

Mitigation of salt stress in radish (*Raphanus Sativus* L.) by plant growth promoting rhizobacteria

ERTAN YILDIRIM*, METIN TURAN MESUDE FIGEN DONMEZ*****

*Ispir Hamza Polat Vocational Training School, Atatürk University, 25900, Ispir, Erzurum, Turkey

**Department of Soil Science, Faculty of Agriculture, Atatürk University, Erzurum, Turkey

***Department of Plant Protection, Faculty of Agriculture, Atatürk University, Erzurum, Turkey

Abstract

The effects of *Staphylococcus kloosii* EY37 and *Kocuria erythromyxa* EY43 on some agro-physical properties such as shoot-root fresh weight, shoot-root dry weight, emergence percent (EP), chlorophyll content, leaf number per plant (LNPP), leaf relative water content (LRWC), electrolyte leakage (EL) and ionic composition of leaves of radish were tested under saline conditions. Seeds were soaked in the bacterial suspension incubated at 27°C for 2 h. After incubation the seeds were air-dried before use. Salinity treatments were established by adding 0 and 80 mM of NaCl to a base complete nutrient solution. In control, salt treatment significantly decreased shoot and root fresh-dry weight, EP, LNPP, LRWC, chlorophyll and mineral content, but increased EL. However, EY37 and EY43 treatments under saline conditions significantly increased shoot fresh weight (79.5%, 56.2%), root fresh weight (100.0%-52.2%), shoot dry weight (108.1%-97.0%), root dry weight (150.6%-157.4%), LNPP (34.1-12.8), LRWC (37.0%-20.6%), EP (17.2%-14.1%) and chlorophyll content (21.2%-24.9%), but decrease EL (19.5%-26.9%) compared to the control (without plant growth promoting rhizobial bacteria (PGRB)). Ionic compositions of the leaves of radish plants were significantly affected by salinity and bacterial inoculations. All nutrient element contents investigated were significantly decreased except for Na and Cl under salt stress, which were significantly increased by salt treatment. Bacterial inoculation under salinity conditions usually increased plant nutrient element (PNE) contents of the leaves. It can be concluded from the study that treatment with EY37 and EY43 strains can ameliorate the deleterious effects of salt stress on nutrition and on the growth parameters of radish plants under salinity conditions; PGRB treatment could offer an economical and simple application technology to alleviate the moderately salt sensitive radish production problems in arid soil caused by high salinity.

Keywords: Ameliorative effect, radish, *Staphylococcus kloosii*, *Kocuria erythromyxa*, salinity stress

Introduction

Cultivated soils worldwide are becoming more saline from marginal irrigation water, excessive fertilization, and desertification processes. Impacted soils are a major limiting production factor worldwide for every major crop [1]. Furthermore, reallocation of fresh water from agriculture to cities and industries limits irrigated crop production in many important food-production regions. Under these conditions, production is dependent on the use of alternative water sources including saline or waste water for irrigation. Salinity is a major abiotic stress reducing the yield of a wide variety of crops all over the world [2]. Globally, more than 770.000 km² of land is salt-affected by secondary salinization: 20% of irrigated land, and about 2% of dry agricultural land [3]. The restriction of plant growth and productivity due to salinity is especially acute in arid and semi-arid regions around the world. The salinity and alkalinity are also a problem in some part of Turkey. The global estimate for

agricultural land threatened by or already lost to salinity exceeds 900 million ha [4]. In Turkey, about 2 million ha of cultivated land is affected by salinity and alkalinity [5].

Salinity may occur when there is irregular irrigation, inadequate drainage, wrong fertilizer application, and it extremely increases particularly in protected cultivation [6]. Generally plants growing in saline media come across with major drawbacks. The first is the increase in the osmotic stress due to high salt concentration of soil solution that decreases water potential of soil. The second is the increases in concentration of Na and Cl, exhibiting tissue accumulation of Na and Cl and inhibition of mineral nutrients uptake, thus causing ionic imbalance [7].

Many attempts have been made to reduce the drastic effect of salt stress on growth and productivity, most focusing on chemical amelioration, that involve developing salt-resistant cultivars, leaching excess soluble salts from upper to lower soil depths, flushing soils that contain salt crusts at the surface, reducing salt by harvesting salt-accumulating aerial plant parts in areas with negligible irrigation water or rainfall for leaching, and amelioration of saline soils under cropping and leaching [8].

Breeding for tolerance to salinity in crops has usually been limited by a lack of reliable traits for selection. Multiple genes seem to act in concert to increase salinity tolerance, and certain proteins involved in salinity stress protection have also been recognized [9]. Therefore, the developments of methods and strategies to ameliorate deleterious effects of salt stress on plants have received considerable attention. Recently a biological approach using plant growth promoting rhizobacteria (PGPR) inoculation was attempted. The most appropriate solution in such conditions is to use salt tolerant bacterial inoculants that may prove useful in developing strategies to facilitate plant growth in saline soils [10].

