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ORIGINAL PAPER

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USE OF PLANT GROWTH PROMOTING RHIZOBACTERIA AGAINST SALT STRESS FOR TOMATO (*Solanum lycopersicum* L.) SEEDLING GROWTH

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ABSTRACT

Salt stress affects many aspects of plant metabolism and as a result, growth and yield are reduced. The aim in this study was to determine the effects of plant growth promoting rhizobacteria (PGPR) on tomato plants under salt stress. With this aim, the 'Interland F_1' cv. and bacterial isolates of *Bacillus thuringiensis* CA41/1, *Pseudomonas putida* 18/1K, *Pseudomonas putida* S5/4ep, and *Pseudomonas putida* 30 were used. Salt application was completed in two different doses of 25 and 50 mM NaCl when seedlings reached the stage of 3 true leaves. At the end of the study, in addition to seedling development criteria, some nutrient element contents and rates (K, Ca, Na, K/Na and Ca/Na), superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) enzyme activities, malondialdehyde (MDA) and photosynthetic pigment contents were determined. In the stress environment, PGPR inoculation increased K content by up to 10%, while apart from isolate *P. putida* no.30, the other isolates lowered Na content by up to 18%. Additionally, 18/1K and S5/4ep isolates were identified to reduce membrane injury index by up to 97%. It was identified that CA41/1, 18/1K and S5/4ep isolates were more effective against salt stress, especially. In general, the plant tolerance levels induced by the bacteria were identified to increase with the increase in salt stress.

Key words: *Solanum lycopersicum*, PGPR, salt stress, enzyme, chlorophyll, seedling growth

INTRODUCTION

Tomato is the second most commonly produced vegetable after potato globally, with nearly 182 million tons of production [Faostat 2017]. Especially in arid or semiarid regions with little precipitation, mistaken applications of excessive irrigation and fertilizers along with the effect of high surface evaporation leads to soil salinity, one of the increasingly important abiotic stress factors [Yıldız and Balkaya 2016]. During salt stress, firstly the plant experiences water stress due to low osmotic pressure occurring in soil solutions [Munns 2005], additionally ion instability and toxicity occur due to accumulation of high amounts of Na^+ and Cl^- ions [Aktas et al. 2009]. Generally salt stress due to Na^+ and Cl^- ions disrupts the structure of proteins [Tuteja et al. 2012], and some enzymes also lose functions in high Na^+ concentrations [Agarwal et al. 2013].

One of the most common microorganism groups in the rhizosphere region is bacteria [Kaymak 2010]. In general, among these bacteria *Pseudomonas*, *Enterobacter*, *Bacillus*, *Variovorax*, *Klebsiella*, *Burk-*

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holderia, *Azospirillum*, *Serratia* and *Azotobacter* genus encourage plant development and include strains called plant growth promoting rhizobacteria (PGPR) [Nadeem et al. 2014]. It is thought that PGPR comprise 2–5% of soil microflora [Antoun and Prévost 2006]. PGPR directly or indirectly affect the growth and development of plants [Pieterse et al. 2014]. They ease iron intake with siderophore production, in addition some compounds they secrete transform phosphorus into intake forms and fix elementary nitrogen in the air providing nitrogen for plants [Seymen et al. 2014]. They can release a variety of antibiotics and enzymes to inhibit deleterious organism, and also, can reduce the development of pathogens in the rhizosphere region via competition [Ashrafi and Seiedi 2011]. Another effect mechanism of rhizobacteria is the production of hormones with direct effects on plant growth like Indole-3-acetic acid (IAA), cytokinin, auxin and gibberellic acid or they may reduce the harmful ethylene levels formed under stress [Loon 2007]. These mechanisms may provide increases in seed germination, root and shoot development [Yeole and Dube 1997] and chlorophyll content [Singh et al. 1990] and additionally may increase plant tolerance to biotic stress [Nagarajkumar et al. 2004] and abiotic stress conditions like drought and salinity [Lucy et al. 2004].

In this study, the effect of *Bacillus thuringiensis* CA41/1, *Pseudomonas putida* 18/1K, *Pseudomonas putida* S5/4ep, and *Pseudomonas putida* 30 isolates, with PGPR properties determined in previous studies, on tomato seedlings exposed to salt stress at 25 and 50 mM levels was investigated in terms of development parameters, nutritional element content and enzyme activation related to salt stress in studies completed in a growth chamber.

MATERIAL AND METHODS

Plant material, bacterial growth and salt application. Plant material used in the study was the tomato (*Solanum lycopersicum* L. cv. 'Interland F_1'). Bacterial isolates were obtained from the bacteriology laboratory of Ege University Faculty of Agriculture Department of Plant Protection. With efficacy on plant development determined in previous studies, the *Bacillus thuringiensis* CA41/1, *Pseudomonas putida* 18/1K, *P. putida* S5/4ep, and *P. putida* 30 PGPR isolates were used [Bora et al. 2004, Özaktan et al. 2015].

PGPR isolates were inoculated on plants twice, with the seed coating and drenching methods. For seed coating, 48-hour PGPR cultures developed on King's B (Peptone 20 $g \cdot L^{-1}$, K_2HPO_4 1.5 $g \cdot L^{-1}$, $MgSO_4$ $7H_2O$ 1.5 $g \cdot L^{-1}$, Glycerol 10 $mL \cdot L^{-1}$, Agar 15 $g \cdot L^{-1}$) media was suspended in 1.5% carboxymethyl cellulose (CMC). Seeds that removed residue of pesticide were mixed for 30 min to coat with this suspension and left overnight at +4°C. The drenching method; prepared suspensions with 10^8 $CFU \cdot mL^{-1}$ density from 48-hour bacterial cultures developed on King's B medium [King and Raney 1954]. When the first true leaves formed while the second true leaves were opening, PGPR suspension was inoculated on the root collar with the drenching method (20 $mL \cdot seedling^{-1}$) [Akköprü et al. 2018].

