 1.5 µl of 10 mM each primer; 800F (GGATTAGATACCCTGGTA) and 1500R (TACCTTGTTACGACTT), 10 µl of 5×FIREPol® MasterMix (SolisBioDyne, Tartu, Estonia). The nucleotide sequences of PCR-amplified fragments were determined by PCR-direct sequencing. The nucleotide sequences determined were compared with those in GenBank. All sequence data, including newly obtained and retrieved sequences, were aligned using the computer program BioEdit (available at http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Distance-based phylogenetic trees were generated using the model of Jukes and Cantor (1969) and a neighbor-joining algorithm (Saito and Nei, 1987). The topology of phylogenetic trees was evaluated by bootstrap resampling (1000 replicates). Clustal W, provided by the DNA Data Bank of Japan (available at http://www.ddbj.nig.ac.jp/Welcome-j.html), was used for the analyses.

## Results

### Pot experiment

The pot experiment was conducted to confirm the Na uptake and allocation by *S. grandis*. The Na uptake by *S. grandis* was compared with wheat in this study. The mean dried weight after the growth period of 82 days was 12.4 mg and 6.36 mg for *S. grandis* and wheat, respectively. The growth of stems and leaves in *S. grandis* were higher than those of wheat, but root biomass showed the opposite result (Table 2). Under Na uptake, *S. grandis* transported high amounts of Na to the stem and leaves from the roots (Table 2), whereas wheat retained Na in the roots.

Table 2. Na uptake by *Salsola grandis* over the short-term cultivation (82 days)

| | Dry biomass (mg) | | Na accumulation (mg $kg^{-1}$) | |
|---|---|---|---|---|
| | Stem & Leaves | Root | Stem & Leaves | Root |
| *S. grandis* | 12.3 | 0.16 | 15447 | 1459 |
| Wheat | 1.73 | 4.63 | 346 | 1563 |

### Isolation and screening of Na-resistant bacteria

The population density of rhizosphere bacteria averaged to $5.38 \times 10^6$, $3.17 \times 10^6$ and $1.10 \times 10^7$ cfu $g^{-1}$ in the rhizosphere soil of wheat (Aksaray soil), *S. grandis* (Aksaray soil) and *S. grandis* (Nallıhan soil), respectively.

A total of 131 strains—50, 50 and 31 strains from rhizosphere of wheat, *S. grandis* (Aksaray soil) and *S. grandis* (Nallıhan soil), respectively—were found during the screening of Na-resistant bacteria. In the screening of Na-resistant bacteria, a total of 36 strains were able to grow more than 80% above the growth levels of the control in the medium with 750 mM NaCl. 14 strains were grown in the medium with 750 mM of NaCl than the control (Figure 1).

