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Araştırma Makalesi/Research Article

Effect of Plant Growth Promoting Rhizobacteria on Growth, Nutrient Uptake and Physiological Parameters in Sugar Beet under Different Irrigation Levels

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Abstract

Two experiments were conducted to investigate the of nine ACC deaminase-containing, IAA-producing, N_2-fixing and/or P-solubilizing bacteria, on the growth, yield, chlorophyll, macro- and micro-nutrient content, and selected morpho-physiological parameters of sugar beet, under five irrigation levels (150%, 100%, 75%, 50%, and 25% of water-holding capacity). The research was established according to factorial arrangement in randomized block experimental design of five water regimes, eleven treatments and five replications. The experiment was set up in two sets; and the first set was harvested after 65 days and the second set was done after 130 days. Inoculation with multi-traits bacteria stimulated overall plant growth, including sugar content, root and leaf yield and the leaf chlorophyll contents, and macro- and micro-nutrient uptake, which might partly contribute to the activation of the processes involved in the alleviation of the effect of water stress. According to the results, under water constraint at the level of 75, 50 and 25% of water-holding capacity, beet yield parameters BF4, BF6, Bio-organic and mineral fertilizer was found effective. As an average of the five water regimes, bacterial formulations increased dry storage root weight by 6.5-27.7% and 9.1-27.3% and dry leaf weight by 6.1-26.7% and 3.9-25.8% at the first and second trials, whereas, mineral fertilizers (NP) and bio-organic fertilizers increased dry storage root weight by 24.5% and 9.3-15.5% and 20.2 and 9.2-15.2%, and dry leaf weight by 23.5% and 11.7-23.2% and 22.2% and 3.3-21.9%, respectively, compared with control. Moreover, water stress in sugar beet plants was alleviated partially by the inoculation with bacterial strains. Our results provide strong evidence that the role of bacteria in the performance of sugar beet plants in the stressful environment of soils not only the improved plant growth, yield, and macro- and micro-nutrient content, but also the alleviation of water deficit and waterlogging stress.

Keywords: Water stress, nutrients uptake, bacteria, enzyme activity, hydrogen peroxide, malondialdehyde

Farklı Sulama Rejimlerinde Bitki Gelişmesini Teşvik Eden Bakterilerin Şeker Pancarı Gelişme, Besin Alımı ve Fizyolojik Parametreleri Üzerine Etkisi

Öz

Bu araştırma; beş farklı sulama rejimi altında (su tutma kapasitesinin %150, %100, %75, %50 ve %25'i), ACC deaminaze içeren, IAA üretici, Azot fikseri ve Fosfat çözücü bakteri uygulamalarının şeker pancarının gelişimine, makro ve mikro besin elementi alımına ve bazı morfo-fizyolojik özellikleri üzerine olan etkisini belirlemek amacıyla yürütülmüştür. Araştırma, tesadüf bloklarında faktöriyel düzenlemeye göre beş su rejimi, on bir uygulama ve beş tekerrürlü olarak kurulmuştur. Denemeler biri 65 gün, ikinci ise 130 günlük olmak üzere iki set halinde yürütülmüştür. Çoklu özelliğe sahip bakteri aşılamaları; şeker oranı, kök ve yaprak verimi, yaprak klorofil içeriği, makro ve mikro element alımı dahil gelişmeyi teşvik etmiş ve ayrıca su stresinin etkisinin hafifletilmesinde yer alan süreçlerin aktivasyonuna kısmen katkıda bulunabilmiştir. Sonuçlara göre, su tutma kapasitesinin %75, %50 ve %25'i seviyesinde su kısıtı altında şeker pancarı verim parametreleri bakımından BF4 ve BF6 kombine bakteri, Biyo-organik ve mineral gübre uygulamaları etkin bulunmuştur. Beş sulama rejimi ortalaması olarak, bakteri formülasyonları, birinci ve ikinci deneme setinde sırasıyla, kuru kök-gövde ağırlığını %6,5-27,7 ve % 9,1-27,3 ve kuru yaprak ağırlığını ise % 6,1-26,7 ve % 3,9-25,8 oranında artırırken; kontrole kıyasla, sırasıyla, mineral gübre ve biyo-organik gübre uygulamaları sırasıyla kuru depo-kök ağırlığını % 24,5 ve % 9,3-15,5 ve %20,2 ve 9,2-15,2 ve kuru yaprak ağırlığını ise % 23,5 ve % 11,7-23,2 ve% 22,2 ve% 3,3-21,9 artırmıştır. Bakteri aşılamaları ile şeker pancarında su stresi kısmen hafifletilmiştir. Bu araştırma sonuçları, bakteri aşılamalarının stres koşullarındaki performansının sadece bitki gelişmesi, verim ve besin alımının iyileştirilmesinde değil, aynı zamanda su kısıtı ve fazla sudan kaynaklanan stresin hafifletilmesinde de güçlü kanıtlar sağlamıştır.

Anahtar Kelimeler: Su stresi, besin alımı, bakteriler, enzim aktivitesi, hidrojen peroksit, malondialdehit

Introduction

Sugar beet (*Beta vulgaris* L.) is a very important industrial sugar crop, and fertilizers are the most important inputs for beet production. Adequate sugar beet production requires supplementary irrigation, but drought stress has recently become a major constraint to sugar beet cultivation, causing serious reductions in productivity. Drought inhibits the photosynthesis of plants, and thus reduces growth and development. Water stress reduces the production rate of dry matter, leaf and taproot growth of sugar beet. Water stress is considered as one of the major limiting factors for sugar beet root and leaf yield (Bloch and Hoffmann, 2005; Pidgeon et al., 2006; Romano et al., 2013). As evidenced, sucrose concentration and photosynthesis are highly sensitive to drought, since its efficiency decreases with the increasing water deficit (Bloch et al., 2006). Plant-growth-promoting rhizobacteria (PGPR) improve many nutritional, biochemical, physiological and morphological plant responses, and thus, it enhances the plant resistance to biotic and abiotic stresses. The inoculation of selected drought-tolerant strains can reduce the yield limitation caused by water deficit and improve the ability of plant tolerance to drought stress (Marulanda et al., 2009; Castillo et al., 2013). They have the ability to fix N_2, solubilize inorganic P, produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase and indole-3-acetic acid (IAA), and promote plant growth. In addition to providing plant nutrients, plant growth promoting bacteria directly stimulate plant growth by reducing plant ethylene levels through ACC deaminase activity (Glick, 1995; Glick, 2005). Bacteria containing ACC deaminase reduce the negative effects of ethylene causing water stress (Glick et al., 1998; Safronova et al., 2006). If bacteria containing ACC deaminase can reduce the production of increased "stress ethylene" under stress conditions, it is expected to protect against the inhibitory effect of stress in plants (Glick et al., 1998; Glick, 2005; Çakmakçı et al., 2009). In this way, in the researches carried out using bacteria with ACC deaminase activity in laboratory and field conditions, protection is provided to plants against stresses such as water (Farwell et al., 2007), organic pollutants (Reed and Glick, 2005), heavy metals (Nie et al., 2002; Reed and Glick, 2005; Farwell et al., 2006; Safronova et al., 2006; Pordel et al., 2019), high salinity (Mayak et al., 2004a; Cheng et al., 2007; Saravanakumar and Samiyappan, 2007), and drought and water deficit (Mayak et al., 2004b; Erdoğan et al., 2016; Karagöz et al., 2018).

Soil bacteria providing benefits to plants are defined as PGPR. According to a general definition, bio-fertilizers are substances containing one or more beneficial live microorganisms, which, when applied to seeds, plant surfaces or soil, colonize the rhizosphere or the interior of the plant, and promote plant growth by increasing the supply or availability of primary nutrients (Vessey, 2003; Çakmakçı, 2014). Biofertilizers are the preparations containing cells of microorganisms, which may be nitrogen fixers, phosphorus and potassium solubilizers and/or mobilizers (Chandra, 2015; Okur, 2018).