The study was designed with 3 repeats according to a completely randomized experimental design, with each repeat including 10 plants planted in drainage-free pots of sterile perlite. When seedlings reached the stage of having three true leaves, NaCl was applied 3 times at one day intervals to reach final concentration of 25 and 50 mM. To provide the nutritional requirements of plants, seedlings were regularly watered with Hoagland nutritional solution [Aktas et al. 2009] at two-day intervals. The study was ended 12 days after salt treatment.

Seedling parameters. Morphologic parameters of shoot and root length (SL and RL), shoot diameter (SD), leaf number (LN), and shoot-root fresh and dry weights (SFW, RFW and SDW, RDW) were determined at the end of the experiment. Additionally, after determining dry weights, root : shoot ratio (dry weight (DW%)) (R : S) was determined.

Leaf relative water content (LRWC). To determine the proportional water content of tomato plants, three plants chosen at random from each repeat first had fresh weight of 3rd and 4th leaves determined (FW) and then were left in sterile pure water for 4 h to reach maximum turgor weight (TW) with the turgor weight measured at the end of this process. Leaf samples with turgor weight measured were placed in an oven at 80°C to dry and then dry weights (DW) were determined. After measurements were completed, the LRWC was calculated using the following formula [Yamasaki and Dillenburg 1999]:

$$LRWC = ([FW - DW]/[TW - DW]) \times 100$$

Membrane injury index (MII). The membrane injury index represents the electrolyte amount released from cells. Under stress conditions, the amount of electrolyte released from cells was determined according to Długokęcka and Kacperska-Palacz [1978] and Fan and Blake [1994]. Discs taken from 3 leaves of plants were left in deionized water at room temperature for 6 h and then EC (electrical conductivity) values were measured. Later discs were left in water at 100°C for 10 min and then EC values were measured again. MII was calculated according to the following formula:

$$MII = ([Lt - Lc/1] - Lc) \times 100$$

Lt: EC value of the stressed leaf before autoclaving, Lc: EC value after autoclaving

Nutrient content in shoots. Plant samples were dried in an oven at 65°C for 48 h and then burned to ash at 550°C. The ash was dissolved in 3.3% HCl with readings for Na, K and Ca completed in an atomic absorption device [Aktas et al. 2009].

Enzyme activation. Frozen leaf samples were homogenized in 5 mL cold 50 mM potassium phosphate and 0.1 mM Na-EDTA mixture (pH 7.6) and then the homogenate was centrifuged at 4°C for 30 min at 709 g. All stages of enzyme extraction were completed at +4°C.

Superoxide dismutase (SOD) was identified by inhibition of nitro blue tetrazolium (NBT) at 560 nm wavelength [Jebara et al. 2005]. SOD activity was determined as the reducing activity for 50% of NBT. Catalase (CAT) activity was determined by observing the loss of H_2O_2 at 240 nm wavelength according to the method of Cakmak and Marschner [1992]. Ascorbate peroxidase (APX) activity was measured as reduction of H_2O_2 linked to ascorbic acid at 290 nm wavelength. APX activity was defined as the amount of enzyme necessary to consume 1 µmol ascorbate per minute [Cakmak and Marschner 1992].

Lipid peroxidation (MDA). Samples of 0.5 g taken from plant leaves were homogenized in 0.1% trichloroacetic acid (TCA) and then the homogenate was centrifuged at 492 g for 15 min. From the centrifuged sample, 1 mL was taken from the clear section and dissolved in 2 mL 20% TCA with 0.5% thiobarbituric acid (TBA) added. The mixture was left for 30 min at 95°C, then rapidly cooled in an ice bath, and centrifuged at 219 g for 10 min. The clear portion had absorbance determined at 532 and 600 nm and the MDA content was calculated using the molar absorption coefficient of 155 $mM^{-1} \cdot cm^{-1}$ [Madhava Rao and Sresty 2000].

Photosynthetic pigments. Samples of 0.25 g taken from leaves were homogenized in 80% acetone in a dark room without direct light, filtered and then the extract was brought to 25 mL with acetone. Samples were read at 663, 645 and 470 nm wavelengths with calculations according to the following equation [Amira and Qados 2011]:

$$\text{Chlorophyll-}a\ (mg \cdot g^{-1}) = (12.7 \times OD_{663}) - (2.69 \times OD_{645}) \times V/W \times 1000$$

$$\text{Chlorophyll-}b\ (mg \cdot g^{-1}) = (22.91 \times OD_{645}) - (4.68 \times OD_{663}) \times V/W \times 1000$$

$$\text{Total Chlorophyll}\ (mg \cdot g^{-1}) = (20.2 \times OD_{645} + 8.02 \times OD_{663}) \times V/W \times 1000$$

$$\text{Carotenoid}\ (\mu g \cdot mL^{-1}) = 1000 \times OD_{470} - 3.27 \times Chl_a - 104 \times Chl_b/227$$

W: the fresh weight for extracted tissue (g), V: the final volume of the extract in 80% acetone, OD: optical density

Statistical analysis. For evaluation of data from measurements and observations in the study, with the aim of determining the effect of PGPR isolates on salt stress, variation rates for 0, 25 and 50 mM applications were based on the control group and comparisons used the following formula;

$$\text{Percent change} = ([\text{PGPR treatment} - \text{Control}]/\text{Control}) \times 100$$

To assess the variance analysis of data obtained, mean PGPR were grouped with the Duncan multiple comparison test using the SPSS program.

RESULTS

Seedling parameters. While the differences in shoot length were significant for PGPR inoculations at 0 and 50 mM ($p \leq 0.05$), the 25 mM dose of NaCl was insignificant (Tab. 1). Based on the effect rates com-

pared to control group of each salt dose, 0 and 25 mM salt application generally reduced the shoot length for all isolates. All isolates except no.30 encouraged increase in shoot length which was not significantly with 50 mM salt application. The highest increase rate was 18.90% with CA41/1 isolate (Tab. 1). The differences in mean root length were significant for 0 and 25 mM and generally PGPR inoculation was identified to increase root length.

