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Growth behavior of tomato (*Solanum lycopersicum* L.) under drought stress in the presence of silicon and plant growth promoting rhizobacteria

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Abstract

Among abiotic stresses, drought is considered as the most important growth limiting factor, particularly in arid and semiarid regions. Plant tolerance to drought is mainly associated with the maintenance of plant water status either by reducing water loss through decreasing transpiration or improving plant root capacity to extract more water through osmotic adjustment. In this regard, adequate regulation of plant nutrients may help to maintain or even improve plant water status and hence plant tolerance to drought stress. In the present study, silicon (Si) and plant growth promoting rhizobacteria (PGPR) containing ACC-deaminase activity were evaluated as a tool to improve drought tolerance of tomato (Solanum lycopersicum L.) grown in pots under natural environmental conditions. Nine treatments including, control (irrigation at 60% of field capacity); drought-1 (irrigation at 45% of field capacity); drought-2 (irrigation at 35% of field capacity); drought-1+50 ppm Si; drought-2+50 ppm Si; drought-1+PGPR; drought-2+PGPR; drought-1+50 ppm Si+PGPR; drought-2+50 ppm Si+PGPR were arranged in completely randomized design with 5 replications. Results revealed that both levels of drought stress caused a significant ($P \leq 0.05$) reduction in plant growth and yield, K^+, Ca^{2+} and Mg^{2+} accumulation as well as relative water content (RWC) while increase in Na^+ concentration and electrolyte leakage. However, supplementation of Si and PGPR inoculation increased plant K^+ accumulation, RWC while reduced Na^+ uptake and electrolyte leakage, and subsequently improved fruit yield by 18.34% with Si, 22.80% with PGPR and 30.44% with Si+PGPR at drought-1 while 31.13% with Si, 35.32% with PGPR and 42.36% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment. Ameliorative effects of Si or/and PGPR were mostly more pronounced at drought-2 compared to drought-1. Furthermore, Si and PGPR were more effective to alleviate drought stress effects when applied in combination compared to their individual application. Silicon and PGPR-induced increase in K^+, Ca^{2+} and Mg^{2+} accumulation, and RWC while decrease in electrolyte leakage were the main factors associated with drought tolerance of tomato.

Key words: Drought, electrolyte leakage, PGPR, proline, relative water content, silicon

Introduction

Tomato (*Solanum lycopersicum* L.) is a popular and nutritive vegetable crop ranking next to potato in world's vegetable production. Tomato is an important source of minerals and antioxidants such as carotenoids, vitamins C, E and phenolic compounds, which have a key role in human nutrition to prevent certain cancer and cardio vascular diseases (Adalid *et al*., 2004). Tomatoes are consumed in a number of ways including sun-dried tomatoes, tomato sauce, tomato juice, tomato soup, tomato ketchup and fresh as salad (Frusciante *et al*., 2007). Currently, tomato is grown on area of 68 thousand hectares with the production of 542 thousand tones in Pakistan (GOP, 2014). However, tomato growth and yield were severely threatened by various biotic and abiotic factors in the recent years.

Drought is considered one of the most important environmental factors that affect plant growth and yield in many parts of the world, particularly in arid and semi-arid regions. Approximately, one-third of the world land area is prone to drought stress which poses severe threat to plant growth and food security (Yang *et al*., 2010). Drought induces various changes in morphological, metabolic, or/and physiological functions of plant. At the initial phase of plant growth and establishment, it negatively affects seed germination, stem elongation and expansion (Yordanov *et al*., 2003). Reduced leaf growth and in turn the leaf areas caused a marked reduction in photosynthetic rate which subsequently decreased plant growth and yield (Baligar *et al*., 2001). Jaleel *et al*. (2009) demonstrated that severe water stress produced deleterious effects on plant water relations, photosynthesis, ion uptake, and nutrient metabolism as well as assimilates partitioning. Taiz and Zeiger (2006) reported

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that drought stress can damage plant cell membranes, and cell wall architecture, as well as inhibit photosynthesis and cell division. Marschner (1995) found that decreasing water availability under drought stress conditions generally resulted in reduced total mineral uptake and frequently reduced the concentration of mineral nutrients in crop plants.