The use of beneficial bacteria as agricultural inputs for increasing crop production needs the selection of competent rhizobacteria with plant growth-promoting attributes. The effectiveness of bacterial inoculation under drought conditions in natural soil has been almost unexplored (Armada et al., 2014). Another alternative strategy is to induce stress tolerance by using beneficial microorganisms. Moreover, few studies have focused on the effects of rhizospheric microorganisms on the amelioration of water stress in plants. Different approaches are required for better water wasting due to the lack of water resources. The objective of this study was to evaluate possible effects of mineral fertilizer, two bio-organic and bio-mineral fertilizer, and seven N_2-fixing, P-solubilizing, IAA-producing and/or ACC deaminase containing microorganism based bio-fertilizers formulations in triple and quadruple strains combinations on the growth, yield, chlorophyll, macro- and micro-nutrient content, and selected morphological and physiological parameters of sugar beet, under five watering regimes (150%, 100%, 75%, 50%, and 25% of water-holding capacity:WHC).

Material and Methods

Bacterial strains

We selected twelve different potential promising PGPRs from a pool of 987 rhizobacterial isolates, strains tested in triple and quadruple strain combinations for seven bioformulations, two bio-organic and bio-mineral fertilizers, and mineral fertilizer under five watering regimes (150%, 100%, 75%, 50%, and 25% of water-holding capacity, WHC) on growth, yield, chlorophyll, macro- and

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micro-nutrient content, and selected morphological and physiological parameters of sugar beet increasing potential under natural soil conditions by conducting greenhouse two experiments trials. The experiment also included inoculation two bio-organic fertilizers, and application mineral fertilizer as well as a control treatment without inoculation and fertilizer application. The bacterial strains *Paenibacillus polymyxa* RC05 was isolated from wheat, and *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 (Çakmakçı et al., 2007a, 2009). The other four isolates of the bacteria used in the study (*Pseudomonas putida* RC310, *Pseudomonas fluorescens* PF8/6, and *Bacillus megaterium* A21/3) were selected through 460 isolates that had been isolated from the tea rhizosphere soil (Çakmakçı et al., 2010). Some features of bacteria used in the bacterial formulations are given in Table 1.

Greenhouse Experiment, Inoculation and Growth Conditions

This study was conducted under natural light. In the first and second trial sets, the seeds of genetic monogerm Z type "Feliçita" sugar beet were sowed in 6-L and 16-L pots at 2.5 cm depth. Sugar beet seedlings were grown in a greenhouse in a day-night cycle of 14-10 h light, 15 to 24 °C, and 60% humidity during the initial period and in 15-9 h day-night, 16–28°C and 55-60% relative humidity during the development period. In the research, two different experiment sets were established according to the factorial arrangement in randomized block experimental design of five water regimes, eleven treatments and five replications. Totally 11 applications existed in both test sets; (1) control (without bacteria inoculation and mineral fertilizers), (2) NP-fertilizers (60 mg of N + 40 mg P/kg soil), (3) Bio-OF (banana residue compost-based bacterial formulations); (4) Bio-MF (zeolite-based bacterial formulation), (5) BF1 (*P. fluorescens* T26 + *P. polymyxa* RC05 + *B. subtilis* RC63), (6) BF2 (*P. fluorescens* T26 + *R. erythropolis* RC9 + *B. megaterium* A21/3), (7) BF3 (*P. fluorescens* T26 + *V. paradoxus* RC21 + *B. subtilis* RC11), (8) BF4 (*P. fluorescens* PF8/6 + *P. polymyxa* RC05+ *B. subtilis* RC11 + *B. megaterium* A21/3), (9) BF5 (*P. fluorescens* PF8/6 + *R. erythropolis* RC9 + *V. paradoxus* RC21+ *B. subtilis* RC63), (10) BF6 (*P. putida* RC310 + *P. polymyxa* RC05 + *B. subtilis* RC63 + *B. megaterium* A21/3), and (11) BF7 (*P. putida* RC310 + *R. erythropolis* RC9 + *V. paradoxus* RC21+ *B. subtilis* RC11), and five water regimes (150%, 100%, 75%, 50%, and 25% of WHC), randomly distributed into pots filled with equal amounts of soil. The applied bio-organic fertilizer consisted of organic fertilizer from banana residue compost, zeolite, and it contained bacteria, namely 1.5×10^7 viable *P. fluorescens* + *P. polymyxa* + *B. subtilis* spores per gram of organic fertilizer and 1.5×10^7 viable of *P. fluorescens* + *V. paradoxus* + *B. megaterium* per gram of zeolite-based bio-mineral formulation.

For the experiment, pure cultures of fifteen single trains were grown in 50% strength tryptic soy broth (TSB) on a rotary shaker (120 rpm; 25°C) for 3 days. Bacteria were then harvested by centrifugation (ca. 3,000× g for 10 min), washed and re-suspended in 10 mM sterile phosphate buffer, pH 7.0 to a density of 10^9 cfu·ml^{-1} for the bacterial strains. For triple and quadruple inoculation, equal volumes (10^9 cfu·ml^{-1} of each inoculant) of bacterial cultures were mixed. For the seven microorganisms based bio-fertilizers, frozen bacterial culture seeded in petri dish Nutrient Agar (NA) containing medium, incubated for 24 hours at 27 °C. Pure colonies were taken from fresh culture and transferred to Nutrient Broth (NB) culture media. Horizontal shaker incubator developed a 24-hour culture, inoculated in NB containing the liquid culture media, previously prepared by fermentors and sterilized by autoclaving at 121 °C for 20 min. Bacteria were developed 24 h optimum pH, oxygen, and temperature values. Bacteria inoculated organic liquid carrier, the optimum growth conditions were incubated in the bioreactor. Counts of viable bacteria per millilitre (cfu) made in bacterial concentration were $1x10^8$ cells/ml at the end of 48 hours, during which time exceeds, packaging made completely sterile conditions, the product has been kept in room temperature at 24 °C. Seeds were then treated with the bacterial suspensions for 30 min. Seeds surface-sterilized by soaking in 25% commercial–grade bleach (sodium hypochlorite) for 5 min, followed by thorough washing under running tap water and air-drying aseptically overnight at room temperature were soaked into the bacterial suspension. Bacterial inoculation of the seeds was carried out according to Sahin et al. (2004). Seeds were then treated with the bacterial suspensions at the concentration of 10^8 CFU ml^{-1} for 30 min under sterilized conditions.

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The bio-organic fertilizer used in this study was obtained by aerobically fermenting a mixed organic fertilizer with triple bacterial suspension (100:1, w/v) for 7 days at <40 °C. The mixed organic fertilizer was prepared from mature compost of banana residue compost, which contained 42.5% organic matter, 3.2% N, 0.27% P_2O_5, 2.8% K_2O and 28.4% H_2O and zeolite. Bacterial isolates were inoculated into NB broth and incubated on a shaker incubator at 150-rpm for 48 h. After 48 h of incubation, the broth containing 10^9 cfu ml^{-1} was used for the preparation of banana residue compost and zeolite-based formulations. For the preparation of bio formulations, to 400 ml bacterial suspension, a mixture of 1 kg of a purified banana residue compost and zeolite, pH was adjusted to 7 by adding calcium carbonate and 10 g carboxyl methyl cellulose (CMC adhesive) was added under sterile conditions, following the method described by Vidhyasekaran and Muthuamilan (1995), Ardakani et al. (2010), Jorjani et al. (2011) and Çakmakçı et al. (2014). Powdered carriers material and CMC mixed well. Then four hundred millilitres of bacterial suspension containing 1 x 10^8 cfu/ml was added to 1 kg of carrier and mixed well under sterile conditions. Bio-organic fertilizers formulations were prepared by mixing equal volume of individual strains and blended with the carrier. Survivability of PGPR was checked at a regular interval of one month for a period of six months using direct plating method in nutrient agar medium. The final bio-organic fertilizer was stored at 4 °C and was used in the experiments only if the population of the bacteria remained at the level of 10^8cfu per gram of dry matter. The bio-fertilizer had 10^8 bacterial cells g^{-1} carrier at the time of application to soil. Soil application of bio-organic fertilizers at the rate of 2.5 kg formulation mixed with 25 kg of well decomposed farmyard manure (150 mg of the formulated product for one seedling in a pot) per ha on one days before sowing.