GARCIA & HERNANDEZ [11] reported that salinity negatively affects biological activity by high osmotic strength (low water potential) which can be attributed to the toxic effect on microbial growth, except tolerant halophytic bacteria. Therefore, salt-tolerant root-colonizing bacteria that have managed to survive adverse environmental factors could greatly help in harnessing them for their beneficial properties in such environments in which other microorganisms hardly survive [12].

These PGPR can also prevent the deleterious effects of one or more stressors from the environment. In addition, the identification, selection and application of suitable beneficial microorganisms can increase the options to deal with growing problems, and can be also environmentally sound [13].

Although some studies have been conducted on effects of PGPR on growth and productivity in different plants such as tomatoes [12] and lettuce [14] no attempts have to date been made to study the effects of *Staphylococcus kloosii* EY37 and *Kocuria erythromyxa* EY43 on growth, productivity and ionic compositions in radish plants under salinity conditions. Radish is considered as a moderately sensitive crop to salinity [15]. A review of literature reveals that no investigations have been carried out in relation to salt acclimation of radish treated with *Staphylococcus kloosii* EY37 and *Kocuria erythromyxa* EY43. Therefore, the aim of the present study was to determine the effect of these bacteria on emergence, plant growth, some physiological properties and resistance of radish grown under salt stress.

Materials and methods

Plant materials and growing conditions

The study was conducted under greenhouse conditions at Atatürk University, in Turkey, during 2008. Radish (*Raphanus sativus* L. cv Cherry Belle) plants were maintained under natural light conditions, a day/night temperature of approximate 23/13⁰C and 60% relative humidity during the span of the experiment.

Mitigation of salt stress in radish (*Raphanus Sativus* L.) by plant growth promoting rhizobacteria**Bacterial isolation, culture and inoculation**

Forty four bacterial strains were originally isolated from the rhizosphere of plants naturally grown on high salty soils in Upper Coruh Valley (N+40° 29' L+41° 01') Erzurum, Turkey. The bacterial strains were tested to determine their salt tolerance. One small colony of each strain was placed in Petri dishes containing agar and 2, 5 and 10% NaCl. *Staphylococcus kloosii* EY37, *Kocuria erythromyxa* EY43 (with MIS similarity index (SIM) of 0.580, 0.600, respectively, based on fatty acid methyl ester analysis using MIDI system), which showed resistance to the 10% NaCl, were selected to test.

Bacteria were grown on Nutrient Agar (NA) for routine use, and maintained in Nutrient Broth (NB) with 30% glycerol at -80 °C for long-term storage. For this experiment, the bacterial strains were grown on nutrient agar. A single colony was transferred to 250 ml flasks containing NB, and grown aerobically in flasks on a rotating shaker (95 rpm) for 24 h at 27 °C. The bacterial suspension was then diluted in sterile distilled water to a final concentration of 10⁸ CFU ml⁻¹. For seed treatments, seeds were soaked in the bacterial suspension amended with sucrose to facilitate the adherence of the bacteria to the seeds and incubated at 27°C for 2 h. After incubation the seeds were air-dried before use. Seeds soaked in distilled water amended with sucrose served as the control.

The bacterial strains were able to grow in N-free basal medium indicating their N-fixing potential. In the present study, P solubilizing activities of the two new (EY 37 and EY 43) were measured according to the qualitative methods [16] (Table 1).

Table 1. Some biochemical characteristics of the bacterial strains tested

Bacterial strain	Gram stain	Catalase	Growth in YDC	P solubilization	Growth in N-free Basal medium
<i>Staphylococcus kloosii</i> EY37	+	+	+	+	-
<i>Kocuria erythromyxa</i> EY43	+	+	-	+	+

Plant growth

Radish seeds were sown into plastic trays filled with a mixture (1/1 v/v) of soil and sand (pH: 7.05, EC:1.82 dS/cm, N:10.2 mg/kg, P:13.22 mg/kg, exchangeable K 1.64 meq/100 g soil, organic matter: 3.1 %). There were four rows in trays and each row contained 50 seeds. Trays were 80x80x20 cm (length x width x height). All trays were randomized on the benches in the greenhouse. There were four replicates per treatment, 24 trays in total.

