ALLEVIATION OF WATER STRESS AND PROMOTION OF THE GROWTH OF SUGAR BEET (*BETA VULGARIS* L.) PLANTS BY MULTI-TRAITS RHIZOBACTERIA

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Abstract. Drought stress may affect the yield and sucrose accumulation of sugar beet by restricting leaf and root growth**.** The effect of inoculation of nine IAA-producing, N_2-fixing, P-solubilizing and ACC deaminase-containing bacteria on the yield and morpho-physiological parameters of sugar beet under five different water regimes was investigated. The diminishing water supply caused a gradual decrease in the plant growth, chlorophyll contents, root and leaf weight and sugar content, accompanied by increasing activities of drought stress markers (GR, GST, G6PD, 6PGD, MDA and H_2O_2). Although it varied depending on the water regimes and bacterial strains, the results of two pot experiments show that inoculation with multi-traits plant growth promoting bacteria stimulated overall plant growth, including enzyme activity, sugar content, fibrous and storage roots development and leaf yield and the contents of leaf chlorophyll, N, P, K, Ca, Mg, Na, Mn and Zn, but decreased MDA and H_2O_2 contents, which might partly contribute to the activation of the processes involved in the alleviation of the effect of water stress.
Keywords: *water stress, enzyme activity, nutrients uptake, plant growth-promoting bacteria, malondialdehyde content (MDA), hydrogen peroxide (H_2O_2)*

Introduction

Beta vulgaris L. (sugar beet) is a very significant industrial sugar product, and the most significant input for sugar beet production is mineral fertilizer. Sugar beet production demands more water, but recently, stress of water has turn into an important limitation to sugar beet production, causing serious reductions in productivity. Drought is described by the decrease of water content. One of the most widespread environmental factors affecting plant fertility is drought. During the drought, level of water in leaf decrease and turgor is diminished. As result, reduces cell growth and yield with stoma closes. Drought inhibits the photosynthesis of plants, and thus reduces growth and development. Water stress reduces the production rate of dry matter and is considered as one of the major limiting factors for sugar beet root and leaf yield (Pidgeon et al., 2006; Romano et al., 2013). As evidenced, sucrose concentration and photosynthesis extremely responsive to drought because of its activity reduces with the growing water shortage (Bloch et al., 2006).

Plant-growth-promoting rhizobacteria (PGPR) improve many physiological, nutritional, morphological and biochemical plant responses, and thus, it enhances the plant resistance to abiotic and biotic stresses. The inoculation of selected drought-tolerant strains can reduce the yield limitation caused by water deficit and improve the

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ability of plant tolerance to drought stress (Marulanda et al., 2009; Castillo et al., 2013). In this regard, the use of PGPR is seen as a different tactic to induce stress endurance in cultivation. PGPR has skill to fix nitrogen, solubilize inorganic phosphate, generate 1-aminocyclopropane-1-carboxylate (ACC) deaminase and indole acetic acid (IAA), and support plant development. The bacteria which having ACC deaminase can decrease the negative effects of ethylene causing stress of water (Safronova et al., 2006). Under stress conditions in plants, while "stress ethylene" production increases if the bacteria prevent the ethylene synthesis and containing ACC deaminase, plants can reduce the level of ethylene, expected to provide protection against the inhibitory effects of stress in plants (Çakmakçı et al., 2009).

For increase the crop production, we need to selected effective bacteria which plant growth-promoting rhizobacteria. In addition to there are a few number of studies which against water stress using PGPR. Different approaches are required for better water wasting due to the lack of water resources for this reason, this study was governed for research the effect of varied ACC deaminase-containing, IAA-producing, N2-fixing and/or P-solubilizing bacterial species on the growth, antioxidant and pentose phosphate oxidative cycle enzymes, root yield and quality, in the sugar beet, under different water regimes (150%, 100%, 75%, 50%, and 25% of water-holding capacity). In this study, under limited water conditions, we aimed to reduce the loss of quality and efficiency depending on the stress and to increase the water stress resistance of sugar beet by using bacteria inducing the plant growth.

Materials and methods

Bacterial species

The bacterial species *Bacillus subtilis* RC11, *Bacillus subtilis* RC63, *Pseudomonas fluorescens* T26, *Rhodococcus erythropolis* RC9, and *Variovorax paradoxus* RC21 were isolated from the rhizosphere of wild red raspberries, and *Paenibacillus polymyxa* RC05 was isolated from wheat (Çakmakçı et al., 2007, 2009). The other four strains (*Pseudomonas putida* RC310, *Pseudomonas fluorescens* PF8/6, and *Bacillus megaterium* A21/3) were isolated from the rhizosphere of tea (Çakmakçı et al., 2010). Some features of bacteria used in the experiment are given in *Table 1*.