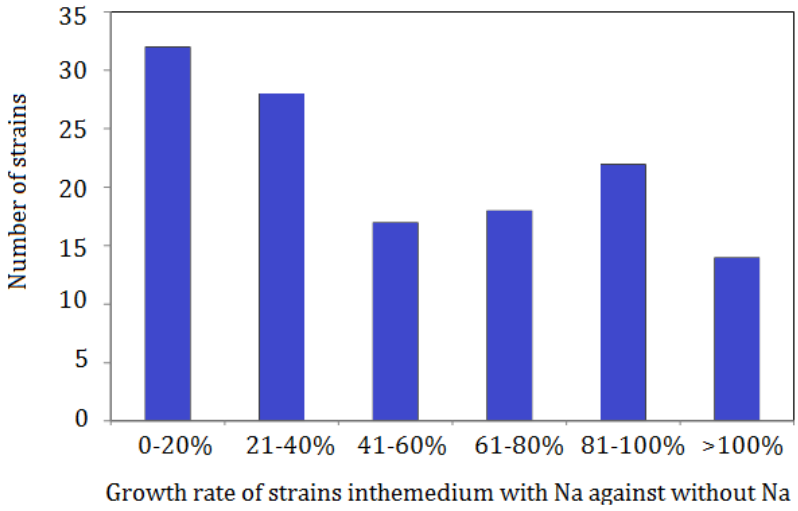


Figure 1. Relative frequency of Na-resistant bacteria from rhizosphere soil

## Screening of PGPR

The plant growth promotion effect of the strains was shown in Figure 2 and Table 3. Total eight strains of bacteria isolated from the rhizosphere of *S. grandis* (strains AP20, B14, A22, B2 and A3) and wheat (strains WN4, WP18 and WP5) enhanced the growth of *S. grandis*, but little variation was observed in bacterial effectiveness among parameters (Table 3). Differences between treatments in the dry weight of stem and leaves were statistically greater than the control in all cases except for strain AP20. The strains A3 and A22 showed significant increases in the fresh weight and dry weight, and means of dried stem and leaves of *S. grandis* in tubes inoculated with these strains increased 15.5% and 17.4%, respectively, above the non-bacterial control. Moreover, the mean dried root weight increased 53.9% and 59.2% in strains A3 and A22, respectively, relative to the non-bacterial control. There was no significant difference between the control and other bacterial treatments (Table 3).

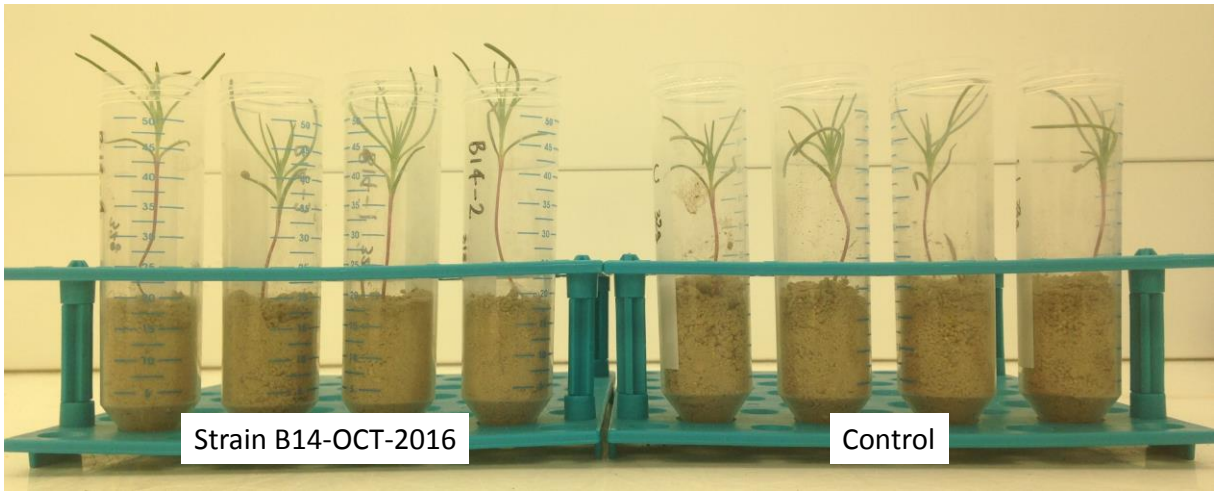


Figure 2. Growth of *Salsola grandis* which was inoculated with strain B14-OCT-2016 (left) and uninoculated control (right)

For PGPR traits, all strains showed a negative activity on the phosphate solubility, but there was a positive activity on the siderophore and IAA production in most strains. Strains A22, WP5, B14 and AP20 produced more than 19 mg $L^{-1}$ of IAA after the 4-day incubation (Table 4). All strains were either siderophore producers or IAA producers. Four strains out of the eight strains (A22, WP5, B14, AP20) showed both traits of siderophore and IAA producers.

Table 3. Growth and Na uptake of *Salsola grandis* by inoculating bacterial strains. These values showed means ± standard deviation. Asterisks indicate significant differences from the control values (*t*-test, $P < 0.05$)