Amelioration of injurious effects of abiotic stresses particularly drought and salinity could be achieved through different approaches including breeding, and biological approaches as well as adequate and balanced supply of mineral nutrients. According to Sakamoto and Murata (2002), plants have adopted different morphological and physiological mechanisms to protect themselves against adverse environmental factors. For instance, certain inorganic ions such as K^+ and Ca^{2+} (Munns and Tester, 2008) and organic osmolytes like proline (Taiz and Zeiger, 2010) can help to regulate different metabolic processes and subsequently improve plant growth under drought stress. Proline found to protect macromolecules such as protein against denaturation, stabilizes cell membrane structure by interacting with phospholipids and acts as a hydroxyl radical scavenger under different abiotic stresses (Claussen, 2005). Likewise, inorganic ions, particularly potassium (K^+) and calcium (Ca^{2+}) may also involve in turgor maintenance through osmotic adjustment under drought stress conditions.

Silicon (Si) is the second most abundant element existing in the Earth's crust (Epstein, 1999). Although, it is not considered as an essential element for plant growth and development, however, many studies have suggested its beneficial effects on plant growth and yield in stress environment (Gong *et al*., 2005; Ashraf *et al*., 2010). Under drought stress conditions, Si acts not only as a physical or mechanical barrier to minimize transpiration losses but also participates in many metabolic and physiological processes which subsequently improve drought tolerance (Hattori *et al*., 2005). Agarie *et al*. (1998) found that deposition of Si in the cell wall reduced transpiration and increased internal storage of water under drought stress. Moreover, Si is precipitated as $SiO_2.nH_2O$ in cell wall and cell lumens of plant tissues which could help to maintain plant water status and protect tissues from the damaging effects of drought stress (Gong *et al*., 2005).

Microbial activities in the rhizosphere may also help in the uptake of water and hence improve plant's ability to survive under drought stress. Plant-growth-promoting rhizobacteria (PGPR) may help to improve root growth and activities that results in improved plant water status and nutrient uptake under drought stress. Different PGPR including associative bacteria such as *Azospirillum, Bacillus, Pseudomonas and Enterobacter* have been used under stress conditions for their beneficial effects on plant growth and metabolism (Kohler *et al*., 2007).

Past studies mostly addressed the individual role of Si or PGPR in plant tolerance to different abiotic stresses in field crops but very little is known about the integrated use of Si and PGPR on drought tolerance of plants, particularly vegetables including tomato. The present study, has therefore, been conducted to investigate the individual and interactive effects of Si and PGPR on plant growth, ionic relations and physiological characteristics of tomato (*Solanum lycopersicum* L.) under drought stress conditions.

Materials and Methods

The present study was conducted to evaluate the effects of drought stress on plant growth and role of Si and PGPR having ACC-deaminase for improving drought tolerance in tomato (*Solanum lycopersicum* L.). For this, a pre-isolated bacterial strain (*Citrobacter freundii* – J118) was taken from Soil Microbiology and Biochemistry Lab, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan.

Characterization and identification of bacterial strain

The bacterial strain was characterized for different plant growth promoting characteristics (Table 1) such as ACC-deaminase activity of the rhizobacterial strain was determined by measuring the amount of α-ketobutyric acid produced due to cleavage of ACC (Penrose and Glick, 2003). Root colonization ability was studied under axenic conditions as described by Simons *et al*. (1996). Chitinase activity was examined through method illustrated by Chernin *et al*. (1998) with various adjustments. Siderophores production assay was done as described by Schwyn and Neilands (1987). Auxin compound expressed (Indole-3-acetic acid equivalents) was determined by digital spectrophotometer using Salkowoski cooling reagent as described by Sarwar *et al*. (1992). Phosphate solubilization test was carried out to evaluate the capability to solubilize inorganic phosphate (tricalcium phosphate), hence it was utilized in this assay with agar media (Goldstein, 1986). The PGPR strain was identified by using standard morphological and biochemical tests (shape, color, Gram staining, methyl red, indole production, H_2S production, gelatin liquefaction, catalase and oxidase production) and was further confirmed by Biolog® identification system (Microlog™ System Release 4.2, Hayward, CA, USA)