Table 1. *Some features of bacteria used in the experiment*

Used bacterial isolates	IAA-production (μg mL^{-1} OD_{600} $unit^{-1}$)	Nitrogenase activity (nmol C_2H_4, 10^7 cfu h^{-1})	P- solubilization (μg P mL^{-1} d^{-1})	ACC deaminase activity (nmol α-ketobutyrate mg^{-1} protein h^{-1})
Rhodococcus erythropolis RC9	22.6 ± 1.5	0.55 ± 0.11	27.8 ± 1.5	577.8 ± 26.7
Pseudomonas fluorescens T26	23.9 ± 2.1	0.61 ± 0.13	27.7 ± 1.2	796.1 ± 35.2
Paenibacillus polymyxa RC05	32.8 ± 2.6	0.68 ± 0.14	10.1 ± 0.9	682.1 ± 33.7
Bacillus subtilis RC11	29.4 ± 1.8	0.32 ± 0.12	16.6 ± 0.4	539.2 ± 21.2
Variovorax paradoxus RC21	19.4 ± 1.2	0.47 ± 0.12	35.7 ± 2.1	332.6 ± 17.4
Pseudomonas putida RC310	25.9 ± 2.4	0.56 ± 0.15	26.8 ± 1.8	746.2 ± 46.8
Pseudomonas fluorescens PF8/6	20.7 ± 1.5	0.47 ± 0.09	113.5 ± 12.7	223.6 ± 21.7
Bacillus subtilis RC63	29.7 ± 1.9	0.74 ± 0.17	34.6 ± 0.08	972.0 ± 28.3
Bacillus megaterium A21/3	19.5 ± 1.1	0.48 ± 0.16	74.3 ± 1.9	276.3 ± 16.7

Data were means ± standard error of three replicates

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Experiments and growth conditions

This study was established under natural light in green house Department of Field Crops, Faculty of Agriculture, Atatürk University of Erzurum during 2010-2011 production season. Sugar beet seedlings were grown in a greenhouse during the initial period in a night- day cycle of 10-14 h light, 15 to 24 °C, and 60% humidity and during the development period in 15-9 h day-night, 16-28 °C and 55-60% relative humidity. Two different trial sets were established as a factorial arrangement (treatments x water regimes) in randomized complete block design with five replications. Totally 11 applications existed in both test sets; (1) control (no bacteria and no mineral fertilizers), (2) NP-fertilizers (55 mg of N + 40 mg P/kg soil), (3) *R. erythropolis* RC9, (4) *P. fluorescens* T26, (5) *P. polymyxa* RC05, (6) *B. subtilis* RC11, (7) *V. paradoxus* RC21, (8) *P. putida* RC310, (9) *P. fluorescens* PF8/6, (10) *B. subtilis* RC63 and (11) *B. megaterium* A21/3, and five water regimes, randomly distributed into pots filled with equal amounts of soil. The soil was a sandy clay loam with an organic substance content of 3.2%, pH 7.6, present Olsen-phosphorus content of 16.2 mg kg^{-1} and total N content of 15.7 mg kg^{-1}. Replaceable K, Ca, Mg and Na were 448, 3420, 472 and 78 mg kg^{-1}; present Fe, Mn, Zn and Cu contents were 5.9, 9.6, 1.2 and 1.7 ppm. Bacterial inoculation of the seeds was carried out according to Sahin et al. (2004).

Two pots essay were made on sugar beet fully watered (100% of water-holding capacity (WHC)), under waterlogging (150% of the maximum WHC), and continuous moderate (75% and 50% of WHC) and severe water stress (25% of WHC). At the beginning of the experiment, pots were saturated with water to determine the water-holding capacity (WHC) per pot; pots were covered to prevent evaporation and they were left for free drainage. After the drainage was stopped, pots were weighed and WHC was found. After sugar beet was planted, all the pots were irrigated at the rate of 65 ± 5% of WHC to provide the seedling emergence and seedling hold for 3 weeks after planting. After three weeks from planting, different irrigation levels were started to be applied in both sets of experiment. Irrigation applications were determined by the amount of available moisture in the soil. By this method, to determine the available moisture in the soil, the amount of moisture, which can bring WHC to the same soil, was determined and different ratios were discussed for the consummation of the lost moisture. Amount of irrigation water that would be given for each pot determined that difference as coefficient (150% 100, 75%, 50%, 25%) multiplying. The pots were measured every three days), the amount of irrigation water diminishing from each group was completed by adding according to pot's own capacity.

After two weeks from planting, seedlings were thinned in the pots, in each pot, 5 plants were remained in five leaf period for the first and 1 plant was allowed in seven leaf period for the second experiment. Using a chlorophyll meter SPAD-502 (Minolta, Japan), Chlorophyll contents of leaves which the top fourth and fifth was evaluated. The first experiment set was harvested 65 days after sowing and the second set was harvested 130 days after sowing. A t 80 °C, dry weightiness was detected by drying for 48 h. Sugar beets' sugar contents in storage-root were detected during harvesting and it was expressed as a weight per plant.

Definition of enzyme activities and protein concentrations

Leaves which washed three times in total was adjusted as ph 8.0 and Using liquid nitrogen, plant examples were homogenized The activities of glutathione reductase

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(GR; EC 1.8.1.7), glutathione S-transferase (GST; EC 2.5.1.18), glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGD; EC 1.1.1.44) were determined with the according to Carlberg and Mannervik (1985), Habig and Jacoby (1981) and Beutler (1984). Each enzyme activity was detected spectrophotometrically (Shimadzu Spectrophotometer UV-1208, Kyoto, Japan) at 25 °C. Protein concentration was calculated according to the method of Bradford (1976) with help to 595 nm absorbance measurement by using as the standard of bovine serum albumin.