Salt (NaCl) treatments

Salinity treatments were established by adding 0 and 80 mM of NaCl to a base complete nutrient solution (SoFertig). The composition of the SoFertig (Elfatochem Co., Paris, France) was (%): N, 17; P₂O₅, 9; K₂O, 31; Mg, 2; SO₄, 4; Na, 0.001; Fe, 0.02; Zn, 0.002; Cu, 0.002; B, 0.01; Mn, 0.01; Mo, 0.001. The solution was prepared by adding SoFertig to the distilled water. The electrical conductivities of these solutions after adding 0 and 80 mM of NaCl were determined with a conductivity meter, Model 470 (Jenway Limited). Electrical conductivities (EC) of these solutions were 1.82 dS m⁻¹ for 0 mM NaCl and 10.21 dS m⁻¹ for 80 mM NaCl.

Emergence percentage

Normal seedlings were counted after sowing and emergence percentage (EP) determined.

Chlorophyll measurements

A portable chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Japan) was used to measure leaf greenness of the radish plants at 2 days before harvest. For each plant measurements were taken at four locations on each leaf, two on each side of the midrib on all

fully expanded leaves and then averaged. Relative leaf chlorophyll content was expressed in SPAD units defined by manufacturer as “1” equivalent to very pale green coloration (chlorotic) and “50” equivalent to very dark green coloration

Measurement of electrolyte leakage (membrane permeability)

For measurement of electrolyte leakage, 20 leaf discs (10 mm in diameter) from the young fully expanded leaves from four plants per replicate were placed in 50 ml glass vials, rinsed with distilled water to remove electrolytes released during leaf disc excision. Vials were then filled with 30 ml of distilled water and allowed to stand in the dark for 24 h at room temperature. Electrical conductivity (EC1) of the bathing solution was determined at the end of incubation period. Vials were heated in a temperature-controlled water bath at 95°C for 20 min, and then cooled to room temperature and the electrical conductivity (EC2) was measured. Electrolyte leakage was calculated as percentage of EC1/EC2.

Leaf Relative Water Content (LRWC)

To determine LRWC of plant, four leaves were collected from the young fully expanded leaves of four plants per replicate at 2 days before harvest. Individual leaves first detached from the stem and then weighed to determine fresh weight (FW). In order to determine turgid weight (TW), leaves were floated in distilled water inside a closed Petri dish. Leaf samples were weighed periodically, after gently wiping the water from the leaf surface with the tissue paper until a steady state achieved. At the end of imbibitions period, leaf samples were placed in a pre-heated oven at 70°C for 48 h, in order to determine dry weight (DW). Values of FW, TW, and DW were used to calculate LRWC using the equation below [17]:

$$\text{LRWC (\%)} = \frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})} \times 100$$

Growth Parameters

Forty five days after sowing (DAS), twenty plants from each replicate were randomly harvested, and data on plant growth variables, such as shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, and leaf number were collected.

Mineral Analysis

In order to determine the mineral contents of leaves of radish, plant samples were collected from fully expanded leaves at fourth five DAS than oven-dried at 68°C for 48 h and ground and passed 1 mm sieve size. The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Königswinter, Germany) were used to determine total N. Phosphorus and S contents were determined after wet digestion using a HNO₃-HClO₄ acid mixture (4:1 v/v) [18]. Phosphorus and S in the extraction solution was measured spectrophotometrically using the indophenol-blue and ascorbic acid method [18] and a UV/VIS Aquamat Spectrophotometer at 660 nm and at 440 nm respectively (Thermo Electron Spectroscopy LTD, Cambridge, UK). Potassium, Na, Ca, and Mg, Fe, Mn, Zn, and Cu were determined after wet digestion using a HNO₃-HClO₄ acid mixture (4:1 v/v). In the diluted digests, K, Na, Ca, Mg, Fe, Mn, Zn, and Cu analysis were determined by atomic absorption spectrometry (Perkin Elmer 3690) [18].

Statistical Analysis

Experimental design was hierarchical with respect to two factors arranged in a completely randomized design with four replications. The first factor (NaCl levels) had two levels (0 and 80 mM), and the second one (bacterial strains) had three levels (control, EY37, EY43). Data were subjected to analysis of variance (ANOVA) to compare the effects of salt stress treatments and bacterial strains. Percentage data were transformed using arcsine prior to statistical analysis. The differences between the means were compared using least significant difference test (LSD, $p < 0.05$).

Mitigation of salt stress in radish (*Raphanus Sativus* L.) by plant growth promoting rhizobacteria**Table 2.** Radish leaf mineral content as affected by bacteria treatments and salt stress (mean \pm standard error, $n = 4$ each treatment)