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 free drainage. After the drainage was stopped, pots were weighed and WHC was found. Two pot experiments were conducted on sugar beet well supplied with water (100% WHC), under waterlogging (150% of WHC), and continuous moderate (75% and 50% of WHC) and severe drought stress (25% of WHC). After sugar beet was sowed, 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 sowing. After three weeks from sowing, different irrigation levels were started to be applied in both sets of the experiment. For the determination of irrigation water to be given to each pot, pots were weighted to found out the difference between current weights with field capacity weight, immediately before the irrigation. Water application of 150% of the field capacity, the water leaking from the pot accumulated on the pot base and recycled. For the first experiment, after two weeks from sowing, beets were diluted in the pots, in each pot, six plants were allowed in the two-leaf period and five plants were allowed in five-leaf periods. For the second experiment, in each pot, six plants were allowed in the two-leaf period, four plants were allowed in five-leaf periods and one plant was allowed in seven-leaf period. Chlorophyll contents of the top fourth and fifth leaves were measured using a chlorophyll meter SPAD-502 (Minolta, Japan), which is used to measure leaf greenness of the plants. The first experiment set was harvested 65 days after sowing and the second set was harvested 130 days after sowing.

Acetylene reduction assay, phosphate solubilization, IAA production and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity

Nitrogen fixation of the isolates was determined in a nitrogen-free medium by acetylene reduction assay (Hardy et al., 1968). Ethylene production was measured using a Hewlett Packard gas chromatograph (Model 6890, USA). All the pure isolates were tested in triplicate for their phosphate solubilizing capacity in sucrose-tricalcium phosphate agar media (Pikovskaya, 1948) by inoculating 1 ml of 6-day-old culture (density 4×10^9) in 250-ml Erlenmeyer flasks containing 500 µg ml^{-1} of P as rock phosphate at 30±1°C. After incubation for 6 days, water soluble P was determined colourimetrically by the vanadomolybdophosphoric yellow colour method.

The PGPR was tested for indole-3-acetic acid-like auxin production using the method of Bent et al. (2001). The concentration of IAA in the culture medium was measured using Salkowski's reagent [50 ml 35% (v/v) $HClO_4$ + containing 1 ml 0.5 M $FeCl_3$]. The absorbance was measured at 530 nm in a Shimadzu UV-1208 spectrophotometer (Tokyo, Japan). Bacterial cells were separated from the supernatant by centrifugation at 10.000 rpm for 30 min at 4°C. The isolates were assayed for 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity by testing their ability to grow in

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DF minimal salts medium supplemented with 3 mmol ACC as the sole source of nitrogen (Penrose and Glick 2003). The cell extracts and the measure of the ACC deaminase activity were carried out quantifying the amount of *α*-ketobutyrate produced (nmoles mg protein^{-1} h^{-1}) by the procedure of Honma and Shimomura (1978).

Determination of enzyme activities and protein concentrations

Leaf samples from harvested plants were washed three times with 50 mM Tris–HCl+0.1 M Na_2SO_4 (pH 8.0), and each was homogenized by liquid nitrogen, transferred to 100 mM PVP + 10mM NaN_3 + 50 mM Tris–HCl+0.1 M Na_2SO_4 (pH 8.0) buffer, and centrifuged at 4°C, 15.000 g for 60 min (Çakmakçı et al., 2009). The supernatant was used as a crude extract at the measurement of enzyme activity and protein determination. The activities of Glutathione reductase (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 assayed by the method of Carlberg and Mannervik (1985), Habig and Jacoby (1981) and Beutler (1984), respectively. 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. The content of hydrogen peroxide (H_2O_2) was determined according to Sairam and Srivastava (2002) method. Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content in fresh leaf weight according to the method of Heath and Packer (1968).

Plant analysis

Leaf samples were oven-dried at 68°C for 48 h and ground to pass 1 mm. The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N. After extraction, Macro- (P, K, Ca and Mg) and micro-elements (Fe, Mn, Zn and Cu) were determined with an inductively Coupled Plasma spectrophotometer (Optima 2100 DV, ICP/OES, Perkin-Elmer, Waltham MA, USA). Polarimetric sugar contens analysis were carried out in the laboratories of the Sugar Factories in Erzurum using Cold Aqueous Digestion Method (Nouruzhan, 1957).

Statistical analysis

The research was established according to factorial completely randomized design of five water regimes, eleven treatments and five replications. The data acquired from both experiments were subjected to analysis of variance using SPSS13.0 (SPSS Inc.) and the means were separated according to Duncan's Multiple Range Test.

Table 1. Biochemical characteristics of the bacterial strains used in bio-formulations and bio-organic and bio-mineral fertilizers

Bio-formulation	Bacterial strain	Nitrogenase activity (nmol C_2H_4, 10^7 cfu h^{-1})	P-solubilization (µg P mL^{-1} d^{-1})	IAA-production (µg mL^{-1} (OD_{600} unit^{-1})	ACC deaminase Activity (nmol α-ketobutyrate mg^{-1} protein h^{-1})
BF1	*Pseudomonas fluorescens* T26	0.61±0.13	27.7± 1.2	23.9± 2.1	796.1±35.2
	Paenibacillus polymyxa RC05	0.68±0.14	10.07±0.9	32.8±2.6	682.1±33.7
	Bacillus subtilis RC63	0.74±0.17	34.6±0.08	29.7 ± 1.9	972.0 ± 28.3
BF2	*Pseudomonas fluorescens* T26	0.61±0.13	27.7± 1.2	23.9± 2.1	796.1±35.2
	Rhodococcus erythropolis RC9	0.55 ±0.11	27.8±1.5	22.6± 1.5	577.8 ±26.7
	Bacillus megaterium A21/3	0.48±0.16	74.3±1.9	19.5± 1.1	276.3 ±16.7
BF3	*Pseudomonas fluorescens* T26	0.61±0.13	27.7± 1.2	23.9± 2.1	796.1±35.2
	Variovorax paradoxus RC21	0.47±0.12	0.47±0.12	19.4± 1.2	332.6±17.4
	Bacillus subtilis RC11	0.32±0.12	16.6±0.41	29.4±1.8	539.2±21.2
BF4	*Pseudomonas fluorescens* PF8/6	0.47±0.09	113.5±12.7	20.7±1.5	223.6±21.7
	Paenibacillus polymyxa RC05	0.68±0.14	10.07±0.9	32.8±2.6	682.1±33.7
	Bacillus subtilis RC11	0.32±0.12	16.6±0.41	29.4±1.8	539.2±21.2
	Bacillus megaterium A21/3	0.48±0.16	74.3±1.9	19.5± 1.1	276.3 ±16.7

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BF5	*Pseudomonas fluorescens* PF8/6	0.47±0.09	113.5±12.7	20.7±1.5	223.6±21.7
	Rhodococcus erythropolis RC9	0.55 ±0.11	27.8±1.5	22.6± 1.5	577.8 ±26.7
	Variovorax paradoxus RC21	0.47±0.12	0.47±0.12	19.4± 1.2	332.6±17.4
	Bacillus subtilis RC63	0.74±0.17	34.6±0.08	29.7 ± 1.9	972.0 ± 28.3
BF6	*Pseudomonas putida* RC310	0.66±0.15	26.8± 1.8	35.9±2.4	746.2±46.8
	Paenibacillus polymyxa RC05	0.68±0.14	10.07±0.9	32.8±2.6	682.1±33.7
	Bacillus subtilis RC63	0.74±0.17	34.6±0.08	29.7 ± 1.9	972.0 ± 28.3
	Bacillus megaterium A21/3	0.48±0.16	74.3±1.9	19.5± 1.1	276.3 ±16.7
BF7	*Pseudomonas putida* RC310	0.66±0.15	26.8± 1.8	35.9±2.4	746.2±46.8
	Rhodococcus erythropolis RC9	0.55 ±0.11	27.8±1.5	22.6± 1.5	577.8 ±26.7
	Variovorax paradoxus RC21	0.47±0.12	0.47±0.12	19.4± 1.2	332.6±17.4
	Bacillus subtilis RC11	0.32±0.12	16.6±0.41	29.4±1.8	539.2±21.2
Bio-OF	*Pseudomonas fluorescens*	S+	S+	S+	S+
	Paenibacillus polymyxa	S+	+	+	S+
	Bacillus subtilis	S+	+	S+	+
Bio-MF	*Pseudomonas fluorescens*	S+	+	+	+
	Variovorax paradoxus	+	S+	ND	+
	Bacillus megaterium	+	S+	+	S+

"S+": strong positive reaction, "+": positive reaction, "W+": weak positive reaction, Bio-OF: bio-organic fertilizer, Bio-MF: bio-mineral fertilizer, BF: bioformulations; ND: not determined; Data were means ± standard error of three replicates