Measurement of H_2O_2 and MDA

The content of H_2O_2 was assessed according to the method described by Sairam and Srivastava (2002). The density of H_20_2 was determined spectrophotometrically by reading the absorbance of the titanium-hydro peroxide complex and calculated using a standard curve drawn with a known H_2O_2 concentration. Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content in fresh leaf weight according to the method of Heath and Packer (1968), prepared in 10% trichloroacetic acid containing 0.65% 2-thiobarbituric acid (TBA) and it was heated at 95 °C for 25 min, and then quickly cooled. The concentration of MDA was calculated from the absorbance at 532 nm and expressed as nmol g^{-1} FW.

Macro and micro element content of sugar beet leaf

The leaf samples were dried in the oven at 68 °C for 48 h and milled to 1 mm. Total N was measured by The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany). Following the extraction process, Macro- (P, K, Ca and Mg) and micro-elements (Fe, Mn, Zn and Cu) were detected by the inductively Coupled Plasma spectrophotometer (Optima 2100 DV, ICP/OES, Perkin-Elmer, Waltham MA, USA).

Statistical analysis

Analysis of variance from SPSS 13.0 (SPSS Inc.) programme was performed on the data obtained from both trials and then in conformity with Duncan's Multiple Range Test, the means were separated. The level of enzyme activities was evaluated in three samples from each replicate.

Results

In terms of sugar beet root dry weight for both harvests (65-day and 130-day harvest), all treatments, except for *P. fluorescens* PF8/6, gave better results compared to control (*Table 2*). According to the average irrigation level, the highest dry root weight was achieved with *P. putida* RC310 and *B. subtilis* RC63 inoculations in both trials. If sugar beet was harvested at early period, it was found that water restriction importantly reduced the root weight. Inoculation with RC310 and RC63 increased fresh and dry leaf weight compared to control and fertilizers, but other inoculations gave the same result with mineral fertilizer. Strains RC310, RC63, RC11, T26, and RC21 were most effective in promoting fresh and dry leaf weight of sugar beet in both trials set. Moreover, these strains were found to be effective in terms of fresh and dry leaf and root weight at water constraint applications at levels of 75%, 50% and 25% of WHC. In

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the excessive water application of both trial sets, primarily IAA-producing and N_2-fixing *P. polymyxa* RC05 and *R. erythropolis* RC9, all bacteria inoculations importantly increased dry root and leaf weight, and the amount of sugar per plant compared to control. Water-stressed plants inoculated with the effective PGPR recorded improved the plant growth in terms of dry root weight, and sugar per plant compared to the un-inoculated, water-stressed plants (*Table 2; Fig. 1*). As an average of the five water regimes, PGPR inoculation increased dry storage root weight by 0.3-41.5% and 2.1-45.6% at the first and second trials, whereas, NP fertilizer increased root weight by 20.2 and 31.8%, respectively, compared with control (*Table 2*). In the application of water at the rate of 25% of WHC, inoculation with RC310 and RC11 achieved an increase respectively of 37.6 and 35.5%, and 43.0 and 39.5% in the trial first and second compared with control. These were, however, higher than 6.1% and 9.2% increases by NP fertilization over control.

Table 2. *The effect of PGPR and fertilizer application on the dry storage root leaf weight of sugar beet in different harvest and under different water regimes*