Salt Treatment	Bacterial Treatment	N	P	K	Ca	Mg	Na	Cu	Mn	Zn	Fe
(mM)		g 100 g ⁻¹ dW					mg kg ⁻¹ dW				
0	Control	2.37 \pm 0.07c	0.46 \pm 0.02b	3.12 \pm 0.25b	0.77 \pm 0.04b	0.53 \pm 0.02b	1089 \pm 84	22.00 \pm 2.65a	37.67 \pm 2.52b	22.33 \pm 0.58b	105.67 \pm 7.32b
	EY37	3.03 \pm 0.06a	0.63 \pm 0.03a	3.49 \pm 0.03a	0.84 \pm 0.04a	0.50 \pm 0.02b	1000 \pm 20	21.33 \pm 1.16a	55.00 \pm 2.65a	22.67 \pm 2.31b	116.00 \pm 2.65a
	EY43	2.90 \pm 0.05b	0.66 \pm 0.02a	3.41 \pm 0.03a	0.81 \pm 0.01ab	0.62 \pm 0.04a	1013 \pm 12	17.33 \pm 0.58b	54.33 \pm 4.04a	26.00 \pm 1.00a	104.00 \pm 3.61b
	LSD	0.12	0.05	0.29	0.06	0.05	ns	3.40	6.28	2.98	9.74
80	Control	2.12 \pm 0.15b	0.30 \pm 0.01c	2.22 \pm 0.17b	0.61 \pm 0.01b	0.33 \pm 0.02b	3267 \pm 160a	10.33 \pm 0.58b	26.67 \pm 0.58b	17.33 \pm 1.53b	68.00 \pm 2.65c
	EY37	2.66 \pm 0.21a	0.47 \pm 0.02a	2.81 \pm 0.03a	0.74 \pm 0.03a	0.45 \pm 0.04a	2670 \pm 118b	12.00 \pm 1.00a	39.67 \pm 2.52a	19.67 \pm 0.58a	91.67 \pm 5.51b
	EY43									19.00 \pm 0.06a	
	LSD	0.31	0.03	0.20	0.05	0.06	2638 \pm 122b	12.33 \pm 0.58a	37.67 \pm 2.52a	b	100.00 \pm 2.00a

^z: Numbers with the same letters in the same column are not statistically different ($p < 0.05$) n.s: non significant

Results

Effects of bacterial application on EP and growth

EP was significantly influenced by NaCl and PGRB strains. There were no significant differences between treatments in terms of EP in salt stress absence whereas PGPR strains significantly ($p < 0.05$) increased the EP values compared to the control (non-inoculated plants) under stress conditions. When evaluated with and without PGPR treatment in salt stress conditions, the lowest value of EP was obtained without PGPR, while the highest was recorded in the EY37 treatment (Figure 1). Salt stress significantly ($p < 0.05$) decreased leaf number of radish plants regardless of PGPR treatments compared to the non-saline conditions. However, plants treated with PGPR had more leaf number than the control under salt stress. E37 gave the highest leaf number in both salt stress and salt stress absence (Figure 1).

The results of this study showed that growth parameters were significantly affected by salinity and bacterial applications (Figure 1). Fresh-dry shoot and root weight significantly ($p < 0.05$) were decreased by 80 mM NaCl treatment. An increase in NaCl concentration from 0 mM to 80 mM resulted in dry shoot and root weight reduction of 77.9% and 85.6%, respectively. Nevertheless, bacterial treatments under salinity conditions have positive effects on plant growth. EY 37 inoculation gave the highest fresh shoot (10.1 g) and root (5.1 g) weight and dry shoot- root (6.0g-2.2 g) weight. EY43 bacterial strains had also higher fresh and dry shoot-root weight than the control under salt stress.

Effects of bacterial application on chlorophyll content, LRWC and EL

Figure 1 shows the effect of salt stress and PGRB treatments on chlorophyll content, LRWC and electrolyte leakage of radish plants. Salinity and bacterial applications significantly ($p < 0.05$) affected chlorophyll content, LRWC and EL in this study. Chlorophyll contents of plant leaves were significantly ($p < 0.05$) decreased with the salt stress. Effects of PGRB application on chlorophyll contents of leaves was not observed in salt stress absence, but significant ($p < 0.05$) increase was obtained with PGRB application under salt stress conditions (Figure 1). The lowest and highest chlorophyll reading values were obtained from without PGPR (32.1) and EY43 (40.1) treatments in 80 mM NaCl, respectively.

Increasing the concentration of NaCl from 0 to 80 mM lowered LRWC of radish plants. The average percentage of LRWC decrease was 36.3% under salt stress. Bacterial applications under salinity condition elevated the LRWC level (61.93% for EY37, 54.5% for EY43) over to the control (Figure 1).

Salinity induced significant ($p < 0.05$) increases in EL compared with no salinity (0 mM NaCl) but plants treated with PGPR strains showed lower values than non-treated ones. There were no significant ($p < 0.05$) differences between treatments in regard to electrolyte leakage under the salt absence (Figure 1).

