Sakarya University Journal of Science

ISSN 1301-4048 | e-ISSN 2147-835X | Period Bimonthly | Founded: 1997 | Publisher Sakarya University | http://www.saujs.sakarya.edu.tr/

Title: Effects of Plant Growth Promoting Rhizobacteria (PGPR) on Physiological Parameters Against Salinity in Apple Cultivar "Fuji"

Authors: Şeyma Arıkan, Lütfi Pırlak

Recieved: 2018-10-11 14:42:16

Accepted: 2019-10-08 12:36:55

Article Type: Research Article
Volume: 24
Issue: 2
Month: April
Year: 2020
Pages: 281-286

How to cite
Şeyma Arıkan, Lütfi Pırlak ; (2020), Effects of Plant Growth Promoting Rhizobacteria (PGPR) on Physiological Parameters Against Salinity in Apple Cultivar "Fuji". Sakarya University Journal of Science, 24(2), 281-286, DOI: 10.16984/saufenbilder.469522
Access link
http://www.saujs.sakarya.edu.tr/tr/issue/52471/469522

Sakarya University Journal of Science 24(2), 281-286, 2020

Effects of Plant Growth Promoting Rhizobacteria (PGPR) on Physiological Parameters Against Salinity in Apple Cultivar "Fuji"

Şeyma ARIKAN*[1], Lütfi PIRLAK[2]

Abstract

The present study was conducted with the cultivar 'Fuji' grafted on M9 rootstock in both 2014 and 2016 years. The effect of PGPR (*Bacillus subtilis* EY2, *Bacillus atrophaeus* EY6, *Bacillus spharicus GC subgroup B* EY30, *Staphylococcus kloosii* EY37 and *Kocuria erythromyxa* EY43) were investigated under salt stress conditions. PGPR's effects were tested on leaf relative water content (LRWC), membrane permeability, stomatal conductivity, photosynthetic activity and chlorophyll content (by SPAD-502). The saplings were grown in pots filled 2:1:1 peat: perlite: sand. Salinity was obtained by NaCl: Na_2SO_4: $CaCl_2$: $MgSO_4$ (7:9:3:1) solution. The solution was applied twice a week with irrigation during the growing period. When the salinity reached 2.5-3.0 $dScm^{-1}$, the solution application was ended. All bacteria treatments significantly reduced the physiological damage of leaves compared with the salt treatment in both two years. The LRWC range from 13.33 % (salt treatment) to 26.76 % (control). The best result of bacteria treatment was measured in EY43 with 23.93 % LRWC. The highest rate of membrane permeability was found in salt treatment (30.35 %). The stomatal conductivity was decreased in the salt application (154.35 mmol $m^{-2}s^{-1}$) unlike EY43 treatment (234.44 mmol $m^{-2}s^{-1}$). Similarly, EY43 treatment significantly increased photosynthetic activity (15.24 µmol CO_2 $m^{-2}s^{-1}$) compared with the salt treatment (8.22 µmol CO_2 $m^{-2}s^{-1}$). As a result, bacteria strains had been ameliorative of the deleterious effects under salt stress on "Fuji".

Keywords: Apple, Fuji, PGPR, Salt Stress

1. INTRODUCTION

Soil salinity that is the most important abiotic stress factor after drought in world agriculture, limits yield and plant growth especially in arid and semi-arid regions, In such areas, long periods of drought coincide with high temperatures [1]. However, salinity occurs quickly in these regions with irrigation. Salt in the upper layers of soil transported to by capillary during the irrigation and evaporation and accumulates in the rhizosphere. The wrong applications of irrigation, the presence of high soluble salts level in the water, lack of

*Corresponding Author

[1]The University of Selçuk, Faculty of Agriculture, Department of Horticulture, Konya, TURKEY, arikan@selcuk.edu.tr ,ORCID ID: 0000-0002-4328-0263

[2]The University of Selçuk, Faculty of Agriculture, Department of Horticulture, Konya, TURKEY, pirlak@selcuk.edu.tr , ORCID ID: 0000-0003-3630-3591

drainage and are among other causes of salinity. Salinity is one of the major problems in the agricultural area of Turkey. Agricultural fields, about 1.500.000ha, are faced with salinity problem in Turkey [2]. Salinity is one of the most effective factors which limited yield and soil fertility in production areas. Salt stress causes increasing of respiration rate, ion toxicity, changes in plant growth, mineral disorders, damaging of membrane permeability, decreasing of photosynthetic activity. In addition, soil salinity has negative impacts on nitrogen and carbon metabolism. The salinity tolerances of different species or cultivars differ by their different stages of growth and different soil and environmental conditions. The reactions of fruit species to salinity were examined [3] and observed that many fruit species were very sensitive to salinity. (Table-1).