| | Fresh weight (mg) | | Dry weight (mg) | | Length (cm) | | Na conc. in plant |
|---|---|---|---|---|---|---|---|
| Strain No. | Stem & Leaves | Root | Stem&Leaves | Root | Stem& Leaves | Root | (mg kg$^{-1}$) |
| AP20 | 194.2 ± 13.3 * | 16.9 ± 3.35 | 24.8 ± 2.3 | 6.23 ± 1.5 | 9.38 ± 0.28 * | 8.40 ± 1.93 | 25424 ± 2659 |
| WP18 | 207.6 ± 21.3 * | 36.2 ± 11.5 * | 26.8 ± 1.4 * | 11.5 ± 1.0 | 8.40 ± 0.44 | 8.75 ± 2.19 | 23529 ± 422 |
| WP5 | 209.5 ± 18.7 * | 34.7 ± 8.28 * | 27.8 ± 4.9 * | 15.6 ± 7.7 | 8.80 ± 0.42 | 10.6 ± 0.84 * | 21867 ± 1581 |
| B14 | 192.8 ± 23.3 * | 19.4 ± 7.15 | 27.2 ± 2.4 * | 11.7 ± 4.1 | 8.85 ± 0.44 | 8.63 ± 0.85 | 26081 ± 3464 |
| WN4 | 233.3 ± 40.3 * | 19.7 ± 5.05 | 30.2 ± 1.3 * | 10.9 ± 2.4 | 9.03 ± 0.62 | 10.6 ± 1.33 * | 27634 ± 2479 |
| A3 | 206.7 ± 17.6 * | 32.2 ± 7.74 * | 29.8 ± 4.2 * | 14.5 ± 1.9 * | 8.73 ± 0.76 | 8.83 ± 0.99 | 23638 ± 3142 |
| A22 | 233.6 ± 12.5 * | 28.0 ± 2.19 * | 30.3 ± 1.4 * | 15.0 ± 2.3 * | 9.43 ± 0.96 | 8.27 ± 1.01 | 25390 ± 3834 |
| B2 | 207.4 ± 41.9 | 32.6 ± 6.25 * | 26.1 ± 5.2 * | 16.5 ± 4.0 * | 8.40 ± 0.71 | 6.50 ± 1.62 | 21984 ± 851 |
| Control | 163.4 ± 11.5 | 15.9 ± 4.88 | 25.8 ± 3.1 | 9.42 ± 2.1 | 8.10 ± 0.89 | 7.92 ± 1.73 | 24562 ± 2115 |

Table 4. PGPR traits of Na resistant bacteria. +; producing siderophore and solubilizing P, -; no siderophore production and P solubilization, ±; weak P solubilization.

| Strains | Siderophore | P solubilization | IAA (mg L$^{-1}$) |
|---|---|---|---|
| A3 | + | ± | 2.34 |
| WN4 | + | - | 2.55 |
| WP5 | ++ | - | 19.0 |
| A22 | + | - | 17.7 |
| B14 | + | - | 21.7 |
| B2 | + | - | 1.61 |
| AP20 | + | - | 18.7 |
| WP18 | - | - | 33.4 |

### 16SrRNA sequencing analysis

The 16S rRNA partial gene sequences of the eight strains were compared with those of the bacterial sequences in GenBank. Strains AP20, WP18, WP5, B14, WN4, A22 and B2 exhibited high sequence similarities to those of genus *Arthrobacter*, as shown by the constructed phylogenetic dendrogram (Figure 3). The highest sequence similarity of AP20 (GenBank accession no. LC176874), WP18 (GenBank accession no. LC176871), WP5 (GenBank accession no. LC176876), B14 (GenBank accession no. LC176872), WN4 (GenBank accession no. LC176873), A22 (GenBank accession no. LC176870) and B2 (GenBank accession no. LC176875) was found with *Arthrobacter globiformis* (GenBank accession no. KF923428), *A. pascens* (GenBank accession no. X80740), *A. sulfureus* (GenBank accession no. KF923457), *A. nitroguajacolicus* (GenBank accession no. FM213395), *A. agilis* (GenBank accession no. X80748), respectively. In addition, Strains A3 (GenBank accession no. LC176877) was closely related with *Bacillus amyloliquefaciens* (GenBank accession no. KX369577).

## Discussion

In the present study, we revealed that *Salsola grandis* was able to uptake Na from soil and located it in aboveground plant tissues in higher amounts. Moreover, eight new PGPR, seven strains of *Arthrobacter* spp. and one strain of *Bacillus* sp., were isolated from rhizosphere soils.