Results and Discussion

Bacterial inoculations, bio-organic and mineral fertilizer application significantly increased fresh and dry leaf weight, and sugar per plant compared to control in the first and second trials (Table 2). In terms of sugar beet root fresh and dry weight for both harvests (65-day and 130-day harvest), all inoculations except for BF5 bacterial formulation gave better results compared to control. Of the 11 treatments, the maximum fresh and dry storage root and leaf weight in sugar beet was seen in BF4 inoculation, followed by BF6, NP, Bio-OF, and BF1. While determining the highest weight of fresh leaf, BF4 and BF6 microbial formulations, Bio-OF and mineral fertilizer were found to importantly increase fresh and dry leaf weight compared to control and BF5 inoculation gave the same result as the control in terms of fresh and dry storage root weight. On average of the five water regimes, application of sugar beet with BF1, BF2, BF3, BF4, BF5, BF6, BF7, Bio-OF and Bio-MF gave increases over control respectively of by 12.2, 3.9, 6.0, 32.5, 5.2, 30.5, 6.3, 14.3, and 8.8% and 17.7, 11.7, 12.1, 38.6, 6.8, 35.5, 10.2, 17.9 and 6.8% in fresh storage root weight at the first and second trials, by 14.6, 11.0, 16.1, 34.2, 11.9, 27.6, 16.8, 28.5 and 13.4%, and 17.3, 9.3, 12.7, 32.7, 7.1, 27.5, 14.4, 23.4 and 10.4% in fresh leaf weight at the first and second trials. NP mineral fertilizers applications, however, increased fresh storage root weight by 14.6% and 19.1% and fresh leaf weight by 27.4% and 24.9% at the first and second trials, respectively, compared with control.

In the excessive irrigation by water application of both trail sets, primarily Bio-MF and BF, all bacteria inoculations importantly increased fresh and dry root, 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 fresh and dry root weight, and sugar per plant compared to the un-inoculated, water-stressed plants (Table 2, Figure 1). In the application of water at the rate of 100 % of WHC PGPR inoculation increased fresh root weight by 6.4-24.5% and 2.7-28.6% in the first and second trials, whereas NP fertilizer increased the weight by 27.2 and 25.4%, respectively, compared to control. Whereas, in the application of water at the rate of 25% of WHC, inoculation with BF4, BF6 and BF1 increased the sugar beet fresh root weight by 34.3, 32.2 and 25% over control respectively in the first trial and 44.3, 16.8, 31.5% in the second trial. As an average of the five water regimes, PGPR inoculation increased fresh root weight by 2.1-28.3% and 7.1-35.1% in the first and second trials, whereas NP fertilizer increased the weight by 11.3 and 17.9%, respectively, compared to control.

In the application of water at the rate of 100 % of WHC PGPR inoculation increased dry root weight by 1.1-22.0% and 3.4-15.4% in the first and second trials, whereas NP fertilizer increased the weight by 48.1 and 22.3%, respectively, compared to control. According to the average irrigation level, the highest fresh and dry root and leaf weight was achieved with BF4, BF6 and BF1

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formulations inoculations (Table 2). As an average of the five water regimes, PGPR inoculation increased dry root weight by. 6.5-27.8% and 9.1-27.4% in the first and second trials, whereas NP fertilizer increased the weight by 24.5 and 20.2%, respectively, compared to control. Bacterial formulations BF4, BF6, BF1, and mineral and bio-organic fertilizer (Bio-OF) were most effective in promoting fresh and dry leaf weight of sugar beet in both trials set. Moreover, BF6, BF4, BF1, BF3 and Bio-OF applications 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. The fact that inoculation of bacteria encouraged the growth of sugar beet leaves was in line with the results of previous researches (Schmidt et al., 2004; Shi et al., 2009). Sugar beet root, leaf development and yield decreased under the water restrictions.

Under waterlogging stress (150% of WHC), sugar beet chlorophyll contents (SPAD) significantly increased NP fertilizer, BF1, BF3 and BF4 inoculation. The maximum chlorophyll contents in the sugar beet leaves were found after BF4, followed by BF6, BF1 and Bio-OF applications under drought stress applied at 75%, 50% and 25% WHC in both experiments (Figure 1). Our results indicated that water stress decreased chlorophyll content, while bacterial inoculations increased it. In both trial sets, drought stress decreased the chlorophyll contents of sugar beet, and generally, inoculation of the multi traits bacteria under drought stress improved the chlorophyll contents, but responses were strain-specific. A similar result was reported by Abbasi et al. (2013) who showed that PGPR application could contribute to reducing the drought stress effect and significantly increased chlorophyll content of soybean**.** Furthermore, Sahin et al. (2015) reported that PGPR inoculation could alleviate the deleterious effects of lower irrigation conditions on the growth and yield of lettuce plants. When temporary water stress appears at the early period, it can be said that it reduces sugar beet root yield importantly. Indeed, when young beet plants were exposed to the 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). Sahin et al. (2004) determined that the bacterial activity was higher at the early development stages.

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

Treat-ments	Water regimes at the first trial set were harvested 65 days after sowing						Water regimes at the second trial set were harvested 130 days after sowing					
	S1	S2	S3	S4	S5	Mean	S1	S2	S3	S4	S5	Mean
Fresh storage root weight (g/plant)												
Control	32.6	46.9	33.6	27.6	23.6	32.9 d	286	405	305	232	203	286 d
NP	38.5	59.7	34.9	25.7	23.8	36.6 b	311	508	398	263	207	337 b
Bio-OF	35.3	53.7	38.3	32.9	21.9	36.4 b	336	491	336	313	194	334 b
Bio-MF	38.9	49.8	40.6	25.6	21.4	35.3 bc	365	445	257	244	201	302 c
BF1	32.8	52.8	40.8	30.8	29.5	37.4 b	306	419	382	293	267	333 b
BF2	38.8	49.9	30.2	26.7	24.9	34.1 bc	367	428	286	263	237	316 bc
BF3	37.6	51.6	31.6	30.5	24.6	35.2 bc	352	422	300	289	234	319 bc
BF4	37.9	58.4	43.8	38.9	31.7	42.2 a	349	551	408	362	293	393 a
BF5	38.0	49.9	30.2	26.8	22.8	33.5 d	361	416	316	244	195	306 c
BF6	33.8	52.9	49.8	42.2	31.2	42.0 a	316	510	474	382	237	384 a
BF7	36.4	48.8	31.5	29.8	24.8	34.3 bc	340	443	300	283	194	312 bc
Mean	36.4 b	52.3 a	36.8 b	30.7 c	25.4 d	36.3	335 b	458 a	342 b	288 c	224 d	329
Dry storage root weight (g/plant)												
Control	4.24	5.28	3.94	3.54	2.96	3.99 d	52.9	69.8	50.2	37.4	28.6	47.8 d
NP	4.63	7.82	5.46	3.73	2.74	4.97 b	54.3	85.4	69.5	43.6	34.1	57.4 b
Bio-OF	4.41	6.46	4.92	4.15	2.90	4.61 b	55.3	80.8	55.3	51.6	32.0	55.0 b
Bio-MF	4.82	5.87	4.42	3.82	2.79	4.37 c	60.2	75.4	54.3	40.1	33.0	52.6 c
BF1	4.14	5.56	5.09	3.99	3.79	4.52 b	50.4	75.2	62.9	48.2	43.9	56.1 b
BF2	4.88	5.71	3.98	3.48	3.34	4.29 bc	60.6	73.3	48.4	43.7	39.0	53.0 bc