In salt-free environment, all isolates were better than negative controls, with negative effects of salt stress on no.30 isolate at 25 mM (–0.88%) and for S5/4ep isolate at 50 mM (–2.89%) (Tab. 1). All PGPR isolates had increased leaf numbers compared to negative control in a stress-free environment; however, generally salt was observed to cause negative effects at 25 mM. Contrarily, with the increase in salt dose, apart from no. 30 isolate (–3.17%), isolates had an increase in leaf numbers. Shoot diameters were observed to have greatest increase compared to controls for CA41/1 PGPR isolate with 0 and 50 mM salt treatment (2.97% and 11.33%, respectively).

The effect of PGPR inoculation on shoot fresh weight was significant for 50 mM salt application ($p \leq 0.05$) (Tab. 2). While no.30 isolate was the only isolate with increase of 3.03% compared to negative control in a salt-free environment, it was the only isolate with reduction observed compared to positive control with 25 mM and 50 mM salt and the highest increase was observed for CA41/1 isolate (13.18% and 53.96%, respectively). For root fresh weight, there were significant differences between the isolates at 25 and 50 mM salt concentrations. Generally, CA41/1 isolate displayed better performance for root fresh weight compared to other isolates.

Table 1. Effect of PGPR and salt stress applications on some seedling growth parameters

NaCl concentration	PGPR	SL	Change ratio (%)	RL	Change ratio (%)	LN	Change ratio (%)	SD	Change ratio (%)
0 mM	negative cont.	11.06 a*	–	20.36 c	–	4.32	–	4.04	–
	CA41/1	10.47 ab	–5.33	21.13 ab	3.78	4.50	4.17	4.16	2.97
	18/1 K	10.15 ab	–8.23	23.32 b	14.54	4.60	6.48	4.09	1.24
	S5/4ep	9.98 b	–9.76	26.10 a	28.19	4.81	11.34	3.99	–1.24
	30	11.14 a	0.72	21.69 ab	6.53	4.56	5.56	4.03	–0.25
	p value	0.050	–	0.001	–	0.137	–	0.779	–
25 mM	positive cont.	9.05	–	19.42 b	–	4.08	–	3.77	–
	CA41/1	8.96	–0.99	20.80 ab	7.11	4.00	–1.96	3.62	–3.98
	18/1 K	8.35	–7.73	21.52 ab	10.81	3.92	–3.92	3.47	–7.96
	S5/4ep	8.43	–6.85	22.88 a	17.82	4.08	0.00	3.61	–4.24
	30	7.98	–11.82	19.25 b	–0.88	4.04	–0.98	3.53	–6.37
	p value	0.183	–	0.051	–	0.878	–	0.126	–
50 mM	positive cont.	6.71 ab	–	18.44	–	3.79	–	3.09	–
	CA41/1	7.98 a	18.90	20.26	9.86	4.04	6.60	3.44	11.33
	18/1 K	7.11 ab	5.99	20.02	8.58	3.85	1.58	3.04	–1.62
	S5/4ep	6.86 ab	2.18	17.91	–2.89	3.89	2.64	3.18	2.91
	30	6.20 b	–7.64	20.35	10.39	3.67	–3.17	3.06	–0.97
	p value	0.056	–	0.478	–	0.375	–	0.311	–

* There were significant differences among the different letter(s) at $P < 0.05$ level (according to Duncan's multiple comparison test)

PGPR: plant growth promoting rhizobacteria; bacterial isolates: CA41/1: *Bacillus thuringiensis*, 18/1 K: *Pseudomonas putida*, S5/4ep: *P. putida*, 30: *P. putida*; SL: shoot length, RL: root length, LN: leaf number, SD: shoot diameter

Table 2. Effect of PGPR and salt stress applications on some seedling growth parameters

NaCl concentration	PGPR	SFW	Change ratio (%)	RFW	Change ratio (%)	SDW	Change ratio (%)	RDW	Change ratio (%)
0 mM	negative cont.	4.23	–	1.35	–	0.49	–	0.105	–
	CA41/1	4.23	0.00	1.53	12.89	0.47	–4.08	0.102	–2.86
	18/1 K	4.21	–0.57	1.43	5.46	0.52	6.12	0.113	7.62
	S5/4ep	4.01	–5.25	1.31	–3.14	0.49	0.00	0.117	11.43
	30	4.36	3.03	1.39	2.45	0.52	6.12	0.110	4.76
	p value	0.724	–	0.429	–	0.689	–	0.575	–
25 mM	positive cont.	2.58	–	1.54 a	–	0.462 a	–	0.096	–
	CA41/1	2.92	13.18	1.19 b	–22.50	0.392 b	–15.15	0.129	34.38
	18/1 K	2.60	0.78	1.07 b	–30.23	0.348 ab	–24.68	0.084	–12.50
	S5/4ep	2.81	8.91	1.17 b	–23.99	0.368 ab	–20.35	0.089	–7.29
	30	2.41	–6.59	1.00 b	–34.98	0.297 c	–35.71	0.098	2.08
	p value	0.751	–	0.001	–	0.001	–	0.479	–
50 mM	positive cont.	1.39 c	–	0.63 b	–	0.175 b	–	0.050 b	–
	CA41/1	2.14 a	53.96	1.05 a	68.23	0.259 a	48.00	0.084 a	68.00
	18/1 K	1.73 ab	24.46	0.75 b	20.34	0.189 b	8.00	0.054 b	8.00
	S5/4ep	1.84 ab	32.37	0.78 b	24.56	0.221 ab	26.29	0.056 b	12.00
	30	1.34 c	–3.60	0.72 b	14.79	0.178 b	1.71	0.069 ab	38.00
	p value	0.042	–	0.002	–	0.008	–	0.031	–

* There were significant differences among the different letter(s) at $P < 0.05$ level (according to Duncan's multiple comparison test)

PGPR: plant growth promoting rhizobacteria; bacterial isolates: CA41/1: *Bacillus thuringiensis*, 18/1 K: *Pseudomonas putida*, S5/4ep: *P. putida*, 30: *P. putida*; SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight

The effect of PGPR treatment on shoot dry weight was identified to be significant at 25 and 50 mM, similar to root fresh weight ($p \leq 0.05$) (Tab. 2). In a salt-free environment an increase compared to negative control only occurred for 18/1K and no.30 isolate (6.12%), the highest increase was for CA41/1 isolate (48.00%) with 50 mM salt application. The difference in root dry weight for PGPR isolates was significant at 50 mM salt. Under salt stress, generally CA41/1 isolate appeared to provide better effects compared to the other isolates.