Treat-ments	Water regimes at first trial set was harvested 65 days after sowing						Water regimes at second trial set was harvested 130 days after sowing					
	WR1	WR2	WR3	WR4	WR5	Mean	WR1	WR2	WR3	WR4	WR5	Mean
Dry storage root weight (g/plant)												
Control	3.46	5.13	3.72	3.20	2.79	3.66 d	45.9	56.7	44.4	37.4	28.6	43.4 d
NP	4.56	7.01	4.14	3.41	2.96	4.40 bc	55.8	89.3	63.4	43.6	34.1	57.2 b
RC9	4.80	5.85	3.42	3.30	2.79	4.03 c	60.2	73.2	42.3	40.1	33.0	49.8 c
T26	4.41	4.86	4.52	4.15	2.92	4.17 bc	55.3	60.8	55.3	51.6	32.0	51.0 c
RC05	4.85	5.61	3.71	3.46	3.34	4.19 bc	60.6	70.3	43.4	43.7	39.0	51.4 c
RC11	4.02	5.51	5.09	3.99	3.78	4.48 b	50.4	69.2	56.5	48.2	39.9	53.0 bc
RC21	4.63	5.22	4.03	3.96	3.31	4.23 bc	58.0	64.2	49.3	47.7	38.5	51.5 c
RC310	4.77	7.51	5.41	4.88	3.84	5.18 a	57.4	90.7	67.2	59.6	40.9	63.2 a
PF8/6	4.67	4.75	3.18	2.78	2.82	3.67 d	58.7	59.5	39.0	33.6	30.6	44.3 d
RC63	4.15	6.46	6.32	5.47	3.02	5.08 a	52.0	83.6	68.0	60.1	35.0	61.9 a
A21/3	4.46	5.82	3.99	3.84	2.89	4.20 bc	55.8	73.0	49.3	46.6	31.9	51.3 c
Mean	4.44 b	5.80 a	4.32 b	3.86 c	3.12 d	4.31	55.5 b	71.9 a	52.6 c	46.5 d	35.3 e	52.4
Dry leaf weight (g/plant)												
Control	5.68	7.94	5.93	5.23	4.58	5.87 e	37.3	51.6	34.8	26.5	27.4	35.5 f
NP	7.43	11.52	9.05	6.43	5.05	7.89 b	39.6	72.1	45.4	33.2	28.8	43.8 bc
RC9	7.76	10.00	5.54	5.91	4.70	6.78 cd	46.1	60.7	33.1	33.5	26.7	40.0 cd
T26	7.75	8.71	7.62	7.29	5.03	7.28 cd	47.0	52.9	45.8	42.7	28.5	43.4 c
RC05	7.76	8.93	5.84	5.48	5.43	6.69 d	42.1	54.2	34.8	28.7	27.4	37.4 ef
RC11	6.41	9.37	8.09	6.72	5.69	7.28 cd	37.8	56.7	48.5	39.3	31.8	42.8 cd
RC21	8.38	9.00	6.73	6.45	5.29	7.17 cd	49.4	54.6	40.3	37.9	27.6	42.0 cd
RC310	7.76	11.54	9.65	8.02	6.60	8.71 a	47.0	70.1	58.1	47.1	37.9	52.0 a
PF8/6	7.87	9.25	6.35	5.50	4.65	6.73 cd	42.4	56.1	37.8	31.4	28.1	39.2d-f
RC63	6.69	10.01	9.19	8.59	5.89	8.07 b	40.0	60.8	55.0	50.2	30.8	47.3 b
A21/3	7.14	10.22	7.17	6.97	5.00	7.30 c	42.6	61.6	42.69	39.6	27.9	42.9 cd
Mean	7.32 b	9.68 a	7.38 b	6.60 c	5.26 d	7.25	42.9 b	59.2 a	43.3 b	37.3 c	29.3 d	41.8

The difference between the averages shown the same letter not important ($p < 0.01$); WR: water regimes

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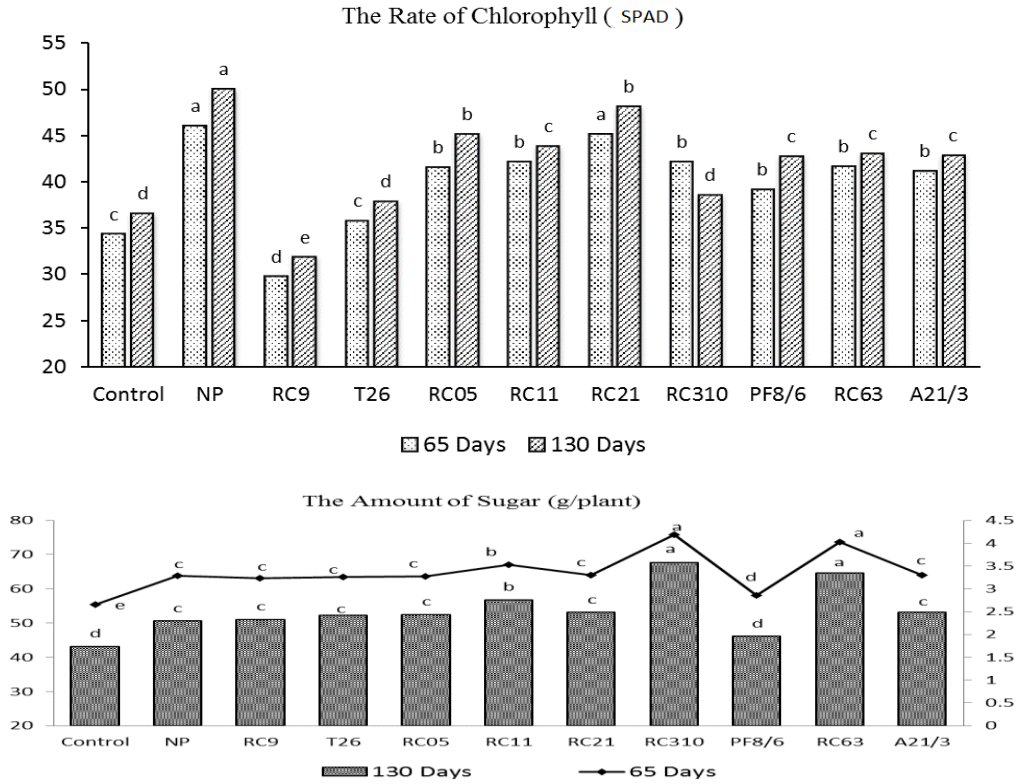

Figure 1. *Effect of PGPR and mineral fertilizer on the chlorophyll content and sugar per plant at the first (65 days) and second trial (130 days) average of five water regimes*

Under waterlogging stress (150% of WHC), sugar beet chlorophyll contents importantly raised NP fertilizer, RC21, A21/3 and RC05 inoculation. The maximum chlorophyll contents in the sugar beet leaves was found after RC21, followed by RC11, RC310 and RC05 inoculations under water stress applied at 75%, 50% and 25% WHC in both experiments (*Fig. 1*). In both trial sets, water stress decreased the chlorophyll contents of sugar beet, and generally, inoculation of the multi traits bacteria under water stress improved the chlorophyll contents, but responses were strain-specific. Chlorophyll content was positively and importantly correlated ($p \leq 0.01$) with dry root weight ($r = 0.35^{**}$), dry leaf weight ($r = 0.36^{**}$) and sugar per plant ($r = 0.34^{**}$) contents.

