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The effect of some probiotic bacteria in induction of drought tolerance in cucumber plants

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Abstract

Water shortage is one of the limiting factors of plant productions in arid and semi-arid areas. Many adaptive strategies such as accumulation of osmotic adjustments, phenolic compounds and antioxidant enzymes activity have been extended in plants for dealing with drought stress. The use of microorganisms, including probiotic bacteria, is a type of soil management that is effective in reducing the effects of stress. This experiment aimed to determine the effects of some probiotic bacterial strains on proline, sugar, total phenolic compounds (TPC), *phenylalanine ammonia lyase (PAL), photosynthesis pigments* and antioxidant activities of cucumber plants under drought stress. A completely randomized design was applied with a factorial arrangement of two factors: irrigation levels and bacteria strains with three replications. The results showed that proline, sugar, TPC, *PAL* and enzymes activity in control and inoculated plants were increased by increasing drought stresses. By contrast photosynthesis pigments significantly decreased under stress. The use of bacterial strains alleviate the harmful effect of stresses by an accumulation of proline, TPC, sugar, PAL activity and enzyme activity. The results also showed that inoculated plants had higher antioxidant activity compared to control plants under drought. It was found that the use of probiotic bacteria is an effective strategy to enhanced drought stress tolerance in plants.

1. Introduction

Cucumber (*Cucumis sativus* L.) belongs to the Cucurbitaceae family. It is one of the most popular and widely used crops in the world. (Korkmaz et al., 2007). Cucumbers after tomato, lettuce and onion are the most popular vegetables in the world (FAO, 2014). Reducing the quality and quantity of water resources has reduced cucumber yields (Mao et al., 2003). Over the last century, drought is well-documented problems affecting agricultural production worldwide, in particular in arid and semiarid conditions. Plants exposure to water stress exhibit a decrease in cell division, leaf area, number of leaves, negative effects on absorption of water and nutrient (Li et al., 2009), and inhibition of photosynthesis and reduces transpiration (Woodward and Bennett, 2005). Reactive oxygen species (ROS) increase during drought stress (Sircelj et al., 2007) that can damage macromolecules, including DNA, proteins, lipids and photosynthetic pigment (Blokhina et al., 2003).

Under stress conditions, plants employ some strategies to relieve disturbing stress effects. One of the strategies is an aggregation of low-

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molecular-mass organic solutes such as soluble sugars, proline, glycine-betaine, polyols and other compounds that lead to increase in the osmotic potential and preservation of cellular turgor (Bates et al., 1973). High antioxidant enzymes activity such as catalases (CAT), superoxide dismutase (SOD), peroxidases (POD), ascorbate peroxidases (APX), glutathione reductase (GR) and non-enzymatic compounds like ascorbic acid (AA), glutathione scavenge reactive oxygen species (Farooq et al., 2009).

Synthesis, accumulation and transportation some phytohormones like abscisic acid (ABA), ethylene and salicylic acid can act as signaling factors under stress condition that induces expression of several genes involved in defense against drought stress (Farooq et al., 2009).

The use of probiotic bacteria can be considered a potential strategy to promoting plant growth under drought stress. These bacteria can enhance plant growth under stress condition by several mechanisms such as facilitating uptake of insoluble elements and essential minerals, nitrogen fixation, phosphate solubilization, siderophore production, phytohormone production, antifungal metabolite production like HCN, phenazines, pyrrolnitrin, 2,4-diacetylphloroglucinol (Glick, 2012) and induction of drought tolerant in plants by stimulating the expression of genes involved in abiotic stress tolerance (yang et al., 2008). The drought-response genes, ERD15 and RAB18 was stimulated in *Arabidopsis thaliana* by strain *Paenibacillus polymyxa* (Timmusk and Wagner, 1999).

Azotobacter, A*zospirillum*, *Bacillus* and *Pseudomonas* are the most important probiotic bacteria. The useful effects of these bacteria have been previously reported in different crop plants (Khalid et al., 2004; Cakmakci et al., 2007; Kidoglu et al., 2008). Abdul Jaleel et al. (2007) reported that the use of *Pseudomonas fluorescens* as native probiotic bacteria enhances biomass and alkaloid content in *Catharanthus roseus* under drought condition. The probiotic bacteria improved drought tolerance in foxtail millet plants by increasing ACC deaminase that inhibited ethylene synthesis (Niu et al., 2018). On the other hand, probiotic bacteria induce drought tolerance by osmolyte accumulation, activation of antioxidant system, regulation of stress-responsive genes and root morphology alterations (Vurukonda et al., 2016).

The purpose of this study was to evaluate the impact of some strains of *Pseudomonas fluorescens* and *Bacillus subtilis* on photosynthesis pigments, osmoregulators and antioxidant enzymes activity of cucumber plants under drought stress.

2.Materials and methods

2.1. Drought tolerance

In order to evaluation drought tolerance of bacterial strains under *in vitro* culture, difference PEG-6000 concentration (0, 203.36, 298.53, 438.40 and 548.83 g) were added to per 1 kg NB medium. After 72 hour bacterial growth was measured by optical density in 600 nm (Michel and Kaufman, 1973).