The root : shoot ratio in situations without stress had 50.00 and 22.73% increase rates compared to negative controls for CA41/1 and 18/1K isolates, respectively, while all isolates had a reduction in root:-shoot ratio compared to positive control in the presence of stress (Tab. 3).

Leaf relative water content (LRWC) and membrane injury index (MII). The leaf relative water content (LRWC) of PGPR isolates in salt-free conditions were identified to increase by 1–7.5% rates. However, at 25 mM salt density, apart from 18/1K all isolates were negatively affected by salt and experienced a reduction of up to 9% compared to positive controls (Tab. 3).

With the increase in salt, the situation inversed and all isolates were observed to have increased leaf relative water content. The membrane injury index (MII) representing the electrolyte amount released under stress conditions, was determined to have significant reductions compared to positive controls for all PGPR isolates at both 25 and 50 mM salt. The lowest rates were for 18/1K at 25 mM and S5/4ep isolate at 50 mM, especially, compared to positive control (–96.91% and –97.15%, respectively) (Tab. 3).

Table 3. Effect of PGPR and salt stress applications on some physiological traits

NaCl concentration	PGPR	LRWC	Change ratio (%)	R : S	Change ratio (%)	MII	Change ratio (%)
0 mM	negative cont.	92.96	–	0.22	–	–	–
	CA41/1	99.97	7.54	0.33	50.00	–	–
	18/1 K	94.07	1.19	0.27	22.73	–	–
	S5/4ep	93.94	1.05	0.22	0.00	–	–
	30	96.30	3.59	0.21	–4.55	–	–
	p value	0.961	–	0.181	–	–	–
25 mM	positive cont.	90.28	–	0.31	–	5.17	–
	CA41/1	82.18	–8.98	0.23	–25.81	1.66	–67.89
	18/1 K	95.45	5.73	0.22	–29.03	0.16	–96.91
	S5/4ep	88.19	–2.31	0.24	–22.58	1.46	–71.76
	30	83.33	–7.69	0.23	–25.81	0.40	–92.26
	p value	0.445	–	0.745	–	0.874	–
50 mM	positive cont.	70.56	–	0.27	–	19.65	–
	CA41/1	86.49	22.58	0.25	–7.41	8.09	–58.83
	18/1 K	75.14	6.48	0.26	–3.70	11.33	–42.34
	S5/4ep	78.33	11.01	0.27	0.00	0.56	–97.15
	30	75.79	7.41	0.24	–11.11	7.49	–61.88
	p value	0.467	–	0.930	–	0.547	–

* There were significant differences among the different letter(s) at $P < 0.05$ level (according to Duncan's multiple comparison test)

PGPR: plant growth promoting rhizobacteria; bacterial isolates: CA41/1: *Bacillus thuringiensis*, 18/1 K: *Pseudomonas putida*, S5/4ep: *P. putida*, 30: *P. putida*; LRWC: leaf relative watercontent, R : S: root : shoot ratio, MII: membrane injury index

Nutrient content in shoot (K, Ca, Na, K : Na, Ca : Na). With an important place in stress conditions, K intake was observed to be different for PGPR applications at 25 and 50 mM salt. In a salt-free environment, apart from no.30 isolate, all isolates had an increase in K intake compared to negative control, with an increase at 25 mM salt concentration for all isolates and an increase at 50 mM for CA41/1 (5.12%) and S5/4ep (9.27%) isolates (Tab. 4). The Ca content of tomato seedlings generally increased with PGPR inoculation at 0 and 25 mM, and there was a reduction of –6.04% only for S5/4ep isolate with 25 mM salt application. Apart from S5/4ep and no.30 isolates (13.54% and 1.23%, respectively), 50 mM salt concentration had negative effects.

In terms of the Na content of plants, there were significant differences identified for PGPR inoculation at 25 mM salt concentration. In salt-free conditions, apart from no.30 PGPR isolate (–4.92%), the Na content of tomato seedlings was identified to increase compared to negative control. Again, with 25 mM, only the S5/4ep isolate was identified to have reduced Na intake of –10.61% compared to control. When stress increased to 50 mM, the Na content of plants without PGPR application reduced from –4.29% to –6.43%, while for no.30 isolate the Na amount increased by 1.61% (Tab. 4).

There were variations in K : Na and Ca : Na ratios, which are important to determine the effect of salt stress (Figs. 1, 2). In the 0 mM application with no salt, CA41/1, 18/1K and S5/4ep inoculations were identified to experience negative changes in K : Na ratio compared to control (–10.14%, –7.83%, and –2.30%, respectively). Contrary to this, at 25 mM salt 18/1K and S5/4ep isolates had increases of 2.33% and 9.88% in K : Na ratios, while no.30 isolate with increase at 0 mM had a reduction of –5.81%. When salt stress reached concentrations of 50 mM, none of the PGPR isolates

Table 4. Effect of PGPR and salt stress applications on K, Ca, Na content

NaCl concentration	PGPR	K (%)	Change ratio (%)	Ca (%)	Change ratio (%)	Na (%)	Change ratio (%)
0 mM	negative cont.	5.06	–	2.79	–	3.05	–
	CA41/1	5.19	2.57	3.38	21.15	3.41	11.80
	18/1 K	5.07	0.20	3.18	13.98	3.29	7.87
	S5/4ep	5.14	1.58	3.18	13.98	3.17	3.93
	30	5.06	0.00	3.53	26.52	2.90	–4.92
	p value	0.620	–	0.670	–	0.170	–
25 mM	positive cont.	4.48 c	0	2.98	–	6.85 b	–
	CA41/1	4.74 ab	5.80	4.05	35.91	6.84 b	–0.15
	18/1 K	4.94 a	10.27	3.27	9.73	6.38 b	–6.86
	S5/4ep	4.57 bc	2.01	2.80	–6.04	5.59 c	–18.39
	30	4.82 a	7.59	3.19	7.05	7.44 a	8.61
	p value	0.010	–	0.212	–	0.001	–
50 mM	positive cont.	4.10 bc	0	3.25	–	9.65	–
	CA41/1	4.31 ab	5.12	3.23	–0.62	9.29	–3.73
	18/1 K	3.99 c	–2.68	3.08	–5.23	9.20	–4.66
	S5/4ep	4.48 a	9.27	3.69	13.54	9.22	–4.46
	30	4.26 ab	3.90	3.29	1.23	9.79	1.45
	p value	0.013	–	0.309	–	0.418	–