Of the bacterial inoculations, RC310 and RC63 produced the highest sugar per plant compared to control and mineral fertilizer while other inoculations gave the same result with mineral fertilizer. The highest ACC deaminase-containing RC63, RC310, RC11, and T26 inoculation were found to be effective according to the amount of sugar per plant at water constraint applications with 75%, 50% and 25% WHC (*Fig. 1*).

On average of five water regimes, inoculation of PGPR significantly raised the N and P content leaves of the sugar beet except RC9 and RC05, respectively, compared to control. On the other hand, all bacterial strains tested importantly raised the K, Ca and Zn content of the sugar beet leaf. N and K content were the greatest with RC310 and RC63, whereas maximal P, Ca and Mg was with strain RC21 and A21/3 inoculations (*Table 3*). In addition, six of the strains (RC05, PF8/6, RC6/3, RC310, RC9, and RC21) importantly raised the Mn content of sugar beet plants, but not Fe and Cu concentrations (*Table 3*).

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Table 3. *Effect of mineral fertilizer and plant growth-promoting rhizobacteria (PGPR) on macro- and micro-nutrient concentrations in sugar beet leaves in the first trial set with the average of five water regimes*

Treatments	Macro-nutrient (g kg^{-1} DW)					Micro-nutrient (mg kg^{-1} DW)				
	N	**P**	**K**	**Ca**	**Mg**	**Na**	**Fe**	**Cu**	**Mn**	**Zn**
Control	21.4 g	2.95 b	20.92 e	8.58 c	3.58 c	1672 b-c	142 a	31.8 a	45.6 f	31.5 g
NP	23.5 ef	3.08 ab	22.30 de	8.88 bc	3.70 bc	1696 a-c	137 a-c	28.7 bc	45.6 f	33.9 f
RC9	22.9 fg	3.18 a	23.10 cd	9.09 ab	3.91 ab	1760 ab	128 cd	28.2 bc	51.5 cd	37.7 e
T26	23.1 f	3.16 a	23.26 b-d	9.34 ab	3.80 a-c	1711 a-c	134 a-d	28.2 bc	48.1 ef	38.6de
RC05	23.9 d-f	3.10 ab	23.71 a-d	9.34 ab	3.84 ab	1616 cd	132 b-d	28.6 bc	56.5 a	39.9cd
RC 11	24.1 c-f	3.20 a	23.88 a-d	9.31 ab	3.87 ab	1713 a-c	137 a-c	27.4 c	47.9 ef	44.5 b
RC21	25.6 bc	3.26 a	23.39 a-d	9.49 a	3.99 a	1804 a	127 d	28.7 bc	50.4 de	41.8 c
RC310	27.7 a	3.21 a	24.98 a	9.41 a	3.91 ab	1544 d	138 ab	28.6 bc	52.4 b-d	38.6de
PF8/6	25.2 b-d	3.18 a	22.97 cd	9.43 a	3.77 a-c	1678 bc	139 ab	28.9 bc	55.1 ab	48.2 a
BS63	26.7 ab	3.20 a	24.74 ab	9.46 a	3.83 a-c	1511 d	132 b-d	28.4 bc	54.2 a-c	45.4 b
A21/3	25.0 c-e	3.24 a	23.95 a-c	9.46 a	3.98 a	1543 d	132 b-d	29.3 b	46.7 f	47.6 a
Mean	24.5	3.16	23.38	9.25	3.70	1659	134	29.0	50.4	40.7

Means followed with the same letter within each column are not significant different ($p < 0.05$)

On average of five water regimes, the highest GR activity was found effective at RC05, RC11, RC63 and mineral fertilizer, whereas the highest GST activity was found effective at PF8/6 and T26. Under waterlogging stress, sugar beet GR activity importantly raised after RC21, RC9 and RC11, while G6PD and 6PGD activity importantly raised after RC21, RC9 and RC63 inoculations. Under continuous moderate and severe water stress, the maximum GR activity in sugar beet leaf were found after RC63 inoculation, followed by A21/3, RC11, RC05 and 28/3, whereas the highest levels of GST activity were determined in treatments with PF8/6, followed by T26 and RC9. Moreover, under these stress, G6PD activity was found effective after RC310, RC05, RC21, and RC63; whereas 6PGD in sugar beet leaf was found effective after PF8/6, RC310, RC63, and RC05. In general, under water stress, there was an importantly raise in the enzyme activity in both inoculated and uninoculated treatments. In addition, enzyme activity in the uninoculated plants was lower than that of inoculated plants (*Fig. 2*).