2.2. Plant material

Cucumber seeds (*Cucumis sativus* L.) were disinfected for 5 min in a 5% sodium hypochlorite (NaClO) solution and then were washed twice with sterile water. The seeds were placed on moist filter paper for germination. Four germinated seeds were sown in a plastic pot containing sandy loam soils, pH 7.6 and EC 1.2 $ds.m^{-1}$. Seedlings were grown under greenhouse conditions with a temperature of 25-27 ºC. Twenty days after planting, drought treatments were applied as completely randomize design based on factorial.

2.3. Drought stress

In order to evaluate the effects of bacterial strains on cucumber plant under drought stress an experiment was arranged as a factorial in the framework of completely randomized design with two factors drought (irrigation at 70 and 30% field capacity) and *Pseudomonas fluorescens* strains (VUPF5, CHA0, T17-4) and *Bacillus subtilis* strains (Bs96, BsVRU, BsVRU1) with three replications. Drought stress application conducted according to Rad et al. (2012) method with 2 levels, irrigating at 70% of field capacity (control) and irrigating at 30% of field capacity (moderate stress) for 25 days. The fresh sample was taken after 25 days for the biochemical analysis.

2.4. Inoculation of bacterial inoculum

P. fluorescens strains (VUPF5, CHA0, T17-4) and *B. subtilis* strains (Bs96, BsVRU, BsVRU1) were retrieved from the Collection of Plant Protection Group of Vali-e-Asr University of Rafsanjan, Iran (these strains were selected based on previous studies (Lagziana et al. 2013; Baradar et al. 2015). The bacterial suspensions were diluted in distilled water and the concentration adjusted to 1×10^{10} CFU/ml (OD 0.5 at 540 nm = 10^{10}) using a spectrophotometer (*U-2000, Hitachi Instruments*, Tokyo, Japan). Ten ml of these suspensions were added to each pot and used for soil drenching.

2.5. Total chlorophyll

Chlorophyll a,b and total chlorophyll was assayed as described by Lichtenthaler (1987). The leaves (0.25 g) were homogenized with 10 ml of 80% acetone. The absorbance of samples was determined at 470, 646 and 663 nm in a spectrophotometer (*U-2000, Hitachi Instruments*, Tokyo, Japan).

2.6. Proline and soluble sugar content

To determine proline concentration, 0.5 g fresh leaf samples were homogenized in 5 ml of 95% ethanol. The sediments were washed two times by 5 ml of 70% ethanol, then the extracted samples was centrifuged at 3500 g for 10 min at 4 ºC and the supernatant was regained and kept at 4 ºC.

One ml of alcohol soluble extract was diluted with 10 ml of distilled water and after adding 5ml of ninhydrin (ninhydrin, phosphoric acid 6 M and glacial acetic acid at 99%) and 5 ml of glacial acetic acid added then was heated in a bath for 45 min at 100 ºC. After cooling, the samples were mixed with 10 ml benzene and absorbance was determined at 515 nm. The proline content was determined from aprolin standard curve and calculated as $\mu g.g^{-1}$ of leaves (Paquin and Lechasseur, 1979).

To determine the content of soluble sugars, 0.1 ml of alcoholic extract was mixed with 3 ml antron (150 mg antron plus 100 ml of 72% sulphuric acid). The tubes were heated in a bath at 100 ºC for 10 min. After cooling, the absorption was measured at 625 nm. The soluble sugars concentration was determined from a standard curve by preparing solutions of soluble sugars and calculated as $mg.g^{-1}$ leaves (Irigoyen et al., 1992).

2.7. Enzyme extraction and activity determination

Fresh leaves (0.5g) were homogenized with potassium phosphate buffer pH 7.2 (50 mM) containing 1 mM EDTA and 1% (w/v) soluble PVP and centrifuged at 20000 g, 20 min at 4 °C. The supernatant was used for enzyme assays.

2-7-1. Total Soluble Proteins

Determination of protein concentration was done using Bradford protein assay (1976). 0.1 ml enzyme extract was added to 5 ml of Bradford reagent, then was mixed by vortexing. The absorbance at 595 nm was measured after 5 min. Bovine Serum Albumin was chosen for the protein standard curves.

2-7-2. Guaiacol peroxidase (GPX) (EC1.11.1.7)

The guaiacol peroxidase activity was determined as described by Plewa et al. (1999). The reaction mixture included 2.77 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.1 ml of 1% hydrogen peroxide, 0.1 ml 4% guaiacol and 30 µl enzyme extract. The increase in absorbance due to formation of tetraguaiacol by measuring the absorbance at 470 nm and using an extinction coefficient of 25.5 mM^{-1} cm^{-1}.

2-7-3. Polyphenol oxidase (PPO) (EC 1.14.18.1)

The PPO activity was determined by the method of Nicoli et al. (1991) with some modifications. The assay mixture contained 2.5 ml of 50 mM potassium phosphate buffer at pH 7.0, 200 µL of 0.02 M pyrogallol and 0.1 ml of enzyme. The activity was expressed as a change in absorbance at 420 nm. The enzyme activity-change was expressed in absorbance min^{-1} mg^{-1} of protein.

2-7-4. Phenylalanine ammonia-lyase (PAL) (EC 4.3.1.5)

Phenylalanine ammonia lyase activity was done by following method reported by D´cunha et al. (1996). The reaction mixture (1 ml of the extraction buffer, 0.5 ml of 10 mM L-phenylalanine, 0.4 ml of deionized water and 0.1ml of enzyme extract) were incubated at 37 °C for 1h, then stopped after by the addition of 0.5ml of 6M HCl. The absorbance was measured at 290 nm and the enzyme activity was expressed as 1µmol cinnamic acid produced in 1 min.