Table-1. The response of fruit species against salinity [3]

Tolerant	Moderately Sensitive	Too Sensitive
Pheoneix dactylifera	*Ficus carica*	*Citrus paradisi*
	Punica granatum	*Citrus limon*
	Olea europea	*Citrus reticulata*
		Citrus sinensis
		Prunus amgydalus
		Prunus armeniaca
		Prunus avium
		Malus communis

In order to improve soil salinity, it needs lots of time and more expense. Therefore, these methods cannot be applied to prevalent countries. Many studies focused on chemical treatments and suitable rootstocks against for saline soil but nowadays, biological treatments with plant growth-promoting rhizobacteria (PGPR) have been started to use for solving this problem [4, 5].

PGPR are free-living microorganisms as colonizing in the rhizosphere of plants [5]. These bacteria which increased root growth and plant pathogens kept under the control have great importance in sustainable agriculture. Such as bio-control of plant disease, plant growth-promoting, bio-fertilizers and growth regulator substances production have been functioning [6]. PGPR affects plants a-symbiotically notwithstanding plant species. PGPR can have beneficial effects on plant growth and yield by two main mechanisms. These are direct and indirect mechanisms. There are different ways in the direct effect mechanism. Direct mechanisms may act on the plant itself and auxins, cytokinin's, and gibberellins or lowering of the ethylene in the plant, solubilization of inorganic phosphate and mineralization of organic phosphate, a-symbiotic fixation of atmospheric nitrogen, and stimulation of disease-resistance mechanisms [6, 7]. In the indirect mechanism; PGPR acts like biocontrol agents reducing disease or stimulate other beneficial symbioses or protect the plant [8]. Additionally, PGPR improves the plant's tolerance to stresses, such as drought, high salinity, metal toxicity, and pesticide load [9].

In this study, we aimed at determining effect of PGPR strains (*Bacillus subtilis* EY2, *Bacillus atrophaeus* EY6, *Bacillus spharicus* GC subgroup B EY30, *Staphylococcus kloosii* EY37, *Kocuria erythromyxa* EY43) which were identified positive effects on vegetables, apple, cherry and crops on leaf relative water content (LRWC), membrane permeability, stomatal conductivity, photosynthetic activity and chlorophyll content of cv. Fuji apple saplings.

2. MATERIALS AND METHOD

2.1. Material

This study was carried out under controlled greenhouse conditions at Selçuk University Department of Horticulture in Turkey. The "Fuji" apple cultivar grafted on M9 rootstock was used as plant materials and saplings were planted to 12-liter pots in March 2014. The pots contained 2:1:1 peat: perlite: sand mixture.

The strains of bacteria, EY2, EY6, EY30, EY37, EY43 were obtained from the University of Iğdır in Turkey (Assos. Prof. Dr. M. Figen Dönmez). These bacteria have been the ability to grow in a saline culture medium [10% sodium chloride (NaCl)] [10].

2.2. Method

2.2.1. Bacterial treatments

All bacteria were inoculated into the roots before planting. Roots were held in bacterial suspensions of the concentration of 10^9 CFU mL^{-1} for 30 minutes. After planting saplings were watered with bacterial suspensions once in a month.

2.2.2. Salinity treatments

Bacteria inoculated saplings started to be irrigated once a week with NaCl: Na2SO4: CaCl2: the MgSO4 solution in a ratio of 7: 9: 3: 1 one month after planting and this application have continued during the growth period. When the EC of soil reached 2.5-3.0 $dScm^{-1}$, solutions application was ended.

2.2.3. Analysis of physiological features

Physiological effects of bacterial treatments were evaluated by leaf relative water content (LRWC), membrane permeability, stomatal conductivity, photosynthetic activity and chlorophyll content (by SPAD-502).

2.2.4. Statistical analysis

Experiment divided into seven application groups each including nine saplings with three replicates in a completely randomized design. The collected data were analyzed statistically using the SPSS 23. All data in the present study were subjected to analysis of variance (ANOVA) and means were separated by Duncan's Multiple Range Tests at 5% level of significance.

3. RESULTS

The highest chlorophyll content was obtained from control saplings (52.95 SPAD unit) while the lowest chlorophyll content value was measured in salt treatment (39.28 SPAD unit). All bacteria applications were increased chlorophyll contents of leaves as compared to salt treatment. But the highest increase occurred the EY43 bacteria strain with 49.58 SPAD unit of chlorophyll reading the value in comparison to salt treatment.