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BF3	4.65	5.34	4.03	3.96	3.34	4.27 bc	58.0	72.2	49.3	47.7	38.5	53.1 bc
BF4	4.67	6.44	5.21	4.92	4.04	5.10 a	57.4	79.7	64.2	54.6	48.2	60.8 a
BF5	4.78	5.49	4.28	3.74	2.93	4.25 d	58.7	72.7	58.9	39.8	30.6	52.1 c
BF6	4.24	6.29	5.52	5.29	3.44	4.99 a	52.0	80.6	73.0	60.1	35.0	60.2 a
BF7	4.48	5.87	4.19	3.86	2.90	4.28 bc	55.8	77.0	49.3	46.6	31.9	52.1 bc
Mean	4.54 b	6.01 a	4.64 b	4.04 c	3.20 d	4.51	56.0 b	76.6 a	57.8 b	46.7 c	35.9 d	54.6
Fresh leaf weight (g/plant)												
Control	69	92	79	51	47	67.5 d	368	473	344	264	233	336 d
NP	90	128	101	58	52	86.0 b	448	627	446	319	259	420 b
Bio-OF	84	117	100	81	52	86.7 b	436	602	405	378	256	415 b
Bio-MF	84	108	80	64	48	76.6 c	426	538	353	304	236	371 c
BF1	71	101	86	70	58	77.3 c	392	515	429	348	290	395 bc
BF2	84	97	82	57	55	74.9 c	427	481	366	291	274	368 c
BF3	91	97	83	68	54	78.4 c	452	484	357	335	268	379 c
BF4	84	121	99	83	66	90.6 a	427	556	494	417	338	446 a
BF5	85	100	83	62	48	75.6 c	432	497	355	283	235	360 c
BF6	72	111	98	89	60	86.2 b	372	541	487	444	304	430 b
BF7	77	110	83	72	51	78.8 c	385	549	382	356	253	385 bc
Mean	81 b	108 a	88 b	68 c	54 d	79.9	415 b	533 a	402 b	340 c	268 d	391
Dry leaf weight (g/plant)												
Control	7.46	8.64	6.98	4.86	4.59	6.51 d	41.6	55.8	39.9	29.8	26.6	38.7 e
NP	8.19	11.64	9.18	5.76	5.05	8.03 b	49.6	72.0	49.6	36.8	28.8	47.4 b
Bio-OF	7.75	10.60	8.82	7.66	5.03	8.02 b	49.9	69.2	45.8	42.7	28.5	47.2 b
Bio-MF	7.76	10.71	7.04	5.91	4.70	7.27 c	46.1	60.7	33.1	33.5	26.7	40.0 d
BF1	6.56	9.45	8.09	6.72	5.72	7.33 c	42.9	58.7	48.5	39.3	31.8	44.2 bc
BF2	7.76	8.95	6.84	5.48	5.44	6.90 c	42.1	54.6	48.5	28.7	27.4	40.3 d
BF3	8.38	9.20	6.73	6.45	5.29	7.22 c	49.4	54.6	41.3	37.9	27.6	42.2 c
BF4	7.76	10.74	8.47	7.39	6.60	8.24 a	47.0	62.8	51.0	45.1	37.9	48.8 a
BF5	7.87	9.54	6.75	5.85	4.77	6.98 c	42.4	56.2	47.8	31.4	28.1	41.2 d
BF6	6.69	10.44	8.69	8.19	5.89	8.02 b	43.6	61.1	55.0	50.2	30.8	48.1 b
BF7	7.14	10.16	7.17	6.97	5.12	7.35 c	42.6	61.7	46.7	39.6	27.9	43.7 bc
Mean	7.57 b	10.0 a	7.71 b	6.48 c	5.29 d	7.44	45.2 b	60.7 a	45.2 b	37.7 c	29.3 d	43.6

*Control: without bacteria inoculation or mineral fertilizers; NP fertilizer (60 mg of N + 40 mg P/kg soil); S1: 150% of water-holding capacity (WHC), S2: 100% of WHC, S3: 75% of WHC, S4: 50% of WHC, S5: 25% of WHC; all strains used in these bioformulations were explained in Table I

**Averages of the same column values (each section separately) followed by the same letter did not differ significantly from Duncan's multiple range tests ($p<0.01$).

Water-restriction caused important yield loses and this effect was excessive in young plants affected by drought. If water stress occurs in young sugar beet plants, it can drastically reduce the growth and yield. Sugar beet root weight reduction decreased significantly under drought conditions, but this situation changed according to inoculated bacteria and irrigation level.

Of the bacterial inoculations, BF4 and BF6 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 bacterial formulations BF4, BF6, BF1, and banana residue compost-based bacterial formulations inoculation were found to be effective according to the amount of sugar per plant at water constraint applications with 75%, 50% and 25% of WHC. Under insufficient water supply, the sucrose concentration was higher than under well-watered conditions. The increase in sucrose concentration caused by adding may only be a result of low water content in the roots. 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 for 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 (Pidgeon et al., 2006). 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

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and the percentage of fresh weight in the root. Bacterial inoculation minimized the drought stress-imposed effects significantly increasing the sugar per plant in sugar beet, but this changed depending on the inoculation bacterial formulations and level of irrigations.

On average of five water regimes, inoculation of PGPR significantly increased the N and P content leaves of the sugar beet except for Bio-MF and BF2, respectively, compared to control. On the other hand, all bacterial strains formulations and bio-organic fertilizers tested significantly increased the K, Ca and Zn content of the sugar beet leaf. N, P, K and Zn content were the greatest with BF4 and BF6, whereas maximal Ca and Mg was with bacterial formulations BF3 and BF7 inoculations, but BF4 and BF6 inoculation were as effective as them (Table 3). In addition, five of the formulations (BF1, BF3, BF4, BF5, and BF6) significantly increased the Mn content of sugar beet plants, but not Fe and Cu concentrations. Apart from BF5 and BF6, the five remaining PGPR formulations tested significantly increased Mg content, while only BF3 inoculants significantly increased the Na content in the leaves of the sugar beet compared to the control (Table 3). Decreasing water availability under drought generally results in the reduced total nutrient uptake, whereas application of PGPR increased the uptake of N, P, K, Ca, Mg, Mn and Zn in sugar beet.

Of the bacterial inoculations, high ACC deaminase-containing bacterial formulations exhibiting better performance under severe drought stress (25% of WHC) conditions was observed to have the highest-level K content in sugar beet leaf, which was correlated well with their increased root and leaf weight and enhanced 6PGD and G6PD enzyme activities (Figure 2), thus protecting the plants from water stress compared to the other bacteria and control. 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. The data in this study show a close relationship between enzyme activities in plant leaves and growth promotion by PGPR formulations and bio-organic fertilizer. Thus, the growth and yield parameters of sugar beet could be enhanced by bacterial formulations inoculation due to increases in the activities of enzymes, which play an important role in nitrate assimilation as well as in water and nutrient-use efficiency (Çakmakçı et al., 2007 b, 2009). Previous studies indicated that PGPR inoculations could improve the yield and growth, nutrient and water uptake of different crops grown under drought stress (Saravanakumar et al., 2011; Heidari and Golpayegani, 2012; Lim and Kim, 2013; Şahin et al., 2015; Mutumba et al., 2018).

On average of five water regimes, the highest GR activity was found effective at BF2, BF1, BF6, BF4 and mineral fertilizer, whereas the highest GST activity was found effective at BF5 and Bio-MF (Figure 2). The maximum 6PGD in sugar beet leaf was found after BF5 inoculation, followed by BF4, BF1, and BF6. The highest leaf G6PD activity was observed after NP fertilization, followed by BF4, BF6 and BF1 inoculations. Under continuous moderate (75% and 50% of WHC) and severe drought stress (25% of WHC), the maximum GR activity was found after NP mineral fertilizer application, followed by BF6, BF2, BF4 and BF1, whereas the highest levels of GST activity were determined in treatments with BF5, followed by bio-organic fertilizers. Recently, our studies demonstrated, for the first time, that PGPR could enhance GR, GST, 6PGD and G6PD activities, together with the growth of wheat and spinach plants (Çakmakçı et al., 2007 a, 2009).