2-7-5. Superoxide dismutase (SOD) (EC 1.15.1.1)

SOD activity was assayed by monitoring the inhibition of nitroblue tetrazolioum (NBT) reduction at 560 nm, according to Giannopolitis and Ries (1997). The reaction mixture contained 50 mM phosphate buffer (pH 7.5), 1.5 mM carbonate sodium, 3 mM EDTA, 60 mM riboflavin, 200 mM L-methionine, 2.25 mM NBT and 50 µL enzyme extract. The reaction mixture was placed below fluorescent lamps (30W) for 10 min and then by switching off the light reaction was stopped.

2-7-6. Total phenolic compounds

The total phenolic content was carried out using the Folin-Ciocalteau method (Roland and Laima, 1999). Five hundred grams of the fresh leaves of pistachio seedlings were ground in 5 ml of 95% ethanol and was kept in dark for 48 h. Then 0.5 ml ethanol was added to the 0.5 ml supernatant and topped to 2.5 ml with distillation water. 0.25 ml of 50% folin reagent and 0.5 ml of 5% carbonate sodium were added to samples and color clenched to black. The samples were placed in the dark for an hour and measured at 725 nm. Gallic acid was used for constructing the standard curve (gallic acid (GA) per milligram of the fresh weight).

2-7-7. Experimental design

In order to evaluated the effects of probiotic bacterial strains on cucumber plants under drought stress the experiments were arranged as a factorial in the framework of completely randomized design with two factors: drought (irrigation at 70 and 30% field capacity) and *Pseudomonas fluorescens* strains (VUPF5, CHA0, T17-4) and *Bacillus subtilis* strains (Bs96, BsVRU, BsVRU1) was done at three replications. The data were analyzed by analysis of variance (ANOVA) with Duncan's multiple range test ($P<0.05$) using the SAS program, SAS Institute, Cary, NC, USA.

3. Results and discussion

3.1. Drought strains tolerance

Results showed that all strains had good performance under drought stress. Induced

stress by PEG decreased growth in all strains than control strains. The minimum and maximum reduction of growth strains were observed in VUPF5 and CHA0 (9.55%) and VRU1 (50.6%), respectively (Figure.1).

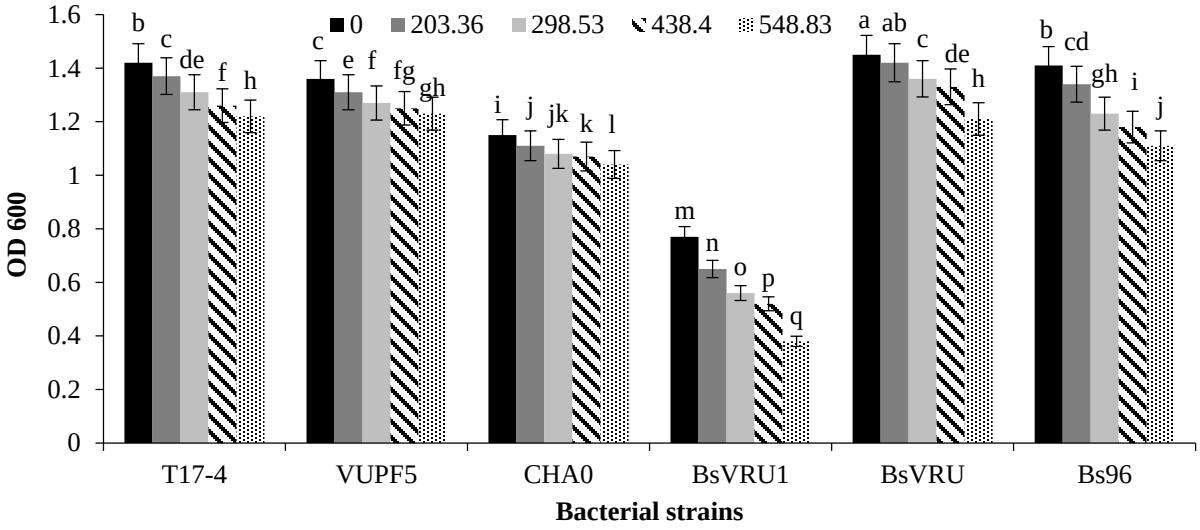

Figure. 1 Evaluation of bacterial strains tolerance to drought induced by PEG. Bar indicate standard error. Columns with different letters are significantly different at $P \geq 0.05$.

Abiotic stresses lead to accumulation of free radicals due to changes in the conformational protein, restricted enzyme and electron transport efficiency (Ngumbi and Kloepper, 2016). Free radicals can damage to biological molecules such as nucleic acids, proteins and lipids, thereby leading to peroxidation of lipids, denaturation of protein and ultimately disturb cell function (Potts, 1999), also abiotic stresses causes changes membrane fatty acids composition of microbial cells (Conlin and Nelson, 2007). Under stress condition soil bacteria employ different physiological mechanisms for protect cell structures and organelles such as production of compatible solutes (osmolytes), exopolysaccharide production and spore formation. Synthesis of osmolytes such as proline, glycine betaine and trehalose increases drough tolerance by inhibiting of proteins denaturation, protecting of the structures of macromolcules and contributing to membrane integrity (Conlin and Nelson 2007).