Plant growth and treatments

For pot experiment, inoculum of PGPR containing ACC-deaminase activity (10^7-10^9 CFU mL^{-1}, 0.6 of OD600) was prepared as described by Shahzad *et al.*

(2010). Two weeks old healthy and uniform tomato seedlings (cv. Aroma) were inoculated by dipping into bacterial inoculum and 10% sugar solution for 6 hours while control was consisted of the seedlings treated with nutrient broth and 10% sugar solution only. After this, tomato seedlings were transplanted in to earthen pots, 28 cm in diameter and 30 cm deep having a basal hole and filled with 12 kg of well-prepared soil. Initially, three seedlings were transplanted in each pot but only two seedlings per pot were maintained seven days after transplanting.

Table 1: Characterization of bacterial inoculant used in the study

Bacterial strain	ACC-deaminase activity (α-ketobutyrate nmol g^{-1} biomass h^{-1})	Chitinase activity (qualitative)	Siderophore production	Phosphate solubilization	Root colonization (cfu g^{-1})	Indole-3-acetic acid (IAA) (µg mL^{-1})	
						Without L-TRP	With L-TRP
Citrobacter freundii – J118	379.7 ± 9.3	++ve	+ve	++ve	4.87×10^5	6.39 ± 1.03	10.29 ± 1.54

±: Standard error of means; Single positive sign means halo size < 2 mm while double positive means halo size > 2 mm, Average of four replications

The experimental soil was sandy clay loam (sand 55.16%, silt 20.19% and clay 24.65%) having pH 7.9, electrical conductivity (EC) 1.12 dS m^{-1}, sodium adsorption ratio (SAR) 8.6 (mmol $L^{-1})^{1/2}$, organic matter 0.84%, N 24.8 mg kg^{-1}, P 3.4 mg kg^{-1} and K 134 mg kg^{-1}. Each pot was supplied with 0.72 g N as urea, 0.48 g P_2O_5 as single super phosphate and 0.48 g K_2O as potassium sulfate while micronutrients were applied through Johnson's solution (Johnson *et al*., 1957) twice, first 10 days after transplanting and then 35 days after transplanting, along with irrigation water. Experimental plan was comprised of nine treatments including T_1 control (irrigation at 60% of field capacity); drought-1 (irrigation at 45% of field capacity); drought-2 (irrigation at 35% of field capacity); drought-1+50 ppm Si; drought-2+50 ppm Si; drought-1+PGPR; drought-2+PGPR; drought-1+50 ppm Si+PGPR; drought-2+50 ppm Si+PGPR. Silicon was applied as sodium silicate while PGPR through root inoculation before transplanting. The experiment was conducted according to completely randomized design (CRD) with five replications. All agronomic practices were adopted uniformly to control insect pest and disease attack during growth period of crop.

Ionic analyses

One plant from each replication of all treatments was harvested 45 days after treatment application and used to determine biomass accumulation and ionic relations. These plant samples were washed, separated into roots and shoots, weighed (fresh biomass), oven dried at 70ºC for 48 hours in an oven (EYELA WFO-600ND; Tokyo Rikaikai Co., Ltd., Tokyo, Japan) and reweighed to obtain dry biomass. Oven-dried plant samples were finely ground in a grinder fitted with stainless steel blades and a chamber (MF 10 IKA-WERKE, GMBH & Co., KG, Germany). A 0.5 g portion of oven-dried samples of roots and shoots was digested in a mixture of concentrated nitric acid and perchloric acid (2:1, v/v) at 250ºC. The K^+ and Na^+ concentrations in root and shoot samples were estimated with a flame photometer (Jenway PFP 7, ELE Instrument Co. Ltd., UK) according to Yoshida *et al*. (1976) while Ca^{2+} and Mg^{2+} were determined by atomic absorption spectroscopy.