Effects of bacterial applications on ionic composition of leaves

The results showed that salinity and bacterial inoculations significantly affected ionic compositions of leaves (Table 2). We have found in this study that compared with 0 mM NaCl treatment 80 mM NaCl treatment decreased plant nutrient element (PNE) contents of leaves, except Na and Cl contents. On the other hand, Na and Cl concentration of leaves increased with increasing NaCl concentration in the nutrient solution. However, generally bacterial applications under salinity condition significantly increased PNE content of leaves except for Na and Cl concentration compared to the control. Na and Cl contents of leaves significantly ($p < 0.05$) were decreased by bacterial applications. Bacterial applications brought PNE contents of leaves near to that of 0 mM NaCl treatment.

Mitigation of salt stress in radish (*Raphanus Sativus* L.) by plant growth promoting rhizobacteria

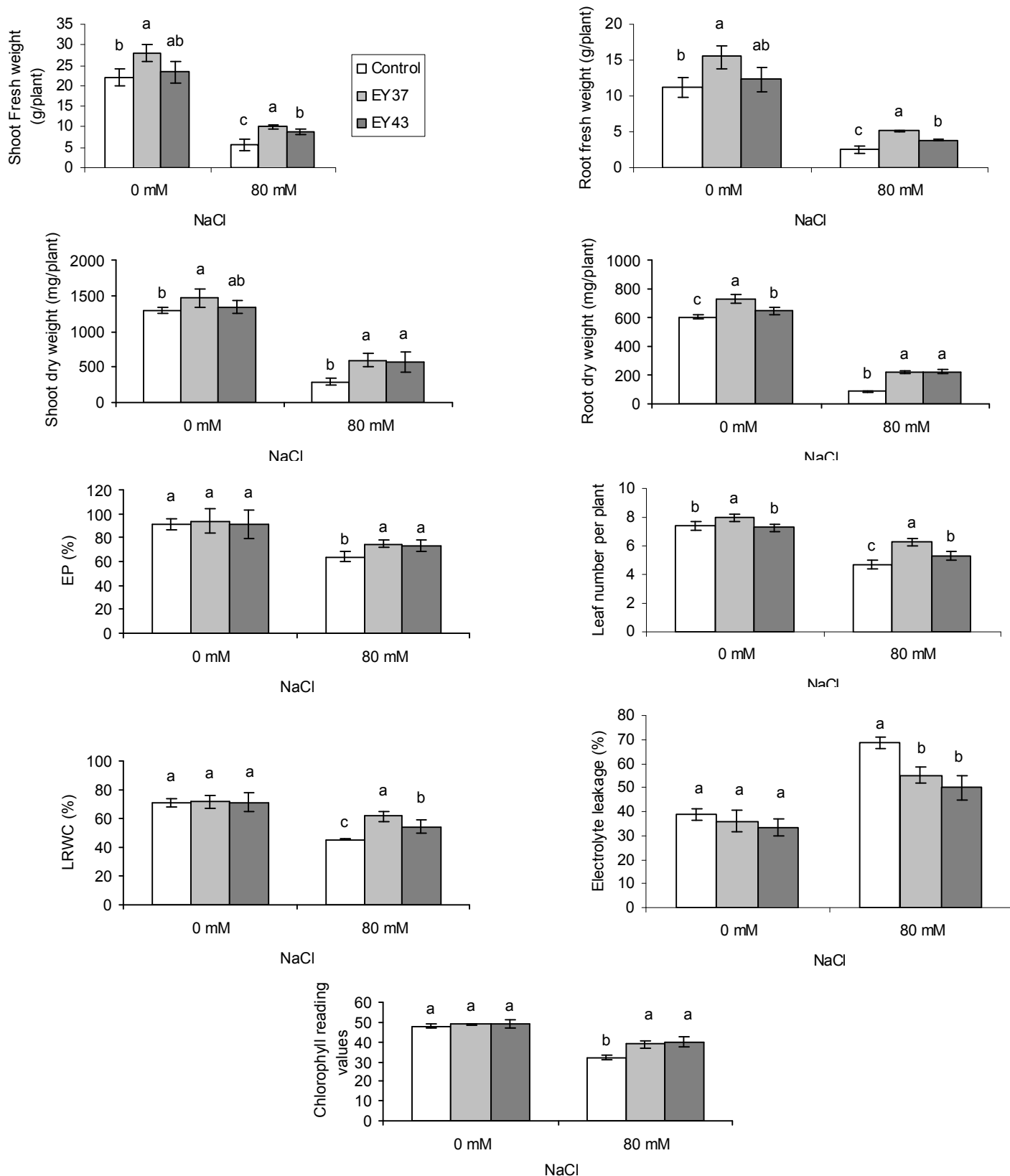


Figure 1. Emergence percentage, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, leaf number per plant, chlorophyll content, electrolyte leakage and LRWC of radish plants in response to different PGRB treatments under salt stress.

Different letters on top of bars indicate significant differences according to LSD test ($p < 0.05$) at each salt level. Vertical bars indicate the mean \pm SE, $n=4$.