3.2. Proline and Sugar content

As shown in table 1, proline content was significantly affected by interaction of drought, bacterial strains ($P \leq 0.05$). Leaf proline content was significantly increased by increasing drought intensity in control plant and inoculated plants with different bacterial strains. The maximum and minimum increase of proline content was observed in T17-4 (27.49%) and VUPF5 (6.58%), respectively (Fig. 2). The results of ANOVA showed significant effect of interaction water deficit and bacterial strains on leaf Sugar content ($P \leq 0.01$) (Table 1).

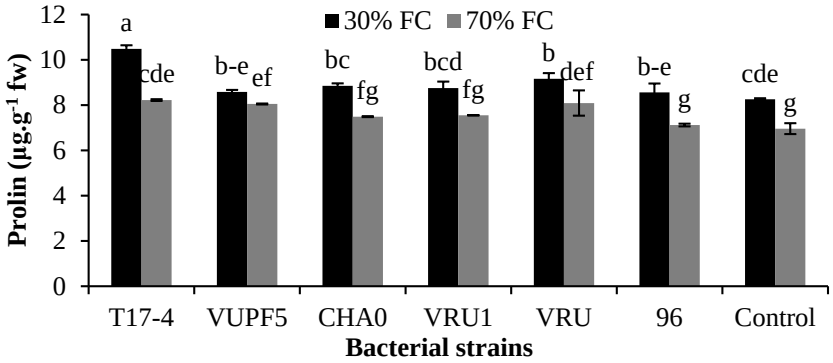

Fig. 2 Effect of bacterial strains on leaf proline content of cucumber plants under drought stress. Bar indicate standard error. Columns with different letters are significantly different at $P \geq 0.05$.

Table 1 ANOVA results of the effect of bacterial strains on proline, sugar, chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (TChl) content of cucumber plants under drought stress

Source of variations	df	Mean squares				
		Prolin	Sugar	Chla	Chlb	TChl
Bacterial strains (B)	6	3.09**	92.08**	0.04**	0.01**	0.10**
Drought stress (D)	1	10.72**	747.13**	0.13**	0.03**	0.34**
B × D	6	0.43*	0.21**	0.003*	0.01**	0.02**
Error		0.16	9.41	0.001	0.0003	0.005
CV%		4.82	6.22	12.97	15.22	19.20

** – significant (P≤0.01), *-significant (P≤0.05)

Leaf Sugar content in control and inoculated plants was significantly increased under drought stress. The maximum and minimum increase of leaf sugar content was recorded in control (13.06 fold) and inoculated plant with BsVRU1 strain (3.29 fold) respectively (Fig. 3).

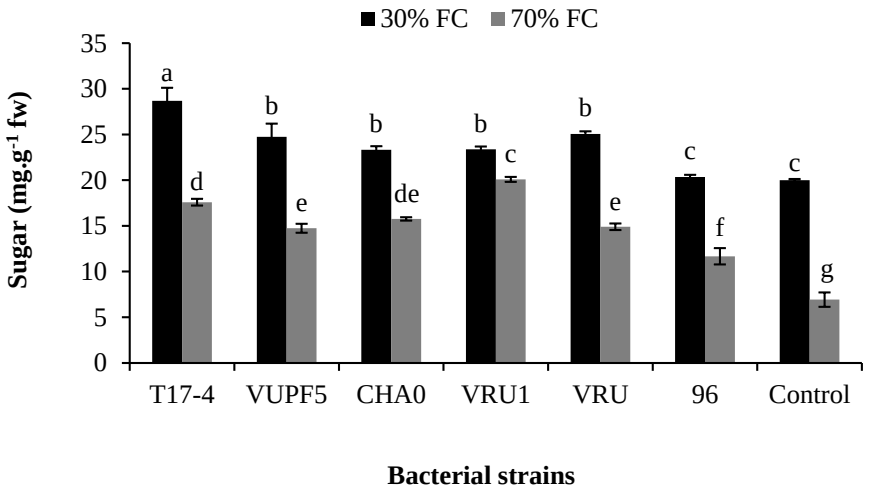

Fig. 3 Effect of bacterial strains on leaf sugar content of cucumber plants under drought stress. Bar indicate standard error. Columns with different letters are significantly different at $P \geq 0.05$.

Plants have evolved several strategies under drought stress such as compatible solute accumulation and antioxidant regulation. Osmoregulators accumulation is a main strategy for osmoprotection and osmotic adjustment under drought stress. In the present

study, we evaluated the effects of probiotic bacteria on the osmolytes and activity of antioxidant enzymes on cucumber plants under drought stress condition. The results indicated that decreasing soil filed capacity induced accumulations of proline and soluble sugar. Inoculated plants with different bacterial strains improved proline and soluble sugars accumulation in leaf cucumber plants during drought stress as compared to non-inoculated plants. Osmolytes secreted by these bacteria can synergistically with osmolytes produced by plants increase the levels of osmolyte accumulation in plant cells (Paul et al., 2008). Inoculation of plant with probiotic bacteria caused up-regulation of prolin biosynthesis pathway and increases proline content in plant hence maintaining cell water potential and turgor and protects cells against oxidative damage induced by ROS under drought stress (Sandhya et al., 2010). Accumulation of osmolytes is highly correlated with stress tolerance. On the other hand, accumulation of osmoregulators under stress conditions protects cell membranes against reactive oxygen species (Szabados and Savouré, 2010). Armanda et al. (2014) reported that PGPR *B. thurigiensis* inoculation enhanced the level of proline accumulation in shoot compared to control plants under drought stress. Tomato plant that inoculated with *Bacillus polymyxa*, known as phosphate solubilization bacteria, had more proline content than control plants (Shintu and Jayaram, 2015). Naz and Bano (2015) also reported that the use of some PGPRs genus like Azospirillum and Pseudomonas on sunflower plants improved salinity stress by more accumulation proline and soluble sugar compared to control plants. Which these results correspond with our results.