While MDA and H_2O_2 decreased, sugar beet growth, root and leaf weight, sugar per plant and chlorophyll contents also increased. Water deficit treatment importantly raised the drought stress markers (MDA and H_2O_2), which showed the degree of oxidative damage posed with during stress. In all applications, levels of MDA and H_2O_2 were raised because of water stress. Also, when drought treatment, in all treatments show that H_2O_2 and MDA increased, whereas effective bacterial strains (RC310, RC63, RC26, RC11 and RC05) decreased the MDA and H_2O_2 content in the leaf (*Fig. 3*).

Discussion

One of the most negative factors of plant growth is certainly scarcity of water. Drought stress can also occur under irrigated conditions when use or availability of

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irrigation water is restricted (Tarkalson and King, 2017). Sugar beet is considered a drought-tolerant species based on the ability of established plants to survive transient periods of drought and recover when water subsequently becomes available (Wedeking et al., 2017). However, the stage of plant growth that emerged from stress is important to come from above the stress (Tatar et al., 2016). Water-restriction caused important yield losses and this effect was excessive in young plants affected by drought. When a temporary water stress appears at early period, it can be said that it reduces sugar beet root yield importantly. Indeed, when young beet plants were exposed to stress of water, it was found that sugar yield, the rate of photosynthesis and assimilation severely decreased Monti et al. (2006) and storage roots showed significant changes (Hoffmann, 2010). Bacterial activity was found higher than the beet harvested in the early period. Sahin et al. (2004) determined that the bacterial activity was higher at the early development stages. The water stress in growth stage may cause decrease of yield. Sugar beet root weight reduction decreased importantly under drought conditions, but this situation changed according to inoculated bacteria and irrigation level.

Bacterial inoculations and fertilizer application importantly raised fresh and dry leaf weight, and sugar per plant compared to control in the first and second trials. Generally, different PGPR applications give dissimilar response with different application of irrigation. The fact that inoculation of bacteria encouraged the growth of sugar beet leaves was in line with the results of previous researches (Çakmakçı et al., 1999; Schmidt et al., 2004; Shi et al., 2009). Similar to previous research findings (Romano et al., 2013), sugar beet root, leaf development and yield decreased under the water restrictions and drought stress.

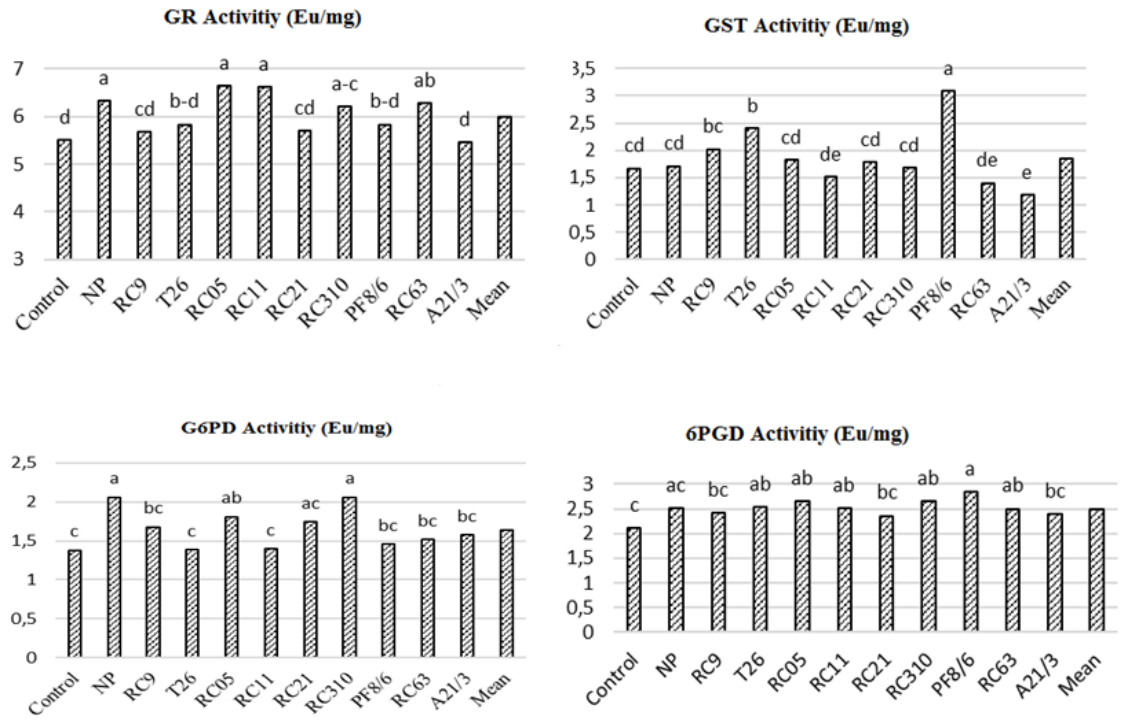

Figure 2. *Effect of PGPR and mineral fertilizer on the activities of anti-oxidant (GR and GST) and pentose phosphate oxidative cycle enzymes (G6PD and 6PGD) in sugar beet leaves in the second trial*