Discussion

PGPR are free-living microorganisms having beneficial effects on plants by colonizing in the rhizosphere of plants. PGPR may improve plant growth and yield by indirect and direct mechanism. In indirect mechanism, PGPR have antagonistic effect against phytopathogenic microorganisms [19].

Direct mechanisms may act on the plant itself and affect growth by means of plant growth regulators such as auxin, cytokinins and gibberellins, solubilizing of inorganic phosphate and mineralization of organic phosphate and asymbiotic fixation of atmospheric nitrogen [20].

In this study, evidence is provided that PGPR improves emergence performance, growth and nutrient uptake of radish and to the best of our knowledge, this is the first report to suggest that *Staphylococcus kloosii* EY37 and *Kocuria erythromyxa* EY43 inoculated onto seeds could induce tolerance to salt stress. Similar findings were reported in the previous studies showing that application of PGPR may stimulate yield, growth and PNE uptake from soil in different crops such as tomatoes [12, 13] and lettuce [14] under salinity conditions. However, *Azospirillum* sp., *Achromobacter* sp., *Serratia* sp., *Rhizobium* sp., *Aeromonas* sp. and *Bacillus* spp. as PGPR was used in these previous studies. *Staphylococcus* and *Kocuria* were not used as PGPR under saline conditions.

The present study demonstrated that salt stress adversely affected emergence and growth of radish (Figure 1). Salinity, as an abiotic hazard, induces numerous disorders in seeds and propagules during germination. Salinity either completely inhibits germination at higher levels or induces a state of dormancy at lower levels [21]. Salinity can also affect germination by facilitating the intake of toxic ions, which can cause change of certain enzymatic or hormonal activities of the seed. Salinity has been reported to cause significant reductions in the rate and final percentage of germination and emergence of radish, which in turn may lead to uneven stand establishment and reduced crop yields [22, 23, 24]. In the study, radish seeds inoculated with PGPR strains showed greater EP than non-primed ones under salt stress. Similarly, PGPR inoculations improved germination of seeds of lettuce [14] in saline conditions. This positive effect of the PGPR on seed germination and emergence could be attributed to the bacterial ability to produce or modify plant hormones including gibberellins, which play a key role in germination [14].

Salt stress also significantly decreased leaf number, fresh and dry weight of shoot and root of radish plants (Figure 1). Similarly, MARCELIS & VAN HOOIJDONK [23] and Jamil et al. [24] reported that growth characteristics such as shoot and root weights, and leaf area were negatively affected when radish plants were grown under salt stress. The present study showed that PGPR improved the growth parameters of radish plants compared to the non-inoculated controls under salt stress as well as non-saline conditions (Figures 1). Our findings are concordant with those of BOCHOW & al. [25] in eggplant, MAYAK & al. [12] in tomato, YILDIRIM & TAYLOR [26] in bean, BARASSI & al. [14] in lettuce, and YILDIRIM & al. [27] in squash, who found that PGPR ameliorated the deleterious effects of salt stress on plant growth. This results also confirmed that the PGPR inoculations may effectively increase the surface area of roots [28] and the root weight [29].

Salt stress significantly decreased chlorophyll reading values compared to the non-saline conditions (Figure 1). Similarly, JAMIL & al. [24] indicated that chlorophyll content was reduced by salt stress. However, PGPR inoculation elevated the chlorophyll reading values compared to the controls under both salt stress and absence of salt stress. There were

Mitigation of salt stress in radish (*Raphanus Sativus* L.) by plant growth promoting rhizobacteria

no significant ($p < 0.05$) differences between treatments in regard to LRWC in 0 mM NaCl while PGRB treatments caused to the increase in LRWC under salt stress. Electrolyte leakage was higher in 80 mM NaCl compared to the non-saline conditions. PARIDA & DAS [30] reported that salt stress led to a significant increase in the level of electrolyte leakage in many crops. In the present study, PGRB inoculated plants had less electrolyte leakage compared to their respective non-inoculated controls. PGRB can trigger a plant-mediated induced systemic resistance (ISR) response [31]. SASKIA & al. [32] reported that PGPR could induce accumulation of the signaling molecules of salicylic acid and jasmonate, which have been shown to be important signal molecules for modulating plant responses to environmental stress [33].

Bacterial inoculations in radish plants could alleviate deleterious effects of salt stress. This phenomenon can be explained by a couple of mechanisms:

-bacterial inoculation can restrict Na and Cl uptake and enhance the uptake of other PNE such as N, P, K and Ca positively. We found that bacteria inoculated plants under salt stress have lower Na and Cl contents and higher PNE contents compared with non inoculated plants. Seed inoculation of radish with the EY37 and EY43 significantly increased concentration of P and N in the leaves of radish plants. The higher total N and P uptake of radish could be aroused by N fixation and P solubilization, thus promoting the plant growth (Table 1). Phosphate-solubilizing and N₂-fixing bacteria can improve the N and P nutrition of plants and stimulate the plant growth [34, 35]. Our data showed that a higher nutrient uptake by PGPR inoculations significantly improved seedling growth.