3.3. Photosynthesis pigments

According to ANOVA results interaction of drought stress and bacterial strains have significantly affected on TChl, Chl a and Chl b ($P \leq 0.01$, $P \leq 0.05$) (Table 1). Chl a significantly decreased in inoculated and control cucumber plants under drought stress conditions. Means comparison showed that the highest reduction in Chl a content in cucumber plants was observed in control plants by 3.85 fold and the lowest reduction was observed in inoculated plants with T17-4 strain by 1.3 fold comparison to control plants (Figure. 4).

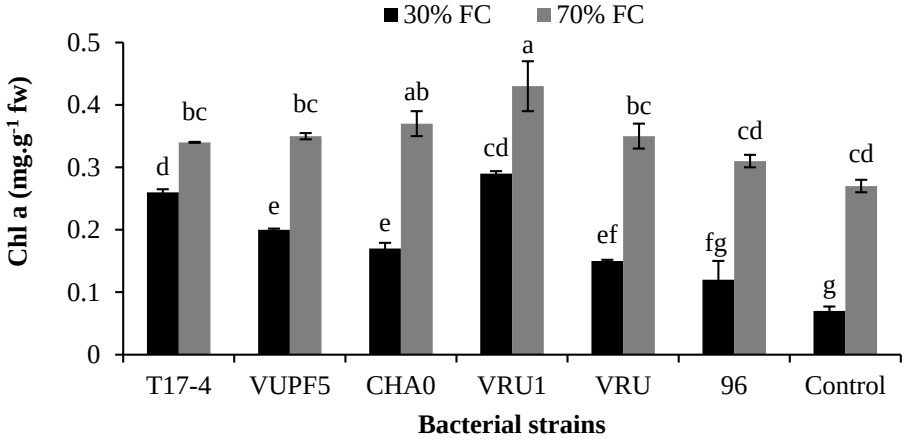

Figure 4. Effect of bacterial strains on leaf chlorophyll a content of cucumber plants under drought stress. Bar indicate standard error. Columns with different letters are significantly different at $P \geq 0.05$.

Drought significantly changed the concentration of Chl b in the leaf of control and inoculated plants. Chl b content decreased by drought stress in the plant (Figure. 5).

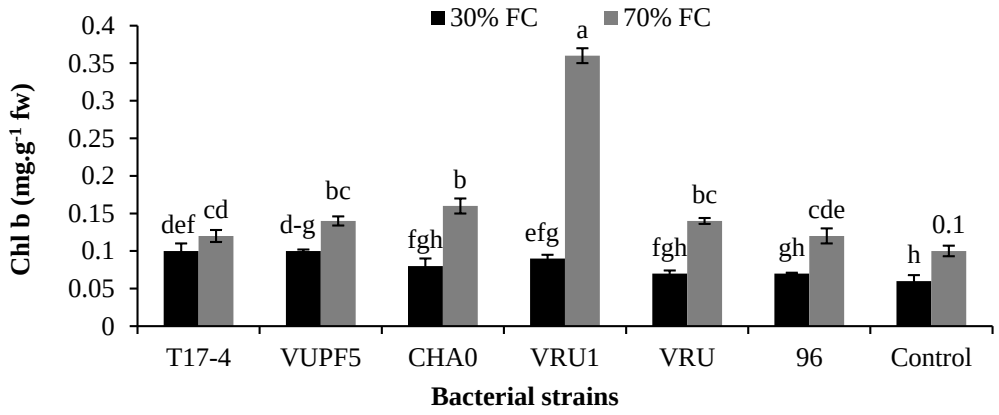

Figure. 5 Effect of bacterial strains on leaf chlorophyll b content of cucumber plants under drought stress. Bar indicate standard error. Columns with different letters are significantly different at $P \geq 0.05$.

The maximum decrease in Chl b content due to drought stress was observed in BsVRU1 strain fallowed by CHA0> BsVRU> Bs96> control> VUPF5> TI7-4 (Figure. 5). Based on results, TChl in inoculated and control cucumber plants were significantly decreased under drought stress. The highest and the lowest reduction of TChl were recorded in BsVRU by 2.63 fold and T17-4 inoculated plants by 1.29 fold comparison to control (Figure. 6).