* There were significant differences among the different letter(s) at $P < 0.05$ level (according to Duncan's multiple comparison test)

PGPR: plant growth promoting rhizobacteria; bacterial isolates: CA41/1: *Bacillus thuringiensis*, 18/1 K: *Pseudomonas putida*, S5/4ep: *P. putida*, 30: *P. putida*

had a reduction in terms of K : Na ratio, with the greatest increase of 6.19% for the S5/4ep isolate (Fig. 2).

The Ca : Na ratio at 0 mM for all isolates and at 25 mM for all isolates, apart from no.30 isolate, was increased compared to control. This increase was recorded from 4.55 to 31.82% at 25 mM, while only S5/4ep isolate had 5.56% increase at 50 mM salt (Fig. 2).

Enzyme activation and lipid peroxidation (MDA). According to Table 5, there is an increase in enzyme activities, especially in CAT and APX with 50 mM, while SOD activity decreased with salt stress. Differences in terms of PGPR inoculation were significant for APX content at 0 and 50 mM, for SOD content at 0 mM and for CAT content at 25 and 50 mM salt administrations ($p \leq 0.05$). For PGPR of tomato seedlings, at 0 mM NaCl application, APX amounts varied from 0.054 to 0.295 mmol·g^{-1} FW, with these amounts identified as 0.464–1.089 mmol·g^{-1} FW at 25 mM salt and 1.286–2.375 mmol·g^{-1} FW at 50 mM. Under stress-free conditions, PGPR inoculation reduced SOD activity compared to negative control while there were increases for CA41/1 (9.39%) and S5/4ep (12.09%) isolates at 25 mM and only 8.57% for 18/1K isolate at 50 mM salt. For CAT content at 0 mM, there were high rates of increases for all isolates compared to negative controls. For the salt application, at 25 mM there was a 27.39% increase for the 18/1K isolate, while at 50 mM salt concentration all isolates had increases in CAT activity compared to positive control and the highest increase rate was identified for 18/1K isolate (858.90%).

MDA content at 0 mM salt varied from 2.41 to 3.61 µmol·g^{-1} FW, with this value observed to be from 1.89 (18/1 K)–2.54 (negative control) µmol·g^{-1} FW at 25 mM salt concentration. However, at 50 mM salt dose, there was higher increase observed for MDA content and it was identified to vary from

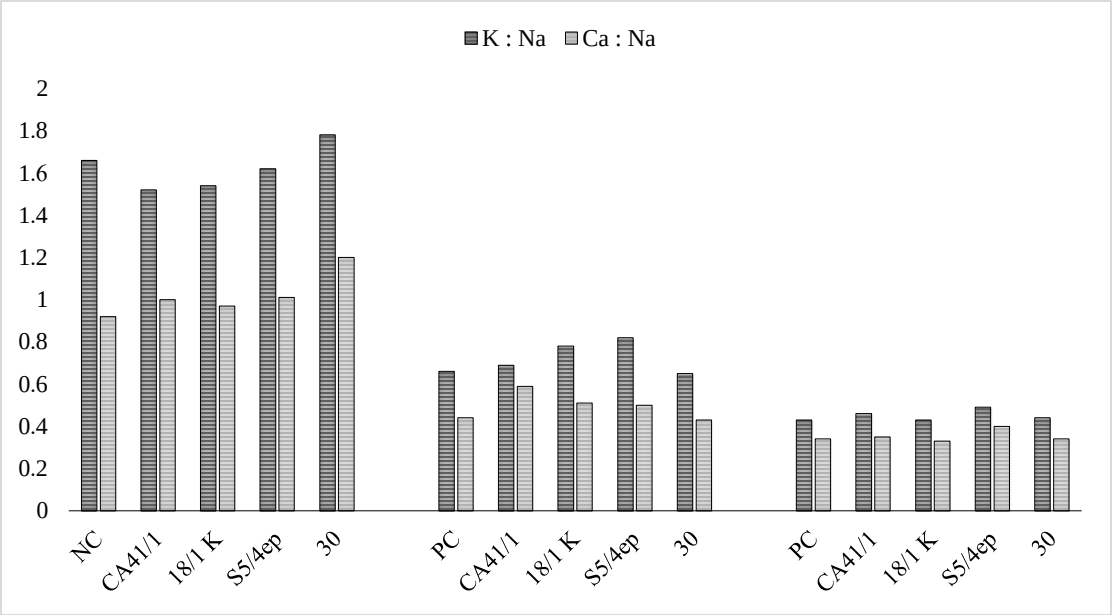

Fig. 1. Effect of PGPR treatments to K : Na and Ca : Na ratio of tomato seedlings (NC: negative control, PC: positive control; bacterial isolates: CA41/1: *Bacillus thuringiensis*, 18/1 K: *Pseudomonas putida*, S5/4ep: *P. putida*, 30: *P. putida*)