*S. grandis* was reported as a new species of genus *Salsola,* and it is an annual halophytic plant which is distributed in Nallıhan region as the region has semi-arid climate conditions and clayey soil environments (Freitag et al., 1999). However, there has been no previous report about the Na uptake from soil by this plant. It is said that the halophytes are associated with a correlation between cation accumulation and plant succulence (Maimaiti et al., 2012), and *S. grandis* is also succulent. Moreover, *Salsola soda*, a relatively closely related species, is also able to uptake Na at the value of around 45,980 mg kg$^{-1}$ (Hasanuzzaman et al., 2014). Thus *S. grandis* also has a high potential to uptake Na. In this study, we revealed that *S. grandis* is able to uptake Na, as shown in Table 2. We tried to show the sodium accumulation of *S. grandis* in comparison with that of wheat. Wheat (*Triticum aestivum* L.) is moderately salt-tolerant and one of the most important staple crops in the world (Maas and Hoffman, 1977). Moreover, it occupies 32.8% (7860,000 ha in 2015) in the cultivated land in Turkey. The comprehensive surveys conducted on salt tolerant crops indicated that wheat can tolerate and grow with no yield loss up to salinity level of 6–8 dS m$^{-1}$ EC corresponding 60–80 mM NaCl.

(Maas and Hoffman, 1977; USDA-ARS, 2005; Munns et al., 2005), which is very close to the level of Nallıhan soil, 9.3 dS $m^{-1}$ EC and 60 mM NaCl, in the present study. Besides, the survival of any plant species under such a stress condition depends primarily on the ability of the plant to develop adaptive response within their own rhizosphere and phyllosphere, which can be achieved by the aid of specific rhizosphere microorganism. Many studies indicate that plants in different living areas contain different microorganisms (Berendsen et al., 2012). Therefore, in the present study, we tried to isolate bacteria from the rhizosphere of both *S. grandis* and wheat to monitor how free-living rhizospheric bacteria of a salt tolerant (wheat) and a salt accumulator (*S. grandis*) plant respond with high salinity.