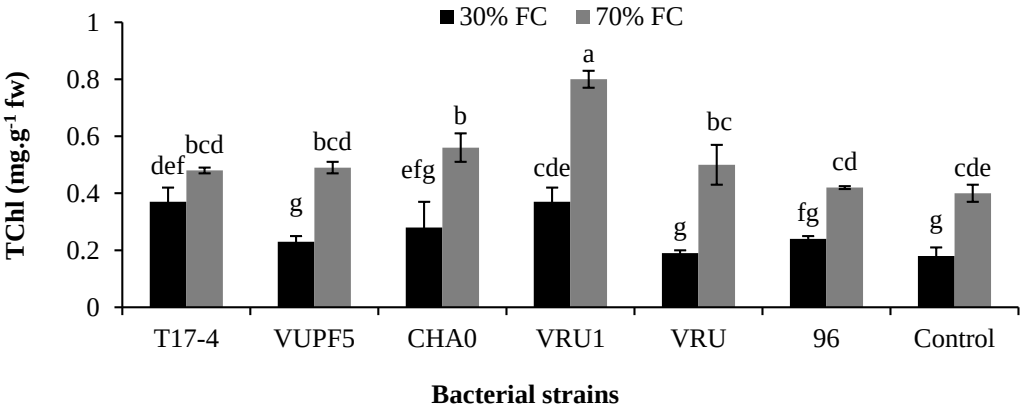

Figure. 6 Effect of bacterial strains on leaf total chlorophyll content of cucumber plants under drought stress. Bar indicate standard error. Columns with different letters are significantly different at $P \geq 0.05$.

Reduction in chlorophyll and other photosynthetic pigments content under drought stress might be due to the chloroplast destruction or change in thylakoid membrane structure, the loss of specific enzyme that plays an important role in biosynthesis of photosynthesis pigments and greater activity of chlorophyllase in stress condition (Tian et al., 2013; Gharsallah et al., 2016). Bacterial strains or PGPRs by improving water potential and increasing water uptake decreased the effect of drought. Therefore improving water potential under drought stress decreased chlorophyllase activity (Ebrahimi et al. 2016).

Rong et al. (2014) reported that PGPR promoted wheat growth under drought stress by increasing proline and Chl content. Leaf photosynthesis pigments of Avena sativa were significantly higher in inoculated plant than control plant under drought stress (Delshadi et al. 2017).

3.4. Total phenolic compounds

According to ANOVA results (Table 2), total phenolic compounds were significantly affected by interactions of drought and bacterial strains ($P \leq 0.01$).

Table 2. ANOVA results of the effect of bacterial strains on total phenolic compounds, *phenylalanine ammonia lyase (PAL)*, poly phenol oxidase (PPO), super oxide dismutase (SOD) and glycol peroxidase (GPX) under drought stress.

Source of variations	df	Mean squares				
		Total phenolic compounds	PPO	PAL	SOD	GPX
Bacterial strains (B)	6	0.54^{**}	0.41^{**}	0.83^{**}	748.93^{**}	1.62^{**}
Drought stress (D)	1	1.24^{**}	0.60^{**}	2.08^{**}	896.63^{**}	2.69^{**}
B × D	6	0.28^{**}	0.13^{**}	0.14^{*}	81.42^{**}	0.15^{**}
Error		0.02	0.03	0.05	8.82	0.03
CV%		17.76	13.91	9.88	3.60	18.51

Total phenolic compounds were significantly increased under drought stress. The most and the least increase of total phenolic compounds were observed in VUPF5 and T17-4 by 3 and 2 fold, respectively under drought stress than control (Figure. 7).

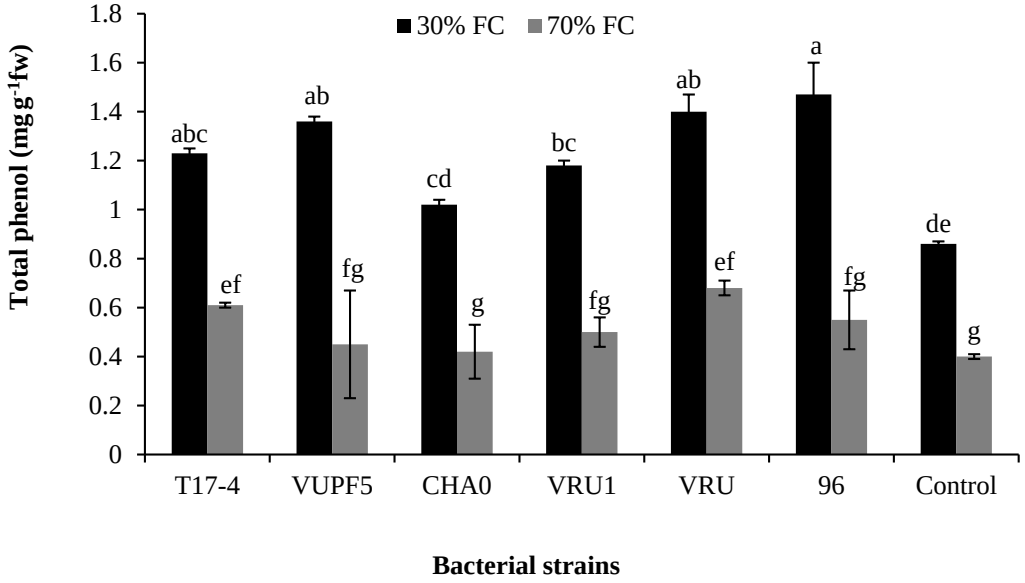

Figure 7. Effect of bacterial strains on total phenolic compounds of cucumber plants under drought stress. Bar indicate standard error. Columns with different letters are significantly different at $P \geq 0.05$.