Proline determination

For proline determination, leaf samples were collected at 40th day after treatment application between 8 and 9 a.m. from tomato plants of each replication in each of the nine treatments. Proline was extracted from 0.5 g of fresh leaf tissue into 10 mL of 3% sulfosalicylic acid and filtered through Whatman No. 42 filter paper. Absorbance was measured at 520 nm with a UV spectrophotometer (Spectron 100, Shimadzu, Japan).

Relative water content

For the determination of RWC, fully expanded leaves of the same physiological age as were collected for proline determination, were detached and weighed immediately to record fresh weight (FW). The leaves were then floated in distilled water inside a closed glass petri dish and weighed periodically after gently wiping water from the leaf surface with tissue paper to get turgid weight (TW). The leaves were then dried at 70 ºC for 48 h to get oven-dried weight (ODW). The RWC was determined using the equation as described by Kaya and Higgs (2003):

$$RWC\ (\%) = [(FW-ODW)/(TW-ODW] \times 100$$

Electrolyte leakage

For electrolyte leakage, 0.2 g of leaf samples were cut into discs 1.0 cm in diameter and placed into plastic tubes containing 50 mL of distilled water. After 24 hours, the EC of water containing the leaf sample was measured (C_1) using an electrical conductivity meter (Jenway 4510, Bibby Scientific Ltd., UK). Plastic tubes were then autoclaved at 120 ºC in an Autoclave (TOMY SX-500E, Japan) for 20 min and their EC was measured (C_2). Electrolyte leakage was determined as:

Electrolyte leakage (%) = $C_1/C_2 \times 100$

Statistical analysis

Statistical analysis was conducted using Mstat-C (Department of Crop and Soil Sciences, Michigan State University, East Lansing, Michigan, USA). The experiment was designed and analyzed according to Completely Randomized Design (CRD) with five replications. Data were subjected to analysis of variance (ANOVA) to compare the effects of treatments (Steel *et al.*, 1997). Differences between means were compared using the least significant difference test (LSD, $P \leq 0.05$).

Results

Plant growth and yield characteristics

There was a significant ($P \leq 0.05$) effect of Si and PGPR on plant growth and yield characteristics in terms of plant height, plant girth, fresh biomass, dry biomass and fruit yield of tomato when grown under both levels of drought stress (Table 2). Maximum plant height of 53.10 cm was found in control treatment when tomato plants were irrigated at 60% of field capacity which was reduced by 46.13% with drought-1 (irrigation at 45% of field capacity) and 58.75% with drought-2 (irrigation at 35% of field capacity) compared to control. However, plant height was improved by 25.87% with Si, 32.16 with PGPR and 42.65% with Si+PGPR at drought-1 compared to dought-1 stressed plants without any amendment. At drought-2 stress level, plant height was improved by 34.70% with Si, 42.46% with PGPR and 57.99% with Si+PGPR compared to drought-2 stressed plants without any amendment. Likewise, maximum plant girth of 14.20 mm was observed in case of

Table 2: Plant growth and yield characteristics of tomato cultivar "Aroma" grown at different levels of drought stress by supplying Si and PGPR (values are the mean of five replications)

Treatment	Plant height (cm)	Plant girth (mm)	Shoot fresh biomass (g plant^{-1})	Root fresh biomass (g plant^{-1})	Total fresh biomass (g plant^{-1})	Shoot dry biomass (g plant^{-1})	Root dry biomass (g plant^{-1})	Total dry biomass (g plant^{-1})	Fruit yield (g plant^{-1})
Control	53.1a	14.2a	34.86a	12.10a	46.96a	6.86a	2.82a	9.68a	122a
Drought-1	28.6e	11.5bc	21.44cd	7.25cd	28.69d	3.62cd	1.35cd	4.97cd	78.5e
Drought-2	21.9f	10.2d	14.27e	4.35e	18.62ef	2.20d	1.01de	3.21d	66.8f
Drought-1+ Si	36.0cd	12.1b	25.62bc	9.72bc	35.34c	4.92bc	1.96bc	6.88bc	92.9d
Drought-2 + Si	29.5de	11.0c	22.74c	7.94cd	30.68cd	3.96c	1.88c	5.84c	87.6de
Drought-1+ PGPR	37.8cd	12.4b	26.81bc	8.98bc	35.79c	5.15b	2.1bc	7.25bc	96.4cd
Drought-2 + PGPR	31.2de	11.6bc	22.96c	7.20cd	30.16cd	4.01c	1.98bc	5.99c	90.4d
Drought-1 + Si + PGPR	40.8c	12.8b	29.8b	10.0b	39.80bc	5.5b	2.42b	7.92b	102.9c
Drought-2 + Si + PGPR	34.6d	11.9bc	25.2bc	8.10c	33.30cd	4.7bc	2.15bc	6.85bc	95.1cd