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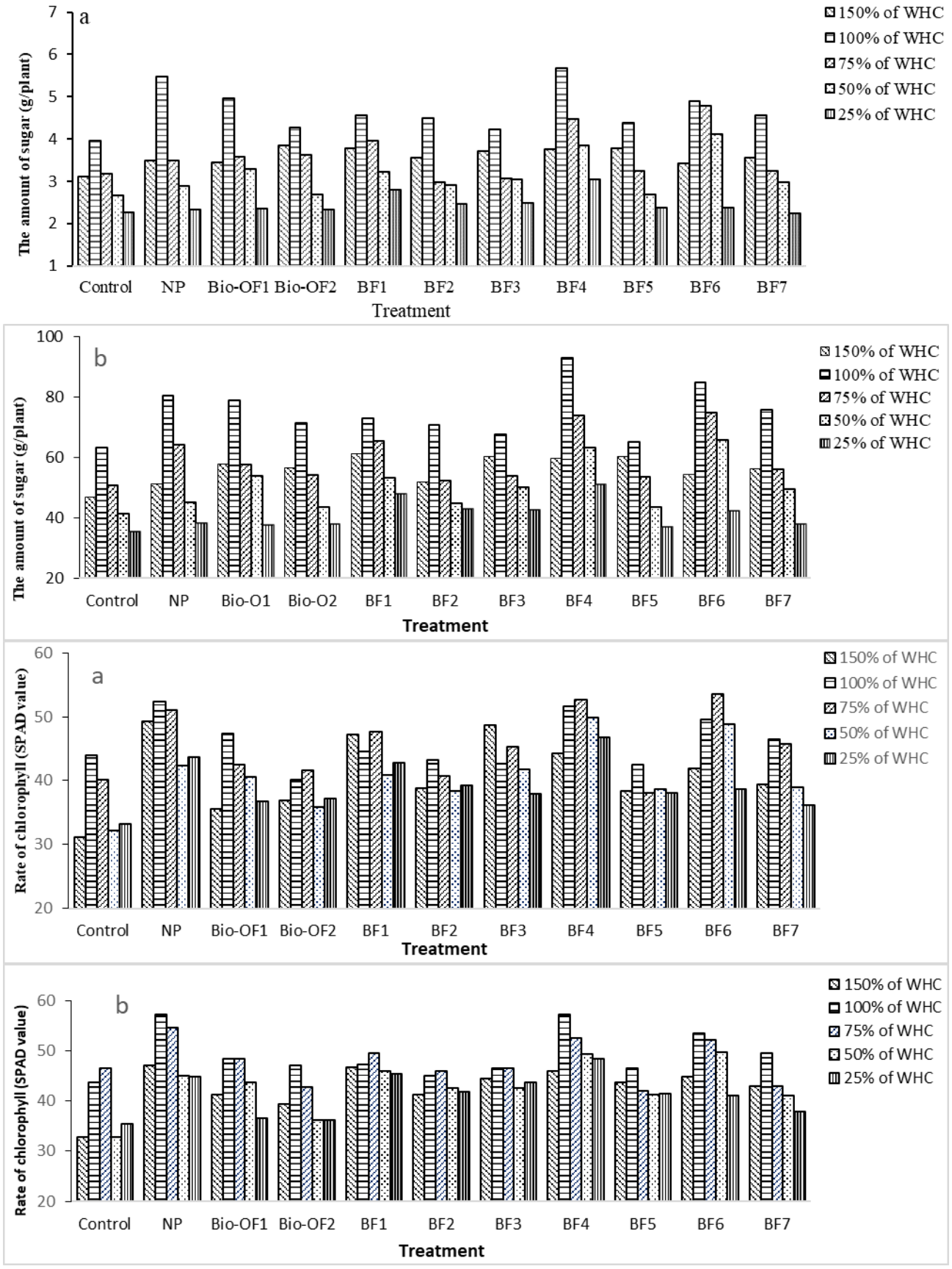

Figure 1. Effect of bacterial formulations and mineral and bio-organic fertilizer on the chlorophyll content and sugar per plant at the first (a) and second trial (b) under different water regimes

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Table. 3. Effect of mineral fertilizer and plant growth-promoting rhizobacteria formulations and bio-organic fertilizers on macro- and micro-nutrient concentrations in sugar beet leaves in the first trial set with the average of five water regimes

Treat-ments	Macro-nutrient (g kg^{-1} DW)					Micro-nutrient (mg kg^{-1} DW)				
	N	P	K	Ca	Mg	Na	Fe	Cu	Mn	Zn
Control	21.6 g	2.98 b	20.9 e	8.61 c	3.58 c	1676 b-c	136 a-c	31.8 a	45.7 f	31.7 g
NP	26.7 ab	3.16 a	24.7 ab	8.85 bc	3.70 bc	1692 a-c	137 a-c	28.6 bc	45.6 f	33.9 f
Bio-OF	25.1 bc	3.19 a	23.3 cd	9.07 ab	3.92 ab	1760 ab	129 cd	28.3 bc	51.4 cd	37.7 e
Bio-MF	22.9 fg	3.15 a	23.0 b-d	9.3 ab	3.80a-c	1709 a-c	134 a-d	28.2 bc	48.1 ef	38.4 de
BF1	25.1 b-d	3.20 a	23.9 a-d	9.34 ab	3.85 ab	1619 cd	133 b-d	28.6 bc	56.4 a	39.9cd
BF2	23.9 d-f	3.12 ab	23.8 a-d	9.32 ab	3.86 ab	1710 a-c	138 ab	27.3 c	47.9 ef	44.6 b
BF3	25.7 bc	3.23 a	23.6 a-d	9.49 a	3.99 a	1807 a	128 d	28.8 bc	50.5 de	41.7 c
BF4	27.9 a	3.26 a	25.0 a	9.40 a	3.90 ab	1534 d	142 a	28.7 bc	52.4 b-d	48.2 a
BF5	24.1 c-f	3.15 a	23.0 cd	9.43 a	3.78a-c	1688 bc	132 b-d	28.8 bc	55.2 ab	38.5 de
BF6	26.7 ab	3.23 a	24.5 ab	9.48 a	3.82a-c	1513 d	138 ab	28.5 bc	54.2 a-c	47.7 a
BF7	24.9 c-e	3.22 a	23.9 a-c	9.44 a	3.97 a	1541 d	133 b-d	29.2 b	46.6 f	45.4 b
Mean	24.8	3.17	23.6	9.25	3.70	1659	134	29.0	50.4	40.7

[a]Means followed with the same letter within each column are not significantly different (Duncan's Multiple Range Test= 0.05)

On average of five water regimes, the highest GR activity was found effective at BF2, BF1, BF6, BF4 and mineral fertilizer, whereas the highest GST activity was found effective at BF5 and Bio-MF (Figure 2). The maximum 6PGD in sugar beet leaf was found after BF5 inoculation, followed by BF4, BF1, and BF6. The highest leaf G6PD activity was observed after NP fertilization, followed by BF4, BF6 and BF1 inoculations. Under continuous moderate (75% and 50% of WHC) and severe drought stress (25% of WHC), the maximum GR activity was found after NP mineral fertilizer application, followed by BF6, BF2, BF4 and BF1, whereas the highest levels of GST activity were determined in treatments with BF5, followed by bio-organic fertilizers. Recently, our studies demonstrated, for the first time, that PGPR could enhance GR, GST, 6PGD and G6PD activities, together with the growth of wheat and spinach plants (Çakmakçı et al., 2007 a, 2009).

MDA and H_2O_2 levels were increased by drought stress both in inoculated and in non-inoculated plants. 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 significantly increased the drought stress markers (MDA and H_2O_2), which indicated the extent of oxidative injury posed by stress conditions. Four of the PGPR formulations (BF4, BF6, BF1 and BF3) and Bio-OF exhibiting better performance under water deficit conditions were observed to have lower levels of MDA content in the leaf, which was correlated well with their decreased H_2O_2 content (Figure 3) and enhanced leaf and root weight (Table 2), thus protecting the plants from lipid peroxidation of membrane systems compared to the other bacteria and control, which had higher levels of MDA content. The diminishing water supply caused a gradual decrease in plant growth, accompanied by the increasing concentrations of drought stress markers (MDA and H_2O_2 content) in sugar beet (Figure 3). The MDA content was measured to determine the extent of lipid peroxidation. The oxidative damage to lipids increased because of drought as measured by the MDA content. After drought treatment, gradual increases of H_2O_2 and MDA contents were observed in all treatments. The MDA content was higher in control plants at all the stress levels. The highest MDA content under severe drought stress (25% of WHC) was observed in the control plants followed by BF5 and BF7 formulations and mineral fertilizer application. As the intensity of drought increased, both H_2O_2 and MDA levels increased. According to the average of 11 applications and two harvests, MDA and H_2O_2 increased approximately three times in severe water restriction (25% of WHC) compared to well-watered plants (100% of WHC). After drought treatment, gradual increases of H_2O_2 and MDA contents were observed in all treatments, whereas effective bacterial strains decreased the MDA and H_2O_2 content. Bacterial inoculation