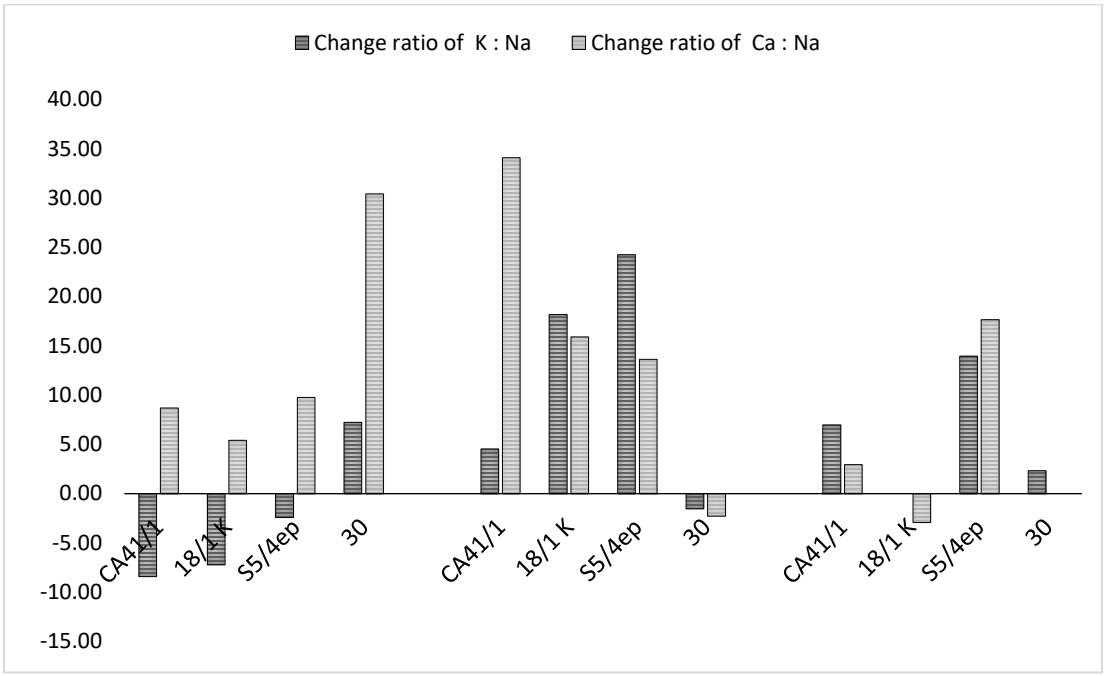

Fig. 2. Change ratios of K : Na and Ca : Na according to control with PGPR treatments (bacterial isolates: CA41/1: *Bacillus thuringiensis*, 18/1 K: *Pseudomonas putida*, S5/4ep: *P. putida*, 30: *P. putida*)

Table 5. Effect of PGPR and salt stress applications on enzyme activation

NaCl concentration	PGPR	APX (mmol·g^{-1} FW)	Change ratio (%)	SOD (U·mg^{-1} FW)	Change ratio (%)	CAT (mmol·g^{-1} FW)	Change ratio (%)	MDA (µmol·g^{-1} FW)	Change ratio (%)
0 mM	negative cont.	0.259 a	–	157.08 a	–	0.0023	–	2.41	–
	CA41/1	0.048 b	–81.47	86.66 b	–44.83	0.0037	60.87	3.36	39.42
	18/1 K	0.054 b	–79.15	77.13 b	–50.90	0.0060	160.87	2.54	5.39
	S5/4ep	0.054 b	–79.15	97.30 b	–38.06	0.0077	234.78	2.84	17.84
	30	0.295 a	13.90	86.29 b	–45.07	0.0057	147.83	3.61	49.79
	p value	0.001	–	0.050	–	0.326	–	0.281	–
25 mM	positive cont.	0.798	–	118.08	–	0.0157 ab	–	2.54	–
	CA41/1	0.655	–17.92	129.17	9.39	0.0037 b	–76.43	2.07	–18.50
	18/1 K	1.089	36.47	116.13	–1.65	0.0200 a	27.39	1.89	–25.59
	S5/4ep	0.464	–41.85	132.35	12.09	0.0080 ab	–49.04	2.54	0.00
	30	1.060	32.83	108.56	–8.06	0.0117 ab	–25.48	2.39	–5.91
	p value	0.570	–	0.297	–	0.050	–	0.449	–
50 mM	positive cont.	1.286 b	–	112.91	–	0.0073 b	–	5.22	–
	CA41/1	1.429 b	11.12	106.94	–5.29	0.0147 b	101.37	5.58	6.90
	18/1 K	1.732 ab	34.68	122.59	8.57	0.0167 b	128.77	5.12	–1.92
	S5/4ep	2.078 ab	61.59	110.18	–2.42	0.0700 a	858.90	4.39	–15.90
	30	2.375 a	84.68	100.59	–10.91	0.0103 b	41.10	4.97	–4.79
	p value	0.047	–	0.385	–	0.050	–	0.421	–

* There were significant differences among the different letter(s) at $P < 0.05$ level (according to Duncan's multiple comparison test)

PGPR: plant growth promoting rhizobacteria; bacterial isolates: CA41/1: *Bacillus thuringiensis*, 18/1 K: *Pseudomonas putida*, S5/4ep: *P. putida*, 30: *P. putida*; APX: ascorbate peroxidase, SOD: superoxide dismutase, CAT: catalase, MDA: malondialdehyde

5.52 (CA41/1) to 4.39 (S5/4ep) µmol·g^{-1} FW. For all isolates, MDA content was not increased compared to positive control at 25 mM, and with the increase in salt dose, only CA41/1 isolate had 6.90% increase in MDA content with 50 mM NaCl administration (Tab. 5).

Photosynthetic pigment content. It was determined that photosynthetic pigment content of plants did not show significant changes with PGPR applications in the presence or absence of stress (Tab. 6). In situations without stress, all PGPR isolates had increases in chlorophyll-*a*, chlorophyll-*b*, total chlorophyll and carotenoid amounts compared to positive and negative controls. Contrary to this at 25 mM salt dose, there were reductions observed apart from carotenoid. The PGPR isolate with greatest reduction in chlorophyll-*a* (–9.05%), chlorophyll-*b* (–11.54%), total chlorophyll (–9.41%) and carotenoid amounts (–3.74%) with 25 mM salt administration was 18/1K isolate. At 50 mM salt concentration, the S5/4ep isolate had increases of 2.50 to 6.67% for all photosynthetic pigment contents compared to positive controls, while no.30 isolate had reductions of –10.67 to –12.92% (Tab. 6).