Phenolic compounds i.e. flavonoids, phenylpropanoids and lignin are large groups of secondary metabolites, which synthesize in different organs of plants under stress condition

(Mechri et al., 2015). Phenolic compounds increase plants resistance under drought stress condition by reducing the ROS formation (Gnanasekaran and Kalavathy, 2017). Erdogan et al. (2016) who reported that use of multi-trait bacteria increased leaf TPC of strawberry under drought stress. Accumulation of TPC in the inoculated plant may be due to the effect of PGPR on increasing PAL activity. These results are in agreement with our finding on cucumber plant growth under drought stress. Increased antioxidant activities and phenolic compounds in cucumber plants help to increase drought tolerance by protecting from oxidative stress.

3.5. PAL activity

ANOVA indicated significant effect of interactions drought and bacterial strains on PAL activity (P≤0.05) (Table 2). PAL activity in control and inoculated plants was enhanced under drought stress. The maximum increase in PAL activity was recorded in VUPF5 by 1.5 fold and the minimum increase was observed in the plants inoculated with T17-4 strain by 1 fold in comparison to control (Figure. 8).

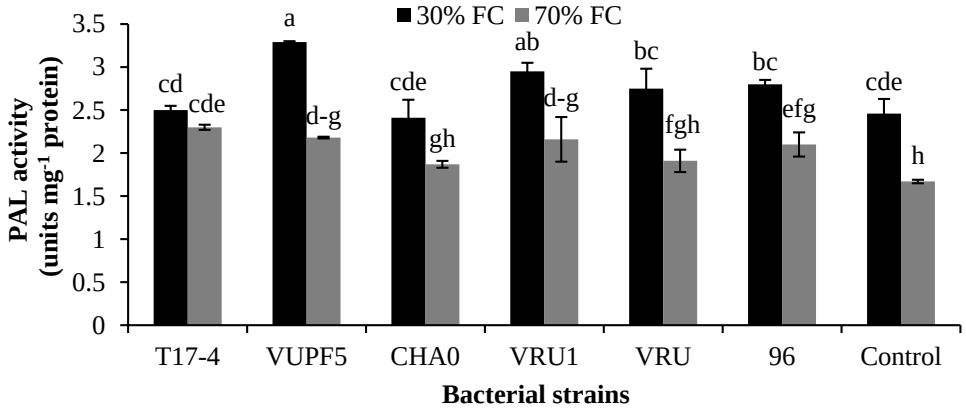

Figure. 8 Effect of bacterial strains on PAL activity of cucumber plants under drought stress. Bar indicate standard error. Columns with different letters are significantly different at P ≥ 0.05.

Stress regulates activity of PAL enzyme, which plays a main role in the first and pivotal steps of the phenyl propanoid pathway. Phenyl propanoid pathway is the pathway of phenols biosynthesis (Tovar et al., 2002). There is a significant correlation between phenylalanine ammonia-lyase activity and soluble phenolic content in roots (Caliskan et al., 2017). Therefore high PAL activity causes more phenolic compound synthesis. Accumulation of TPC in inoculated plant may be due to the effect of PGPR on increasing PAL activity. Basha et al. (2006) reported that use of PGPR on chickpea plant induced synthesis of PAL activity under stress condition.

3.6. Antioxidant activity

In our study, based on ANOVA results, SOD, PPO and GPX were influenced by interaction of drought and bacterial strains (P≤0.05) (Table 2). As shown in figure 8, SOD activity in control plant and inoculated plants were significantly induced by increasing drought stress. The increment in SOD activity due to drought stress was generally higher in the control plant fallowed by CHA0> T17-4> BsVRU1> Bs96> VUPF5> BsVRU (Fig. 9).

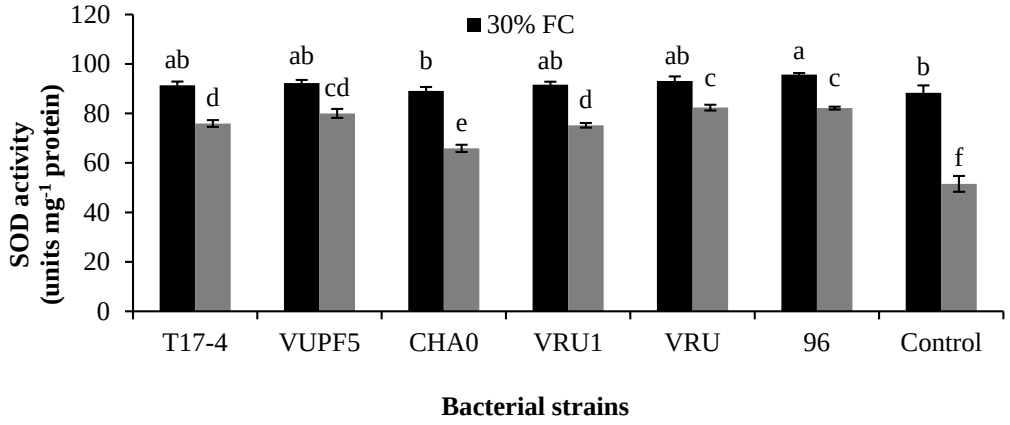

Figure 9. Effect of bacterial strains on SOD activity of cucumber plants under drought stress. Bar indicate standard error. Columns with different letters are significantly different at $P \geq 0.05$.