- PGPR inoculants could hold inorganic ions, especially Na⁺, in roots, thus preventing their transfer to leaves. It is known that halophobic plant species have this mechanism [36]. These bacteria could contribute to increase to salt tolerance via decreasing Na uptake. PGPR can facilitate rooting and growth of plants under saline conditions by enhancing water use efficiency, by providing plants with fixed nitrogen, iron and soluble phosphate [12, 37]. In these ways, plant growth and PNE uptake from soil could be increased by stimulation of root growth. Thus, increasing root growth and PNE uptake and decreasing Na and Cl uptake could supply the stabilization of membrane permeability, and increase chlorophyll reading value and LRWC, stimulating the growth of radish plants under saline conditions. YILDIRIM & al. [27] reported that some PGPR treatments reduced the Na uptake of squash plants and/or increased the K and Ca uptakes compared to the control treatment (non-inoculated plants) under salt stress. ASHRAF & al. [38] determined that PGRB, exopolisaccharide-producing bacteria, could restrict Na influx into roots.

-PGPR can directly affect plant growth through the production of phytohormones. This direct growth-promoting effect appears to involve the plant growth regulators indole-3-acetic acid and cytokinins [39]. PGPR have been also reported to able to control ACC levels or block ethylene biosynthesis in plants, thus stimulating root growth and helping to protect plants from the deleterious effects of the salt stress (12).

Conclusion

Saline soils and saline irrigations constitute a serious production problem for vegetable crops as saline conditions are known to suppress plant growth. The present study demonstrates that salinity stress induced lower plant fresh-dry weight, lower chlorophyll and macro and micro element content in radish plants. The assessment of the effect of salinity on the growth parameters by different salinity levels enabled us to conclude that all of the considered parameters were affected by salinity. To conclude the

present study, we suggest that PGPR seed treatments can ameliorate the deleterious effects of salt stress. The addition of PGPR could offer an economical and simple treatment to salt sensitive radish plants, helping to solve the production problems caused by high salinity. The study does not provide evidence for induction of salt stress tolerance at plant tissue, cell or molecular level. Thus, further studies are required in order to determine the effect of PGPR tested at these levels and the efficiency of these PGPR under natural field condition and for other plant species. E37 treatment could show better effect to alleviate the salt stress in radish than the E43.

Acknowledgments

We are very grateful to The Atatürk University, Scientific Research Projects Foundation for generous financial support (Project Number 2007/70).