DISCUSSION AND CONCLUSION

Soil salinity leads the list of abiotic stress factors limiting plant development and negatively affecting

Table 6. Effect of PGPR and salt stress applications on photosynthetic pigments quantity

NaCl concentration	PGPR	Chl.-*a*	Change ratio (%)	Chl.-*b*	Change ratio (%)	Total chlorophyll	Change ratio (%)	Carotenoid	Change ratio (%)
0 mM	negative cont.	1.79	–	0.64	–	2.43	–	2.24	–
	CA41/1	2.00	11.73	0.71	10.94	2.71	11.52	2.45	9.38
	18/1 K	2.00	11.73	0.69	7.81	2.69	10.70	2.52	12.50
	S5/4ep	1.81	1.12	0.65	1.56	2.46	1.23	2.33	4.02
	30	1.91	6.70	0.68	6.25	2.59	6.58	2.54	13.39
	p value	0.535	–	0.365	–	0.495	–	0.540	–
25 mM	positive cont.	2.10	–	0.78	–	2.87	–	2.14	–
	CA41/1	2.06	–1.90	0.74	–5.13	2.80	–2.44	2.24	4.67
	18/1 K	1.91	–9.05	0.69	–11.54	2.60	–9.41	2.06	–3.74
	S5/4ep	1.99	–5.24	0.71	–8.97	2.69	–6.27	2.14	0.00
	30	2.07	–1.43	0.74	–5.13	2.81	–2.09	2.14	0.00
	p value	0.425	–	0.280	–	0.359	–	0.372	–
50 mM	positive cont.	2.08	–	0.75	–	2.83	–	2.40	–
	CA41/1	1.89	–9.13	0.67	–10.67	2.56	–9.54	2.23	–7.08
	18/1 K	2.09	0.48	0.75	0.00	2.84	0.35	2.43	1.25
	S5/4ep	2.18	4.81	0.80	6.67	2.98	5.30	2.46	2.50
	30	1.85	–11.06	0.67	–10.67	2.52	–10.95	2.09	–12.92
	p value	1.01	–	1.03	–	1.01	–	0.892	–

PGPR: plant growth promoting rhizobacteria; bacterial isolates: CA41/1: *Bacillus thuringiensis*, 18/1 K: *Pseudomonas putida*, S5/4ep: *P. putida*, 30: *P. putida*

yield. Salt stress causes many negative effects on plant growth and development and leads to a range of negative effects limiting product quality. The use of biological methods against abiotic stress has attracted attention in recent years and has become a hopeful approach. For example, one of the alternative agricultural inputs of PGPR is a cheaper and more environmentally-friendly application compared to physicochemical methods used with the aim of removing the effect of stress caused by negative environmental conditions [Singh et al. 2018].

This study observed that PGPR inoculation caused positive effects in terms of seedling development for tomato plants grown under salt stress. As is known, PGPR may protect against disease and pests via "induced systemic resistance (ISR)" [Pieterse et al. 2014]. This protection is known to be effective for abiotic stress factors [Sarma et al. 2012]. Yang et al. [2008] named the increase in tolerance to abiotic stresses via physical and chemical changes in plants inoculated with PGPR is "induced systemic tolerance (IST)". PGPR with the ability to trigger ISR has the potential to stimulate IST simultaneously. In addition, several of ISR phenomena have been found to be associated with IST. Although there are several common linkages between ISR and IST pathways, several mechanistic differences also exist [Sarma et al. 2012].

In our study, CA41/1 isolate was more effective for shoot length, shoot diameter and leaf number at 50 mM salt concentration, while CA41/1 and S5/4ep isolates were better at 25 mM. For fresh and dry weights, the best effect was identified to be created by CA41/1 isolate at both 25 and 50 mM salt (Tabs. 1, 2). Soil salinity increases ethylene synthesis affecting physiological activity of root cells and causing regression of shoot and root development [Bal et al. 2013]. As is known, PGPR encourages development of

plant hormones and metabolites like cytokinin, auxin and gibberellin which are produced or regulated in plants and are known to increase tolerance of salt stress [Loon 2007, Forni et al. 2017]. Additionally, PGPR producing ACC deaminase reduces synthesis of harmful ethylene which increases under stress and prevents the suppression of root and shoot development [Glick 2014]. PGPR properties like production of siderophores with important duties in Fe intake and the ability to solubilize phosphate, may not directly contribute to tolerance of salinity or drought; but, they make important contributions to the general health of the plant [Forni et al. 2017]. However, there are different opinions about whether these abilities of PGPRs are maintained under salt stress. For example, though one study identified that siderophore production reduced in high salt concentrations [Argandona et al. 2010], another identified an increase in siderophore production by *Streptomyces* sp. in CAS media with salt stress induced [Sadeghi et al. 2012]. Additionally, another important characteristic of PGPR is that IAA production may increase under salt stress [Sadeghi et al. 2012]. Tank and Saraf [2010] showed that PGPRs which can dissolve phosphate and produce phytohormones and siderophores, encouraging growth of tomato plants under 2% NaCl stress. In our study, under salty conditions, shoot and root length increased by 19% while fresh and dry weights increased by 68% compared to plants without PGPR inoculation. Kumar et al. [2018] provided nearly 29% increase in shoot length with PGPR inoculation under salt stress, while Mayak et al. [2004] identified an increase in fresh and dry weights.

Due to the osmotic effect of salt stress, water intake reduces and additionally, injury occurs to membranes due to ion toxicity [Munns 2005]. The reduction in LRWC under limited water conditions is represented by low turgor pressure [Katerji et al. 1997] and in this context; salt tolerance is an important marker in cultivated plants [Sarabi et al. 2017]. Apart from 18/1K isolate, PGPR isolates did not create a positive effect on LRWC at 25 mM salt dose, while all isolates were observed to increase LRWC compared to controls with increasing salinity stress at 50 mM. In this context, PGPR treatment appears to create an advantage at increasing stress doses especially (Tab. 3).