PPO activity was also increased under drought condition. The maximum and minimum increase of PPO activity was recorded in control and BsVRU1 inoculated plants by 78.66% and 72.56%, respectively (Fig. 10). With increasing drought severity, the GPX activity increased considerably. The maximum increase in GPX activity was observed in control plants by 6.6 fold and the minimum increase was recorded in the plants inoculated with 96 strain by 1.75 fold in comparison to control (Fig. 11). Another response of drought-stressed plants is reactive oxygen species (ROS) production. Cucumber plants inoculated with bacterial strains significantly had higher activity of antioxidant enzymes and phenolic compounds as compared to un-inoculated plants. These bacterial strains can induced stress related enzymes (SOD, GPX, and PPO) in cucumber plants. In plants, fast removal of hydrogen peroxidase is critical for cell function; otherwise, hydrogen peroxidase can diffuse across membrane to react with singlet oxygen resulting in destructive hydroxyl radicals production (Sanchez-Rodrıguez et al., 2012).

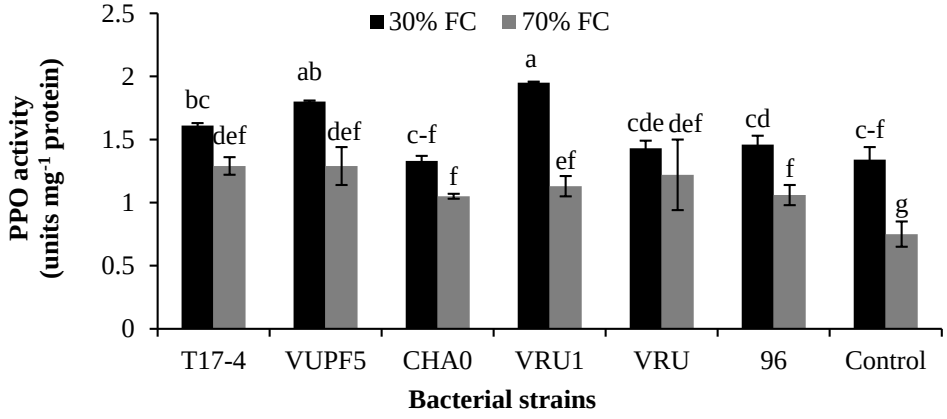

Figure 10. Effect of bacterial strains on PPO activity of cucumber plants under drought stress. Bar indicate standard error. Columns with different letters are significantly different at $P \geq 0.05$.

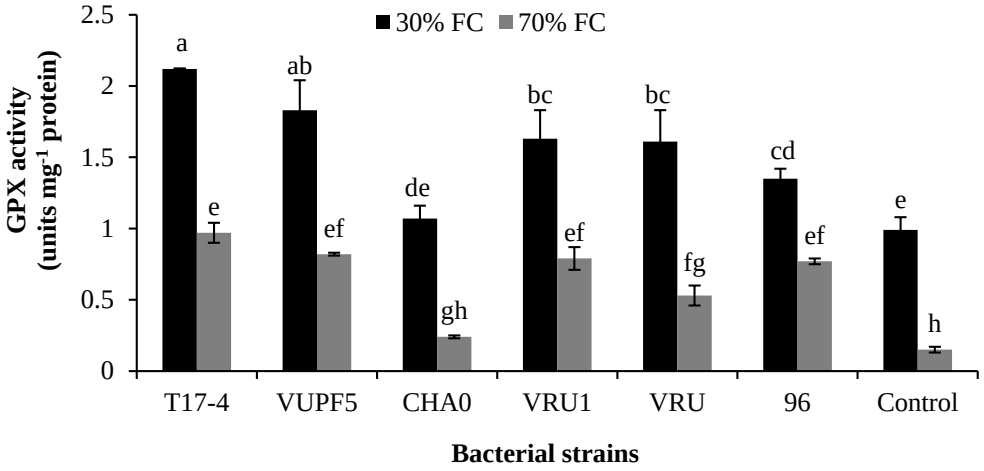

Figure. 11 Effect of bacterial strains on GPX activity of cucumber plants under drought stress. Bar indicate standard error. Columns with different letters are significantly different at $P \geq 0.05$.

Enzymatic antioxidants scavenge reactive oxygen species and reduce the oxidative damage under environmental stress (Miller et al., 2010). Peroxidase can decompose hydrogen peroxidase to H2O and O2. It uses hydrogen peroxidase as an electron donor to metabolize phenolic compounds (Caverzan et al., 2012). Kumar et al. (2016) showed that use of PGPR induced antioxidant activity in chickpea plant compared to control plants. Li and Jiang (2017) indicated that inoculation of maize plant with *B. aquimaris* DY-3 reduced the salt stress by activating of the antioxidant enzymes and the non-antioxidant systems that increase the plant tolerance. Generally, difference between strains on proline, sugar, TPC, and antioxidant activity may be due to following reasons; (i) production of phytohormones like abscisic acid (ABA), gibberellic acid, cytokinins, and indole-3-acetic acid (IAA); (ii) lowering ethylene levels by ACC deaminase production; (iii) The bacterial release compounds induced systemic tolerance; (iv) Exopolysaccharides (EPS) production by bacteria, which change physiological responses.

4. Conclusion

Our results showed that probiotic application could alleviate drought stress effect by increasing in proline, sugar, TPC, PAL and antioxidant activity and chlorophyll content. Probiotic bacteria have a critical role in induction of tolerance and adaptation of plants to environmental stresses. These results suggest that when cucumber roots were treated with probiotic bacteria, they can stimulate plant defense system and alleviate the harmful effect of drought stress by increasing osomoregulators and antioxidant enzymes in plants.

Conflict of interest

The authors declare no conflict of interest.

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