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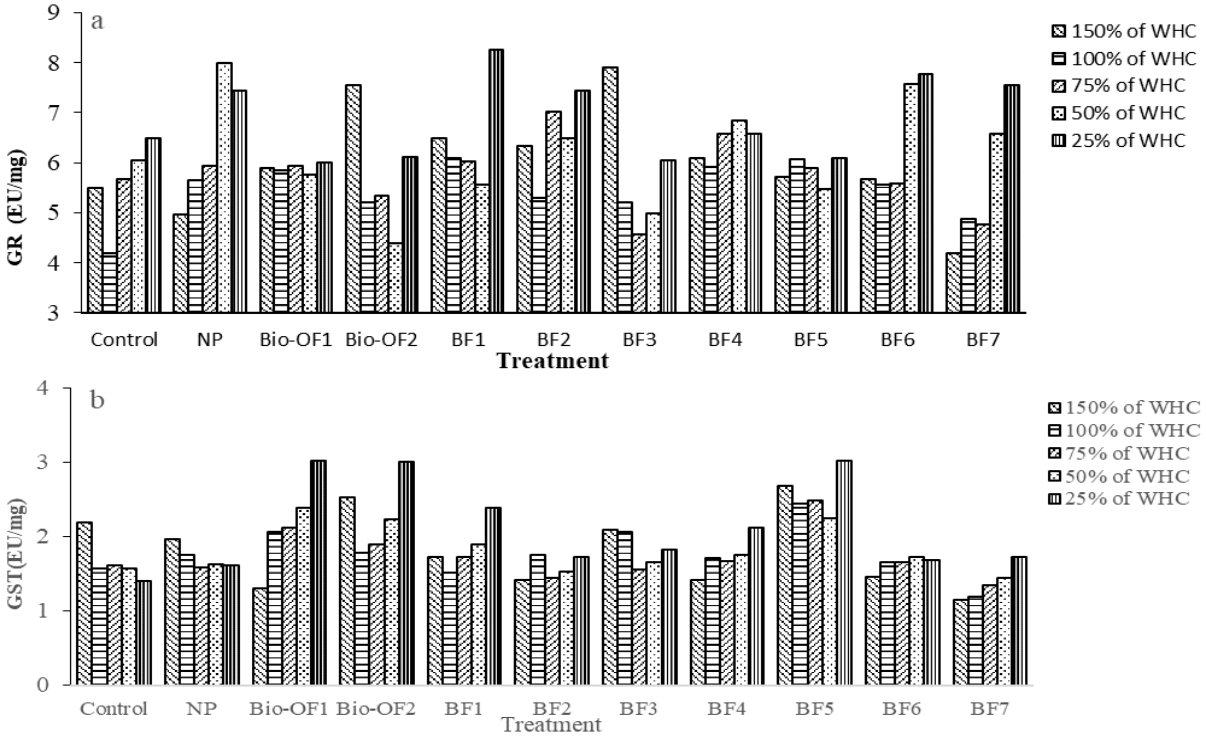
elevated the cold stress deleterious effect and decreased H_2O_2 values non-cold stress and cold stress condition (Turan et al. 2013). Of the bacterial inoculations, high ACC deaminase-containing formulations BF4 and BF6 exhibiting better performance under moderate and severe drought stress (50 and 25% of WHC) conditions were observed to have the highest level N and K content in sugar beet leaf, which was correlated well with their increased root and leaf weight, 6PGD and G6PD enzyme activities and decreased both H_2O_2 and MDA content (Figure 3), thus protecting the plants from water stress compared to other bacteria and control. Earlier studies suggested that inoculation with multi-traits bacteria proved to be the most effective treatment to enhance tolerance to water in in wheat genotypes (Mutumba et al., 2018), increase leaf relative water content, stomatal conductance, and plant nutrient element content (Şahin et al., 2015), and could improve stress tolerance and water use efficiency of plants under water deficit conditions (Sandhya et al., 2010; Lim and Kim, 2013; Mutumba et al., 2018).



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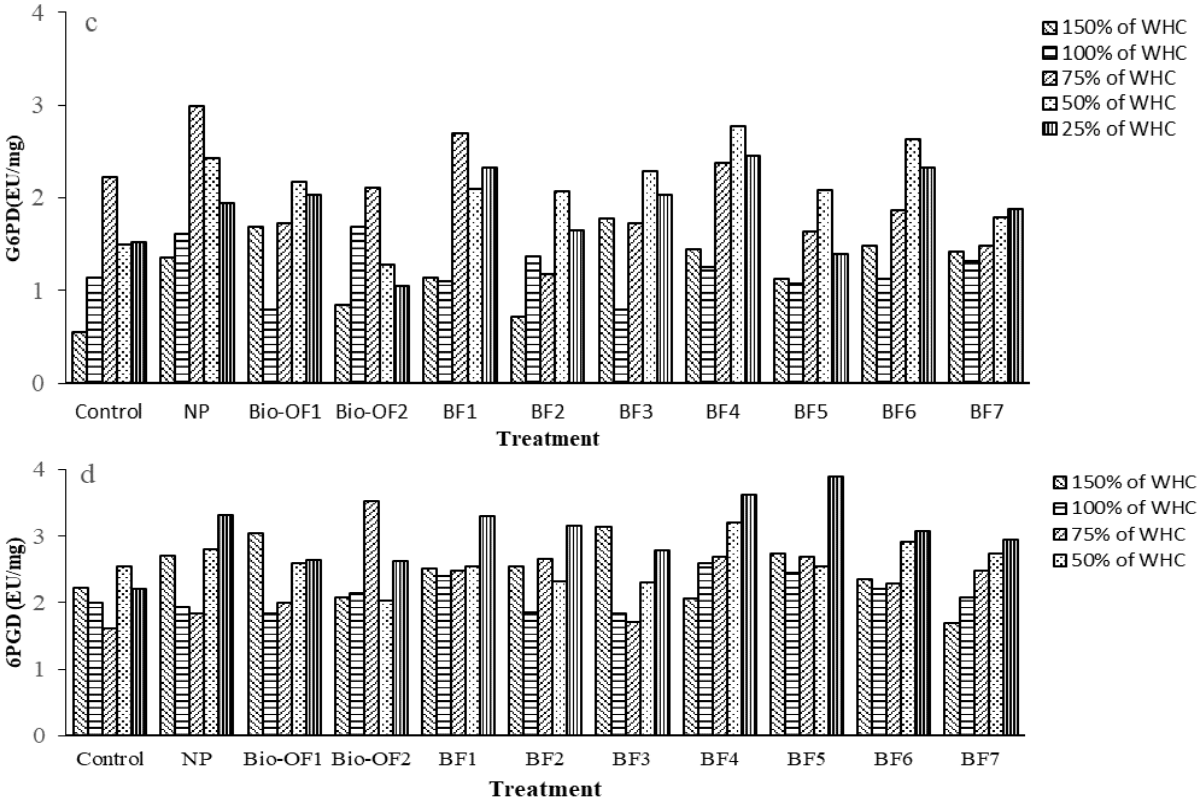
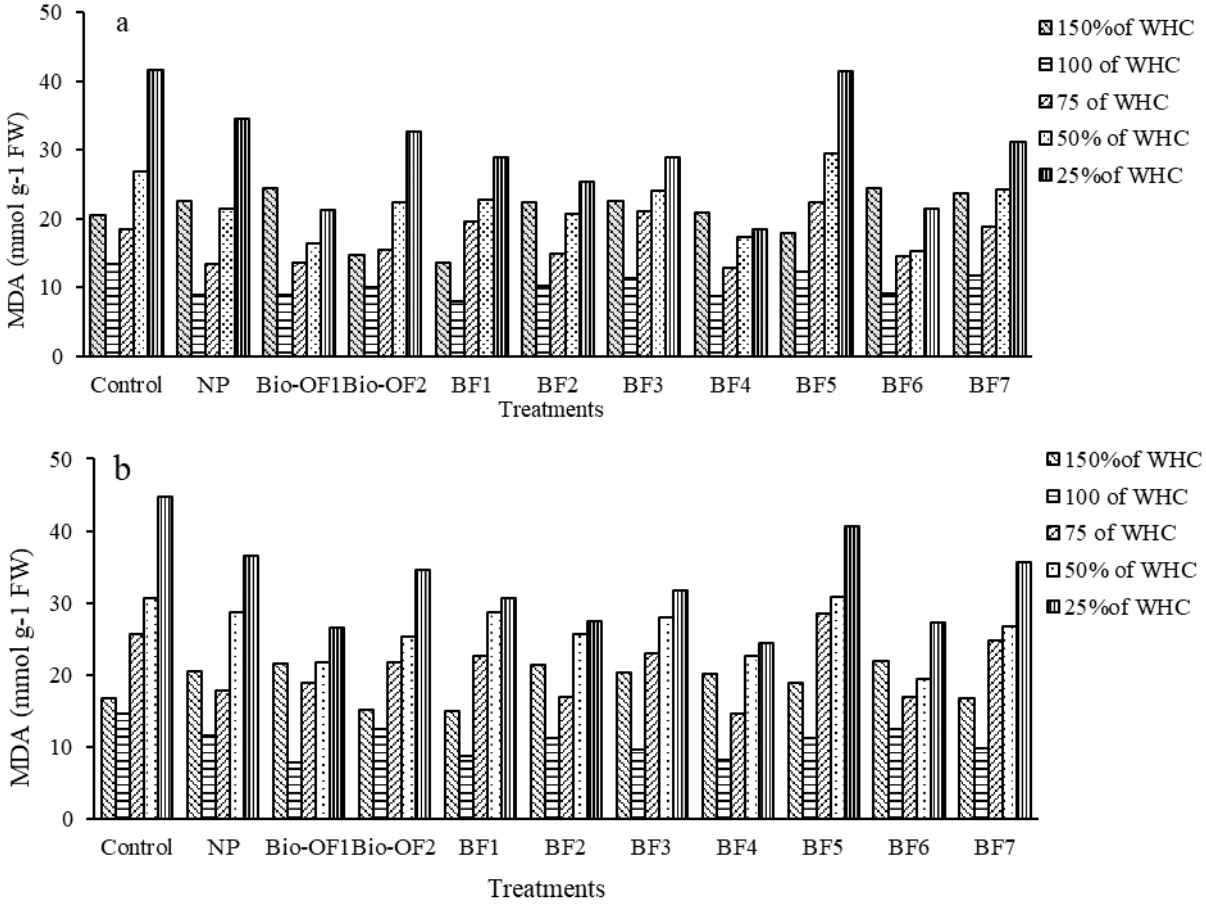