Though the root system is directly exposed to salinity, leaf growth is more sensitive to salt stress compared to root growth and as a result, salt stress increases the root : shoot ratio in plants [Çulha and Çakırlar 2011]. Orsini et al. [2013] reported that the root : shoot ratio increased in plants under salt stress compared to control conditions. According to the results obtained in this study, PGPR isolates reduced the root : shoot ratio at both salt doses and thus, may be said to limit the negative effect of salt stress in terms of root : shoot ratio (Tab. 3).

Membrane injury forms with ion imbalance due to osmotic incompatibility occurring between the interior and exterior of a cell under stress conditions like salinity and drought [Ghoulam et al. 2002]. Membrane injury is lower in tolerant genotypes compared to sensitive ones [Farooq and Azam 2006]. Studies have revealed that membrane injury increases with salt stress and in this context, MII may be used to determine the degree of effect of stress on plants [Jamil et al. 2012]. All PGPR isolates had MII reduce by 58–97% at both salt concentrations (Tab. 3).

PGPR inoculation was observed to increase K^+ and Ca^{2+} intake compared to the negative control group with 25 and 50 mM salt doses. Apart from PGPR isolate 30, all isolates had reduced Na intake and this situation was determined to cause an increase in K : Na and Ca : Na ratios (Tab. 4, Figs. 1, 2). There is a continuous interaction of K^+ [Wu et al. 1996], with a vital role for plant growth, stoma movement, osmoregulation and enzyme activation, and Na^+ intake [Tester and Davenport 2003]. The competition between monovalent Na^+ and K^+, especially, resulting in favor of K^+, will cause an elevation in K : Na values and plants may protect themselves better against salt stress in this situation [Yoshida 2002]. Increasing xylem pressure balance with salt stress reduces with PGPR inoculation increasing the root hydraulic conductivity [Groppa et al. 2012]. In this situation the Na^+ toxicity lessens and the speed of the flow of K^+ from roots to trunk increases [Wang et al. 2016]. Additionally, PGPR inoculation increases production of exopolysaccharides which hold the Na^+ element in saline soils preventing uptake by roots. Though plants uptake sodium along with other nutrients, PGPR preserves most of the sodium in soils and thus, was revealed to reduce the harmful effects of Na accumulation [Tank and Saraf 2010].

Antioxidant enzymes like SOD, CAT and APX directly remove reactive oxygen species (ROS), causing oxidative damage under many stress factors including salt stress, via glutathione and ascorbate accumulating in the cell [Baltruschat et al. 2008]. Under saline conditions, PGPR regulates the activity of enzymes like superoxide dismutase, catalase and ascorbate peroxidase and activates the antioxidative defense mechanisms of the plant [Jha and Subramanian 2014]. APX and CAT enzymes are reported to be important for detoxification of H_2O_2, especially [Azevedo Neto et al. 2006]. According to the current study results, especially at 50 mM salt, APX and CAT activity show a clear increase with bacterial inoculation, with SOD activity identified to increase in S5/4ep at 25 mM and in 18/1K isolate at 50 mM compared to controls (Tab. 5). Some PGPR isolates were observed to have reduced enzyme levels in 25 mM salt in this study. Some studies have reported PGPR inoculation causes a reduction in enzyme levels at low intensity stress [Armada et al. 2014, Kang et al. 2014]. Additionally, production of plant growth regulators increases in the presence of PGPR isolates which reduces the toxic ion intake and formation of stress-specific proteins which are stated to increase the growth of plants [Vivas et al. 2003].

The malondialdehyde occurring with oxidation of lipids and destruction of cell membranes as a result of environmental stress conditions like salinity may provide information about the reaction of the plant to stress [Bharti et al. 2016]. In fact, some studies have found that PGPR inoculation suppresses lipid peroxidation [Jha and Subramanian 2014]. PGPR inoculation reduced MDA accumulation by nearly 26% under salt stress. At both 25 mM and 50 mM salt doses, isolates (apart from CA41/1) were identified to have lower MDA content compared to negative controls.

The increase in photosynthetic pigments with salt stress may explain the increase in antioxidant enzyme activities. Sevengor et al. [2011] reported that antioxidant enzyme activity prevented the degradation of chlorophyll. With the 25 mM NaCl application, there was no increase in photosynthetic pigment content compared to negative controls, while at 50 mM, especially, the S5/4ep and 18/1K isolates were observed to have increases in photosynthetic pigment content in parallel with the increase in APX and CAT enzymes. It is reported that *Bacillus* and *Pseudomonas* species especially encourage synthesis of chlorophyll-*a*, chlorophyll-*b* and carotenoid [Kumar et al. 2018].

One of the interesting results of our study is that while at 25 mM salt doses, there was no increase in development properties of seedlings; it is noteworthy that more pronounced positive effects were seen with the increase in stress dose to 50 mM salt. Some studies have observed similar effects of PGPR application in plants under biotic and abiotic stress. Hardoim et al. [2008] stated that the contributions of PGPR on plant health were more pronounced under stress conditions. Barka et al. [2006] observed that with endophyte bacteria used as PGPR more contribution to the plant was observed as cold stress increased. The reactions of plants to abiotic stress are similar to the reactions formed against a pathogen attack and the two systems may be associated [Barka et al. 2006]. Similar situations were observed for tomato and pepper under biotic stress and the contribution of PGPR to plant health was stated to be greater for plants under disease pressure [Akköprü et al. 2018]. Additionally, Walters et al. [2009] stated that activation of different resistance pathways with saccharin was more clearly observed as the stress pressure on the plant increased.

At the end of the study, it was determined that *Bacillus thuringiensis* CA41/1, *B. thuringiensis* 18/1K and *Pseudomonas putida* S5/4ep isolates increased shoot development, leaf relative water content, nutrient element intake, and antioxidative enzyme activity and reduced membrane injury and lipid peroxidation in tomato plants growth under salt stress. The Na intake was reduced which helped to preserve the K/Na and Ca/Na balance in plants. However, no.30 isolate was not identified to be as effective against salt stress as other bacterial isolates in general.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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