All bacteria applications decreased membrane damaged increased by the salt treatment in leaves. The EY37 and EY43 bacteria strains were the most effective against the reduction of membrane damage. The membrane permeability rate of salt treatment was measured as 30.35%, which was the highest, in salt treatment (Table-2.). The lowest membrane permeability rate was found in the control (18.05%) followed EY43 treatment with 19.21%.

The leaves of the control saplings had the highest LRWC with 26.76 %, while the lowest LRWC was found in the salt treatment with 13.33 %. All bacteria strains have enhanced significantly LRWC as compared to the salt applications, EY43 and EY37 were the highest bacteria applications that increased LRWC ratio by 23.92 % and 21.93% respectively, compared to the salt application (Table-2.).

The stomatal conductivity was found lower in salt treatment than other applications. Although the highest stomatal conductivity was measured in control (275.13 $mmolm^{-2}s^{-1}$), EY43, EY37, and EY2 bacteria applications were increased significantly with 234.44 $mmolm^{-2}s^{-1}$, 215.03 $mmolm^{-2}s^{-1}$ and 208.40 $mmolm^{-2}s^{-1}$ stomatal conductivity values respectively, compared to the salt application (Table-2.).

The photosynthetic activity was increased all bacteria applications in comparison with the salt application. The lowest activity was found in salt treatment with 8.22 µmol CO_2 $m^{-2}s^{-1}$. The highest photosynthetic activity was obtained from EY43 bacteria strain with 15.24 µmol CO_2 $m^{-2}s^{-1}$ followed the control (17.01 µmol CO_2 $m^{-2}s^{-1}$) group.

Table-2. Effect of bacterial applications and solution treatments on physiological parameters of Fuji saplings

	C. C.	M. P.	LRWC	S. C.	P. A.
Control	52.95 a	18.05 d	26.76 a	275.13 a	17.01 a
Salt	39.28 f	30.35 a	13.33 f	154.35 e	8.22 f
EY2+Salt	44.57 e	23.54 b	18.57 d	208.40 c	12.08 e
EY6+Salt	45.01 e	22.81 b	15.85 e	190.13 d	13.57 d
EY30+Salt	46.03 d	22.18 bc	19.16 d	189.62 d	14.42 c
EY37+Salt	47.36 c	19.81 cd	21.93 c	215.03 c	14.68 bc
EY43+Salt	49.58 b	19.21 d	23.92 b	234.44 b	15.24 b

* **Difference between averages in the same column with different lower case letters are significant (P <0.05).**

C.C: Chlorophyll Content (SPAD Unit); **M.P:** Membrane Permeability (%); **LRWC:** Leaf Relative Water Content (%); **S.C:** Stomatal Conductance ($mmolm^{-2}s^{-1}$); **P.A**: Photosynthetic Activity (µmol CO_2 $m^{-2}s^{-1}$)

4. DISCUSSION

In our study, all bacteria strains significantly increased chlorophyll values in comparison with the salt application. Moreover, Bashan, et al. [11] reported that the quantity of photosynthetic pigments significantly increased by *Azospirillum brasilense* inoculation in wheat.

Salinity has an adverse effect on photosynthesis by affecting different parameters on plants. Indeed, many researchers have reported that photosynthetic activity was decreased in salt stress [12-15]. However, it has been determined that the PGPR applications can inhibit this decrease in photosynthesis caused by salt stress in our experiment. Thus, EY43 (15.24 µmol CO_2 $m^{-2}s^{-1}$) bacteria strain, except the control group had the highest photosynthetic activity while lowest activity had in salt treatment with 8.22 µmol CO_2 $m^{-2}s^{-1}$. Similarly, Kumari, et al. [16], reported that *Pseudomonas sp.* and *Bacillus sp.* species increased photosynthesis in soybean under the salt stress conditions.

Net photosynthesis rate, transpiration rate, and stomatal conductivity decrease while the stoma resistance increases with salt stress. Thus, it limits the availability of CO_2 which is responsible for carbon metabolism with a decrease in the rate of photosynthesis, leading to a reduced in stoma conductance [17]. The lowest values in stomatal conductivity measurements were obtained from the salt applications where no bacterial application was observed. This reduction in stomatal conductivity caused by the salt stress was tolerated in bacteria applications and the highest increase was obtained from EY43 bacteria strain with 234.44 mmol m^2s^{-1}. Studies carried out under salinity stress reported that *Serratia sp*. and *Rhizobium sp*. species in lettuce [18], *Pseudomonades sp.* and *Bacillus lentus* species in basil [19], *Bacillus megaterium* species in tomato [20] and *Enterobacter cloacae* and *Bacillus drentensis* species in mung beans [21] have been shown to improve stomatal conductivity.