Figure 2. Effect of bacterial formulations and mineral and bio-organic fertilizer on the activities of anti-oxidant (GR and GST) and oxidative pentose phosphate cycle enzymes (G6PD and 6PGD) in sugar beet leaves under different water regimes.



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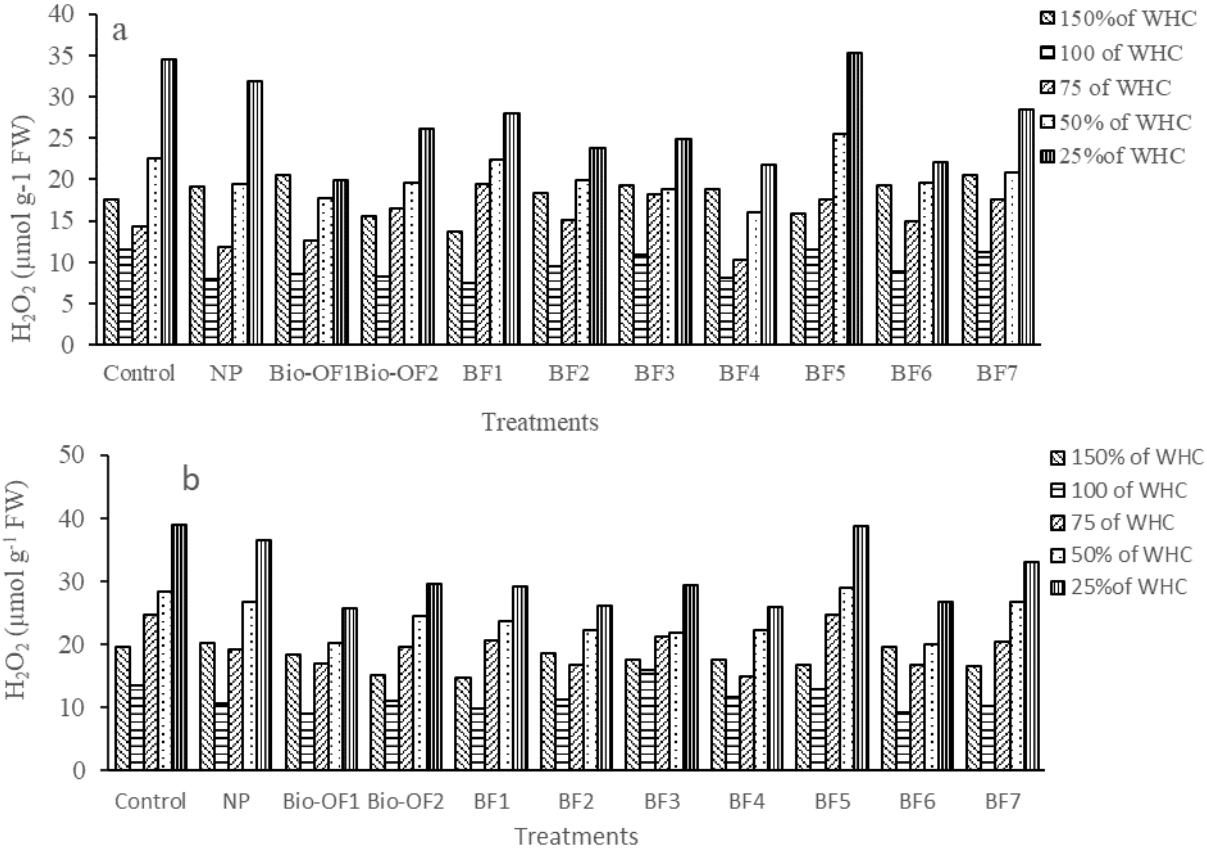

Figure 3. Effect of different water regimes and bacterial formulations and mineral and bio-organic fertilizer on the MDA and H_2O_2 content of sugar beet leaves in the first (a) and second trial (b)

Plant seedlings inoculated with beneficial bacterial strains and exposed to water stress, moreover, showed a better water status than control plants, alleviated drought stress by using alternative mechanisms and higher yields under drought conditions were obtained (Compant et al., 2010). The bacteria also increased total phenolics content (TPC), trolox equivalent antioxidant capacity (TEAC), anti-oxidant enzymes activity (GR, GST, CAT, POD and SOD), and the contents of N, P, K, Ca, Mg, Fe, Mn, Zn and Cu but decreased MDA and H_2O_2 contents which may contribute in part to activation of processes involved in the alleviation of the effect of water stress (Erdogan et al., 2016). The study revealed that screening of bio formulations with multi-traits could be highly effective for improving the growth and nutrient uptake of sugar beet. These beneficial bacterial formulations could play an important role in understanding plant tolerance to stress, adaptation to stress and mechanisms that develop in plants under stress conditions.

Drought caused a significant decrease in leaf weight as well as in root weight. Inoculation with IAA and ACC deaminase-producing bacteria 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 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. The results have shown that by increasing water stress, leaf chlorophyll content decreases leading to less photosynthesis, growth and yield. A large number of studies have suggested that the bacteria that most effectively protect plants against a wide range of different stresses produce both IAA and ACC deaminase (Glick, 2012). Inoculation with IAA-producing bacteria (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. Sugar beet cultivation inoculation with ACC deaminase-containing bacteria can be used to minimize the harmful effects of water stress, eliminated the effects of water stress on growth, and increased the uptake of nutrients (Karagöz et al., 2018).

Conclusions

Under water deficit and waterlogging stress conditions, sugar beet fresh and dry roots and leaf weight reduction were alleviated to a certain extent by using inoculation of bacteria, but it was strongly dependent on the inoculation bacteria and the level of irrigations. While all applications were effective in this study, among the various bio-formulations tested, BF4 (*P. fluorescens* PF8/6 + *P. polymyxa* RC05 + *B. subtilis* RC11 + *B. megaterium* A21/3) and BF6 (*P. putida* RC310 + *P. polymyxa* RC05 + *B. subtilis* RC63 + *B. megaterium* A21/3) were found to be most effective in alleviating the effects of water deficit and waterlogging stress, improving sugar beet growth, increasing yield, quality and enzyme activity. 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. Multi-traits bacterial formulations might also increase nutrient uptake and the antioxidant activities of plants and thereby may alleviate damage induced by abiotic and biotic stresses. 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 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 could induce plant growth and development, reduce stress susceptibility, and may contribute to the concept of biotechnology application in agriculture. Additional field trials are needed to confirm the effects of multi-traits PGPR strains on plant growth, nutrient uptake, enzyme activity, and stress resistance in sugar beet and other plant species under different water deficit and waterlogging stress conditions. There is a need for research focusing on examining and explaining how bacteria and the effective bacterial mechanisms to tolerate stress affect the resistance mechanism of plants against water stress. These studies should also focus on improving the survival of multi character rhizobacteria, their interactions with water and drought-stressed plants, and the ability of PGPR's potential mechanisms to mitigate the effects of drought stress by improving the physiology, growth and yield of crop plants.

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