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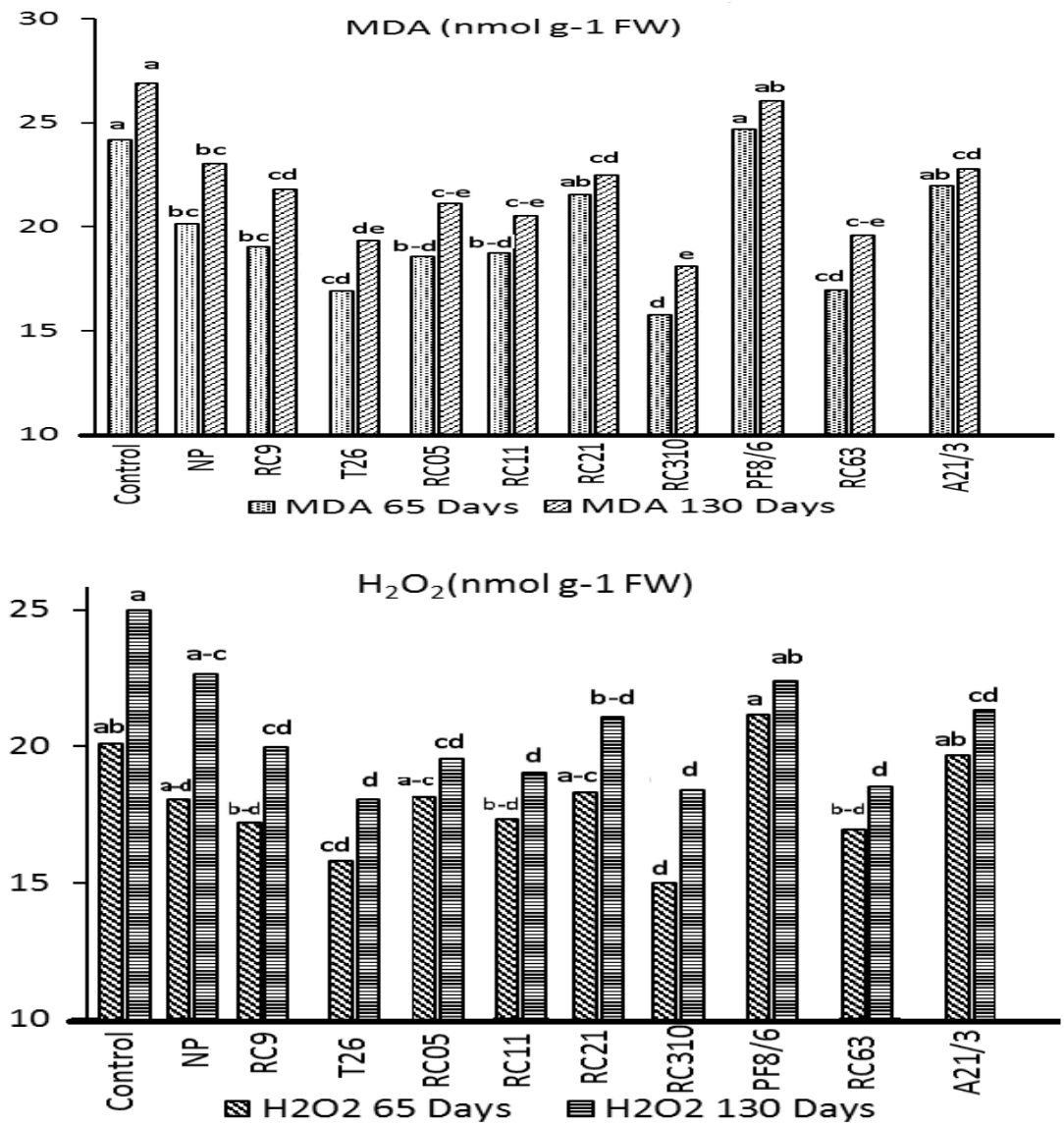

Figure 3. *Effect of PGPR and mineral fertilizer on the malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content of sugar beet leaves in the first (65 days) and second trial (130 days) average of five water regimes*

Under insufficient water supply, the sucrose concentration was higher than under well-watered conditions. In spite of the fact that sugar content ratio is high proportionally, the amount of sugar per plant is reduced at beet under water stress compared to optimum irrigation applications. Moreover, if water stress limits the use of sucrose in the growth at a higher rate than the decrease in photosynthesis, sugar rate increase can be the main reason of the more sugar in the roots. Drought could increase the sugar content in sugar beet, but reduce the root, leaf and sugar yield and the drought is clearly the main reason for the sugar beet yield losses (Jaggard et al., 1998; Pidgeon et al., 2001). Similarly, sucrose concentration increased with reduced water availability (Bloch et al., 2006). Our results showed that water stress reduced the vegetative growth and fresh weight, it increased sugar percentage and the percentage of fresh weight in root. Although there are a few studies in the literature on the main factor limiting the

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drought sugar yield (Pidgeon et al., 2006; Gobin, 2010), sugar beet's sensitivity to water restrictions and the response have not been studied enough (Habibi et al., 2011). Bacterial inoculation minimized the water stress-imposed effects importantly increasing the sugar per plant in sugar beet, but this changed depending on the inoculation bacteria and level of irrigations.