Bacterial inoculation can restrict Na and Cl uptake and enhance the uptake of other nutrients, especially K and NO_3. Thus, it can provide membrane stability in cells [5]. In addition, PGPR reduces electrolyte leakage by protecting plant cell membrane integrity in tissues [22]. In our study supports this information such that the EY37 and EY43 bacteria strains were the most effective against to membrane damage. Karlidag, et al. [5] and Karlidag, et al. [10], EY6, EY30, EY37 bacteria strains in strawberry, Arikan, et al. [23] EY2, EY43 bacteria strains in citrus rootstocks reported that reduce membrane permeability under salt stress. The LRWC result of our study is supposed by Karlidag, et al. [5] in strawberry. They determined LRWC decreased by salt treatment, but all bacteria inoculations were increased LRWC. EY43 bacteria treatment having the best result was found to increased LRWC rate of 23.92%.

The results of this study show that the PGPR applications could be ameliorative of the deleterious effects of salt stress on cv. Fuji apple saplings. This positive effect arose with increasing chlorophyll content, relative water content and reduced membrane injury.

5. CONCLUSIONS

The results of the present study showed that used bacteria strains had been ameliorative of the deleterious effects under salt stress in apple plants. This study was observed to improved physiological parameters and reduced membrane injury with bacterial applications. The highest chlorophyll contents were obtained in the control group but EY43 (49.58 SPAD Unit) bacteria strain had the best effect with compared other bacteria applications.

In stomatal conductivity, control group (275.13 mmol$m^{-2}s^{-1}$) was found the best result, while salt treatment (154.35 mmol$m^{-2}s^{-1}$) was found the lowest result in Fuji saplings. Furthermore, EY43 (234.44 mmol$m^{-2}s^{-1}$) bacteria strains significantly increased stomatal conductivity with compared salt applications. The photosynthetic activity has increased with EY43 (15.24 µmol CO_2 $m^{-2}s^{-1}$) bacteria strain in comparison with the salt application.

The maximum LRWC was obtained from control plants (26.76%) in the study. In addition, EY43 (23.92%) bacteria strain was provided with an increase with respect to the salt application in LRWC. The salt application increased membrane permeability in apple leaves. The highest membrane damage was obtained from the salt application (30.35%), while the control (18.05%) and EY43(19.21%), EY37 (19.81%), bacteria strains had the lowest damage.

It is reported that the use of bacteria that promote ACC deaminase-producing plant growth under stress conditions can be stimulated to plant growth. Even if bacteria do not provide a great benefit to the plant in the bacterial-root association, beneficial effects are observed by reducing ethylene levels. In addition to bacteria exhibiting effective ACC deaminase activity in the future, intensive research should be carried out on the possibilities of increasing the resistance of plants to salinity, low temperature and drought stress so that appropriate and effective bacterial-plant combinations should be demonstrated. In this study, it is foreseen that the identification of beneficial bacteria that can affect the perennial fruit species grown in salty soil conditions may increase the possibility of fruit cultivation in some areas where salinity problems are encountered in our country.

ACKNOWLEDGMENTS

This research was supported by a grant from Selçuk University, Scientific Research Projects Institute-Turkey (Project No: 14101013).

REFERENCES

[1] A. Alizadeh, X. Xlilova, and A. Eivazi, "Biochemical Response of Apple Dwarf Rootstock to Salinity Stress," *Tech. J. Eng. Appl. Sci.,* vol. 1, pp. 118-124, 2011.

[2] Anonymous, "www.fao.org Access date: 2016," 2008.

[3] E. V. Maas and G. Hoffman, "Crop salt tolerance\-current assessment," *Journal of the irrigation and drainage division,* vol. 103, no. 2, pp. 115-134, 1977.

[4] M. Ashraf, S. Hasnain, O. Berge, and T. Mahmood, "Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress," *Biology and Fertility of Soils,* vol. 40, no. 3, pp. 157-162, 2004.

[5] H. Karlidag, A. Esitken, E. Yildirim, M. F. Donmez, and M. Turan, "Effects of plant growth promoting bacteria on yield, growth, leaf water content, membrane permeability, and ionic composition of strawberry under saline conditions," *Journal of plant nutrition,* vol. 34, no. 1, pp. 34-45, 2011.

[6] H. Antoun and D. Prévost, "Ecology of plant growth promoting rhizobacteria," in *PGPR: Biocontrol and biofertilization*: Springer, 2005, pp. 1-38.