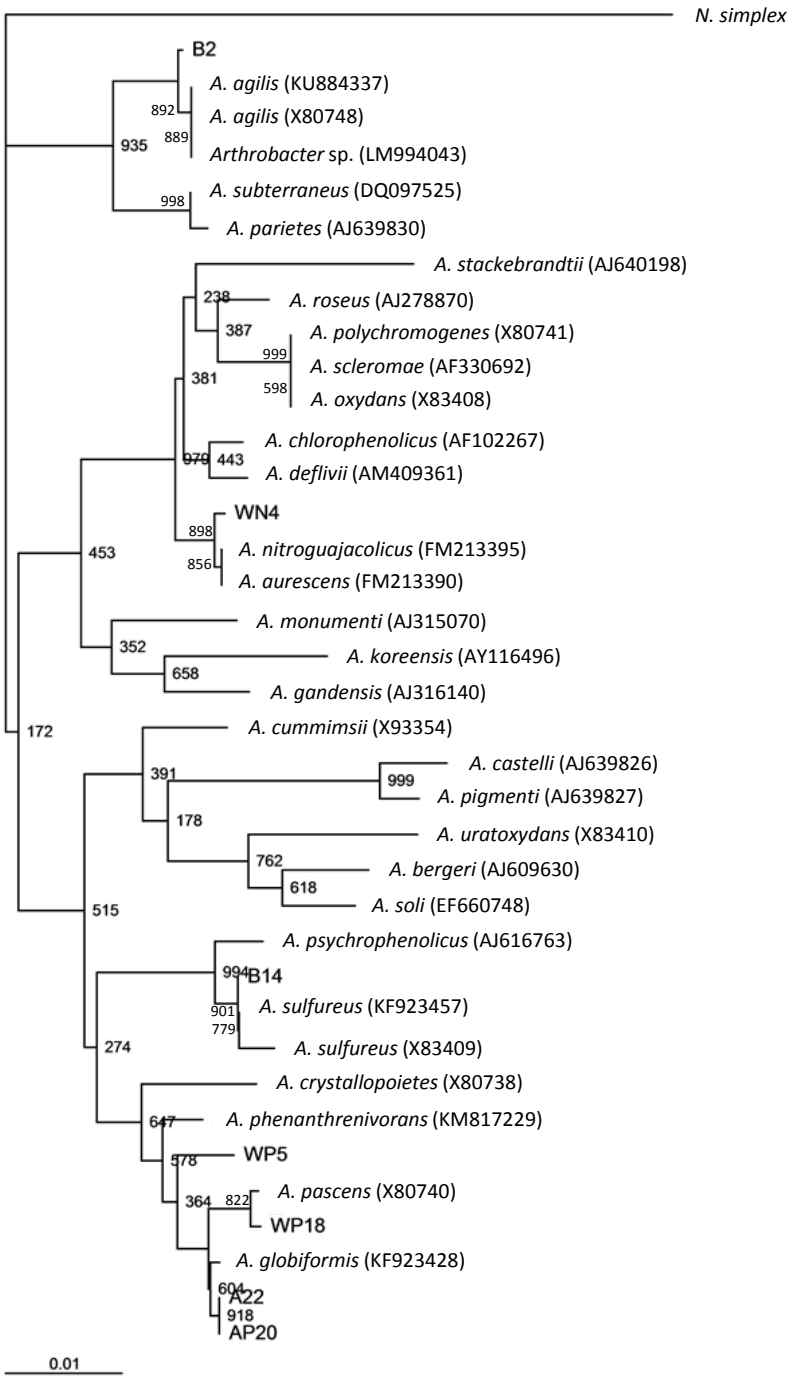


Figure 3. Phylogenetic relationships of strains AP20, WN4, B14, A22, WP5, WP18 and B2 isolated in this study and related species. The phylogenetic tree of 16S rRNA sequences was generated by the neighbor-joining method. The tree was tested for support by performing bootstrap resampling (1000 replicates). Bootstrap values are given at branch points, and the accession numbers of each sequence employed are in parentheses. Bars indicate a relative distance of 0.01 in the 16S rRNA-based tree.

All bacteria isolated and screened as PGPR were able to grow under the medium with 750 mM of NaCl. In the sequencing analysis, seven out of eight strains belonged to genus *Arthrobacter*. Strains of *Arthrobacter* species are among the most frequently isolated indigenous aerobic bacterial genera found in soils. Moreover, there are some reports that indicate *Arthrobacter* spp. has high levels of resistance to not only a variety of toxic metals (Hanbo et al., 2004; Megharaj et al., 2003; Henn et al., 2009) but also NaCl (Upadhyay et al., 2009, 2012). In this study, thus, we suggest that the high isolation of *Arthrobacter* spp. was caused by the high levels of Na stress. In the process of screening, there were many more resistant bacteria in the rhizosphere soil of *S, grandis* than in that of wheat as the frequency was 42.6%, 77.4%, 18% in Aksaray (rhizosphere soil of *S. grandis*), Nallıhan (rhizosphere soil of *S. grandis*), Aksaray (rhizosphere soil of wheat), respectively. The salt concentration might become higher in both soils surrounding of *S. grandis* because of the Na uptake from soil and the non-Na-resistant bacteria might be eliminated from the rhizosphere soil. Therefore, Na-resistant bacteria may be more easily isolated from *S. grandis* than from wheat.

IAA production is an important trait of PGPR (Goswamia et al., 2014), as this phytohormone enables plants to develop their root systems. There are only few reports about IAA production by *Arthrobacter* species (Forni et al., 1992; Siddikee et al., 2010). Our results are in line with these previous findings and indicated that *Arthrobacter* spp. had various types of species with production of IAA even in the genus *Arthrobacter*. Therefore, *Arthrobacter* species in the present study can be considered as potential PGPR. Isolated strains A22, WP5, B14, WP18 and AP20 produced IAA in the presence of L-Tryptophan. Moreover, strains A22, WP5, B14 and AP20 also produced siderophore. The production of siderophores is important and possible to bind with the available form of iron $Fe^{3+}$ in the rhizosphere (Ahmad et al., 2008). This suggests that these strains enhance the growth of *S. grandis* as a result of their enhanced IAA and siderophore production.

We previously expected that Na-resistant PGPR might increase Na uptake of *S. grandis* from soil which may facilitate phytoremediation of saline soils. In this study, however, inoculation with Na-resistant PGPR (AP20, B14, WN4 and A22) did not change Na uptake from soil significantly but they showed higher Na uptake compared to un-inoculated control within 35 days laboratory experiment (Figure 2 and Table 3). This may be attributed to short experimental growth period in the present study. Therefore, experimental studies with longer period should be conducted for a better understanding of the association between Na-resistant PGPR and *S. grandis*.

In conclusion, eight strains appear to be suitable candidates for PGPR of *S. grandis*. These strains belong to genus of *Arthrobacter* and *Bacillus* and this is the first report on the Na-resistant PGPR of *S. grandis*. These PGPR candidates on *S. grandis* have possibility of achievement to remove salt from saline soils using microbe assisted phytoremediation.

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