Reduction in water content due to drought can reduce food intake from soil. whereas application of PGPR increased the uptake of N, P, K, Ca, Mg, Mn and Zn in sugar beet. This parameter showed that using PGPR reduces the negative effects of water constraints in leaf macro- and micro-nutrient contents. Among the bacterial strains, the strains RC310 exhibiting better performance under severe water stress (25% of WHC) conditions was observed to have the highest K content in sugar beet leaf, which correlated well with their increased the amount of sugar per plant and weight of root and leaf (*Tables 2* and *3; Fig. 1*). As reported by Grzebisz et al. (2013), normalization of K intake in case of water stress can provide important advantage against too negative effects of water stress in plant growth and yield. Under drought conditions, increased nutrient uptake could improve water-use efficiency and alleviate drought stress effects on plant growth. Indeed, if nutrient uptake can be increased by using active bacteria, plant growth can be stimulated.

An alternative approach for increasing plant resistance against stress is to raise the plant antioxidant enzymes by using bacteria (Çakmakçı et al., 2007). Our studies show that PGPR can rise GR, GST, 6PGD and G6PD activities, together with the growth sugar beet lıke wheat and spinach plants (Çakmakçı et al., 2007, 2009). Therefore, investigating the ability to enhance enzymes activity in plants by using bacteria, similar to the first and pioneering work is required. Study has clearly demonstrated that some effective bacterial strains can promote plant growth together with antioxidant and oxidative pentose phosphate pathway enzymes in sugar beet under drought conditions. If plant antioxidant enzyme activity can be raised by using bacteria, it is being possible to increase the resistance of plants to stress conditions with plant growth. PGPR inoculation was shown to reduce the negative effects of water stress. Moreover, sugar beet can partly tolerate water stress protecting itself from oxidative damage by the increasing enzymes activities in leaves with the help of bacteria.

Of the bacterial inoculations, high ACC deaminase-containing RC310 and RC63 exhibiting better performance under moderate and severe water stress conditions were observed to have the highest level N and K content in sugar beet leaf, which was correlated well with their increased both fresh and dry weight of leaf and root (*Table 2*), 6PGD and G6PD enzyme activities and decreased both H_2O_2 and MDA content (*Fig. 3*), thus protecting the plants from water stress compared to the other bacteria and control. Increasing potassium and nitrogen by high N_2-fixing, P-solubilizing and for sustain the process of photosynthesis in leaves during drought, bacteria which ACC deaminase-including can decrease lipid peroxidation by rising the activity of antioxidant enzymes and decreasing MDA content. Therefore, Bacteria have nutrient uptake from soil and protect against to pests and diseases, also the bacteria provide protection to plants during water stress and drought.

In our study, drought caused a significant decrease in leaf weight as well as in root weight. Inoculation with PGPR could tolerate a certain degree of slow leaf development caused water constraint and they slowed leaf weight reduction occurred in the mineral fertilization. Positive effects of these selected strains on fresh and dry root and leaf weight, chlorophyll contents, enzyme activities and nutrient uptake of sugar beet plants

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showed the beneficial role of these PGPR, which might be attributed to IAA production, N_2-fixation, P-solubilisation, ACC deaminase activity, or even other non-evaluated PGPR traits that stimulated the plant growth. There are a great number studies that bacteria can provide protection plants against different stresses duo to they have produce IAA and ACC deaminase Glick (2012). Inoculation with IAA-producing Shi et al. (2009) and N_2-fixing and/or P-solubilizing Sahin et al. (2004) bacteria stimulate growth and increased root and sugar yields of sugar beet. Screening of rhizobacteria that show multiple PGP traits suggests that they have better potential in alleviating plant water stress and in improving the growth of sugar beet.

Conclusions

As a result of excess water applications, soil salinization, desertification, the loss of soil due to erosion occur with increasing costs. In addition, programs that provide the maximum production per unit of irrigation water in case of limited water and water constraint conditions in order to reduce the possible decline in production are important. This research has shown that sugar beet cultivation in PGPR can be used to minimize the harmful effects of water stress. Inoculation with ACC deaminase-containing bacteria partially eliminated the effects of water stress on growth, yield and quality of sugar beet. PGPR increases the uptake of plant nutrients (macro and micro). The effective bacterial strain tested in this study improved for enhanced plant growth promotion will be able to reduce the inputs of chemical fertilizers and the negative effect of water stress, and will have a potential to be used as a bio-fertilizer in sustainable and organic sugar beet production. Due to insufficient irrigation water and the high cost of water, in arid and semi-arid regions, inoculation with ACC deaminase containing PGPR can be used to prevent the reduction in yield. The PGPR can encourage plant growth and development, decrease stress sensitivity, and may conduce to the concept of biotechnology application in agriculture. As a result, RC310 and PF8 / 6 bacterial strains used in this study and other useful bacteria can use in organic sugar beet growing and in water-restricted areas. This study will also shed light on researchers working with sugar beets and PGPR. Inspired by this work, researchers can determine how affected of sugar beet by other stress conditions. Also researchers can search to ways to reduce the negative effect of other stress conditions with using PGPR.

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