[7] Z. A. Zahir, M. Arshad, and W. T. Frankenberger, "Plant growth promoting rhizobacteria: applications and perspectives in agriculture," *Advances in Agronomy,* vol. 81, pp. 97-168, 2003.

[8] C. S. Jacobsen, "Plant protection and rhizosphere colonization of barley by seed inoculated herbicide degrading *Burkholderia (Pseudomonas) cepacia* DBO1 (pRO101) in 2, 4-D contaminated soil," *Plant and Soil,* vol. 189, no. 1, pp. 139-144, 1997.

[9] Y. Bashan and L. de Bashan, "Plant growth-promoting," *Encyclopedia of soils in the environment,* vol. 1, pp. 103-115, 2005.

[10] H. Karlidag, E. Yildirim, M. Turan, M. Pehluvan, and F. Donmez, "Plant growth-promoting rhizobacteria mitigate deleterious effects of salt stress on strawberry plants (*Fragaria× ananassa*)," *HortScience,* vol. 48, no. 5, pp. 563-567, 2013.

[11] Y. Bashan, M. Moreno, and E. Troyo, "Growth promotion of the seawater-irrigated oilseed halophyte *Salicornia bigelovii* inoculated with mangrove rhizosphere bacteria and halotolerant *Azospirillum spp.*," *Biology and Fertility of Soils,* vol. 32, no. 4, pp. 265-272, 2000.

[12] P. M. Hasegawa, R. A. Bressan, J.-K. Zhu, and H. J. Bohnert, "Plant cellular and molecular responses to high salinity," *Annual review of plant biology,* vol. 51, no. 1, pp. 463-499, 2000.

[13] R. Munns, "Comparative physiology of salt and water stress," *Plant, cell & environment,* vol. 25, no. 2, pp. 239-250, 2002.

[14] W.-Y. Kao, T.-T. Tsai, and C.-N. Shih, "Photosynthetic gas exchange and chlorophyll a fluorescence of three wild soybean species in response to NaCl treatments," *Photosynthetica,* vol. 41, no. 3, pp. 415-419, 2003.

[15] M. Ashraf and M. Shahbaz, "Assessment of genotypic variation in salt tolerance of early CIMMYT hexaploid wheat germplasm using photosynthetic capacity and water relations as selection criteria," *Photosynthetica,* vol. 41, no. 2, pp. 273-280, 2003.

[16] S. Kumari, A. Vaishnav, S. Jain, A. Varma, and D. K. Choudhary, "Bacterial-mediated induction of systemic tolerance to salinity with expression of stress alleviating enzymes in soybean (*Glycine max* L. Merrill)," *Journal of Plant Growth Regulation,* vol. 34, no. 3, pp. 558-573, 2015.

[17] E. Brugnoli and O. Björkman, "Growth of cotton under continuous salinity stress: influence on allocation pattern, stomatal and non-stomatal components of photosynthesis and dissipation of excess light energy," *Planta,* vol. 187, no. 3, pp. 335-347, 1992.

[18] H. Han and K. Lee, "Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity," *Res J Agric Biol Sci,* vol. 1, no. 3, pp. 210-215, 2005.

[19] A. Golpayegani and H. G. Tilebeni, "Effect of biological fertilizers on biochemical and physiological parameters of basil (*Ociumum basilicm* L.) medicine plant," *Am Eurasian J Agric Environ Sci,* vol. 11, pp. 411-416, 2011.

[20] R. Porcel, Á. M. Zamarreño, J. M. García-Mina, and R. Aroca, "Involvement of plant endogenous ABA in *Bacillus megaterium* PGPR activity in tomato plants," *BMC plant biology,* vol. 14, no. 36, pp. 1-12, 2014.

[21] S. Mahmood *et al.*, "Plant Growth Promoting Rhizobacteria and Silicon Synergistically Enhance Salinity Tolerance of Mung Bean," *Frontiers in Plant Science,* vol. 7, p. 876, 2016.

[22] P. S. Shukla, P. K. Agarwal, and B. Jha, "Improved salinity tolerance of *Arachis hypogaea* (L.) by the interaction of halotolerant plant-growth-promoting rhizobacteria," *Journal of plant growth regulation,* vol. 31, no. 2, pp. 195-206, 2012.

[23] Ş. Arikan, M. İpek, and L. Pirlak, "Effect Of Some Plant Growth Promoting Rhizobacteria (PGPR) On Growth, Leaf Water Content And Membrane Permeability Of Two Citrus Rootstock Under Salt Stress Condition," presented at the VII International Scientific Agriculture Symposium, Jahorina, Bosnia and Herzegovina, October 06 - 09, 2016 2016.