References

- [1] M.C. SHANNON, C.M. GRIEVE, *Scientia Hort.* 78, 5-38 (1999).
- [2] M. ASHRAF, M.A. FOOLAD. *Env. Exp. Bot.* 59, 206–216 (2007).
- [3] FAO, <http://www.fao.org/ag/AGL/agll/spush/intro.htm> (2000).
- [4] T.J. FLOWERS, M.A. HAJIBAGHERI, N.J.W. CLIPSON, *Halophytes*. Quarterly Rev. Biol. 61, (1986), pp 313–337.
- [5] Y. GUNGOR, Z. EREZOL, *Drenaj ve Arazi Islahi*. Ders Kitabı; Ankara University: Ankara, Turkey (1994).
- [6] R. GEORGE, D. MCFARLANE, B. NULSEN, *Hydrogeology Journal* 5, 6-21 (1997).
- [7] H. MARSCHNER, *Mineral Nutrition of Higher Plants* (2nd. Edition) London, GB: Academic Press. Inc. (1995), p. 446.
- [8] M. QADIR, A. GHAFOR, G. MURTAZA, *Land Degradation Dev.* 11, 501–521 (2000).
- [9] B. MURILLO-AMADOR, H.G. JONES, C. KAYA, R.L. AGUILAR, J.L. GARCIA-HERNANDEZ, E. TROYO-DIEGUEZ, N.Y. AVILA-SERRANO, E. RUEDA-PUENTE, *Environ. Exper. Botany* 58, 88–196 (2006).
- [10] M. BACILIO, H. RODRIGEUEZ, M. MORENO, J. HARNENDEZ, Y. BASHAN, (2004) *Biol. Fertil Soils* 40, 188-193.
- [11] C. GARCIA, T. HERNANDEZ, *Plant Soil* 178, 225-263 (1996).
- [12] S. MAYAK, T. TIROSH, B.R. GLICK, *Plant Science* 166, 525-530 (2004).
- [13] M. WOITKE, H. JUNGE, W.H. SCHNITZLER, *Acta Hort.* 659, 363-369 (2004).
- [14] C.A. BARASSI, G. AYRAULT, C.M. CREUS, R.J. SUELDO, M.T. SOBRERO, *Scientia Hort.* 109, 8-14 (2006).
- [15] E.V. MAAS, G.J. HOFFMAN, *Crop salt tolerance - Current assessment*. J Irr Drain Div Proc Am Soc Civ Eng. 103, 115–134 (1977).
- [16] S. MEHTA, C.S. NAUTIYAL, *Curr. Microbiol.* 43, 51–56 (2001).
- [17] C. KAYA, D. HIGGS, F. INCE, B.M. AMADOR, A. CAKIR, E. SAKAR, *J. Plant Nutrition* 26, 807-820 (2003).
- [18] AOAC (Association of Official Analytical Chemists-International), *Official Methods of Analysis*, 18th ed., Dr. William Hortwitz (Editor), Dr. George W Latimer (Assist Edit), AOAC-Int. Suite 500, 481 North Frederick Avenue, Gaithersburg, Maryland 2005, pp 20877-2417, USA.
- [19] D. CUPPELS, F. SAHIN, S.A. MILLER, *Phytopathology* 89, 11-19 (1999).
- [20] A.Z. ZAHIR, M. ARSHAD, W.T. FRANKENBERGER, *Adv. Agron.* 81, 97-168 (2004).
- [21] M.A. KHAN, I.A. UNGAR, *Can. J. Bot.* 75, 835-841 (1997).
- [22] E. YILDIRIM, A. DURSUN, I. GUVENC, A.M. KUMLAY, *Acta Agrobot.* 55, 75-80 (2002).
- [23] L.F.M. MARCELIS, J. VAN HOOIJDONK, *Plant and Soil* 215, 57–64 (1999).
- [24] M. JAMIL, S. REHMAN, K.J. LEE, J.M. KIM, H.S. KIM, E.S. RHA, *Sci. Agric.* 64, 111-118 (2007).
- [25] H. BOCHOW, S.F. EL-SAYED, H. JUNGE, A. STAUROPOULOU, G. SCHMIEEKNECHT, *J. Plant Dis. and Prot.* 108, 21-30 (2001).
- [26] E. YILDIRIM, A.G. TAYLOR, *Annual Report of Bean Improvement Cooperative* 48, 176-177 (2005).
- [27] E. YILDIRIM, A.G. TAYLOR, T.D. SPITTLER, *Sci. Hortic.* 111, 1-6. (2006)

Mitigation of salt stress in radish (*Raphanus Sativus* L.) by plant growth promoting rhizobacteria

- [28] Y. BASHAN, G. HOLGUIN, L.E. DE-BASHAN, *Can. J. Microbiol.* 50, 521–577 (2004).
- [29] H. BERTRAND, R. NALIN, R. BALLY, J.C. CLEYET-MAREL, *Biol. Fertil Soils* 33, 152–156 (2001).
- [30] A.K. PARIDA, A.B. DAS, *Ecotoxicology and Environmental Safety* 60, 324–349 (2005).
- [31] C.M.J. PIETERSE, J.A. VAN PELT, B.W.M. VERHAGEN, J. TON, S.C.M. VAN WEES, K.M. LEON-KLOOSTERZIEL, L.C. VAN LOON, *Symbiosis* 35, 39-54 (2003).
- [32] C.M.W. SASKIA, M. LUIJENDIJK, I. SMOORENBURG, L.C. VAN LOON, C.M.J. PIETERSE, *Plant Mol. Biol.* 41, 537–549 (1999).
- [33] F.V. BREUSEGEM, E. VRANOVA, J.F. DAT, D. INZE, *Plant Sci.* 161, 405-414 (2001).
- [34] H. RODRIGUEZ, R. FRAGA, *Biotechnol Adv.* 17, 319–339 (1999).
- [35] M.E. PUENTE, Y. BASHAN, C.Y. LI, V.K. LEBSKY, *Plant Biol.* 6, 629–642 (2004).
- [36] A. GUREL, R. AVCIOGLU, *Physiology of Resistance to Stress in Plants*. In plant Biotechnology Vol.2: genetic Engineering and Its Applications. Eds. Ozcan S, Gurel E and Babaoğlu M. pp. 288-236. Publishing of S.U. (In Turkish) (2001)
- [37] E. SERGEEVA, S. SHAH, B.R. GLICK, *World J. Microb. Biotech.* 22, 277–282 (2006).
- [38] M. ASHRAF, S. HASNAIN, O. BERGE. T. MAHMOOD, *Biol. Fert. Soils.* 40, 157-152 (2004).
- [39] B. LIPPMANN, V. LEINHOS, H. BERGMANN, *Angew Bot.* 69, 31–36 (1995).