Values in a column followed by the same letter are not significantly different at $P \leq 0.05$. (Control, irrigation at 60% of field capacity; drought-1, irrigation at 45% of field capacity; drought-2, irrigation at 35% of field capacity; drought-1+Si, irrigation at 45% of field capacity+50 ppm Si; drought-2+Si, irrigation at 35% of field capacity+50 ppm Si; drought-1+PGPR, irrigation at 45% of field capacity+PGPR; drought-2+PGPR, irrigation at 35% of field capacity+PGPR; drought-1+Si+PGPR, irrigation at 45% of field capacity+50 ppm Si+PGPR; drought-2+Si+ PGPR, irrigation at 35% of field capacity+50 ppm Si+PGPR

control which was reduced by 19.01% with drought-1 and 28.17% with drought-2 compared to control. Plant girth was improved by 5.21% with Si, 7.82% with PGPR and 11.30% with Si+PGPR at drought-1 while 7.84% with Si, 13.82% with PGPR and 16.66% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment. Maximum shoot fresh biomass of 34.86 g plant^{-1} was found in control that was reduced by 38.49 and 59.06% under drought-1 and drought-2, respectively, compared to control. The shoot fresh biomass was improved by 19.49% with Si, 25.04% with PGPR and 38.99% with Si+PGPR at drought-1 while 59.35% with Si, 60.89% with PGPR and 76.59% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment. Maximum root fresh biomass of 12.10 g plant^{-1} was recorded in control which was decreased by 40.08 and 64.04% under drought-1 and drought-2, respectively compared to control. The root fresh biomass was improved by 34.06% with Si, 23.86% with PGPR and 37.93% with Si+PGPR at drought-1 while 82.52% with Si, 65.51% with PGPR and 86.20% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment. Similarly, maximum total fresh biomass of 46.96 g plant^{-1} was found in control which was reduced by 38.90% with drought-1 and 60.34% with drought-2 compared to control. However, total fresh biomass was improved by 23.17% with Si, 24.74% with PGPR and 38.72% with Si+PGPR at drought-1 compared to dought-1 stressed plants without any amendment. At drought-2 stress level, total fresh biomass was improved by 64.76% with Si, 61.97% with PGPR and 78.83% with Si+PGPR compared to drought-2 stressed plants without any amendment. Maximum shoot dry biomass of 6.86 g plant^{-1} was observed in case of control which was reduced by 47.23% with drought-1 and 67.93% with drought-2 compared to control. The shoot dry biomass was improved by 35.91% with Si, 42.26% with PGPR and 51.93% with Si+PGPR at drought-1 while 80.0% with Si, 82.27% with PGPR and 113.63% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment. Maximum root dry biomass of 2.82 g plant^{-1} was found in control that was reduced by 52.12 and 64.18% under drought-1 and drought-2, respectively compared to control. The root dry biomass was improved by 45.18% with Si, 55.55% with PGPR and 79.25% with Si+PGPR at drought-1 while 86.13% with Si, 88.18% with PGPR and 112.87% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment. Maximum total dry biomass of 9.68 g plant^{-1} was recorded in control that was decreased by 48.65 and 66.83% under drought-1 and drought-2, respectively compared to control. The total dry biomass was improved by 38.43% with Si, 45.87% with PGPR and 59.35% with Si+PGPR at drought-1 while 81.93% with Si, 86.60% with PGPR and 113.39% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment. Maximum fruit yield plant^{-1} of 122 g was observed in control treatment that was reduced by 35.65% with drought-1 and 45.24% with drought-2 compared to control. Fruit yield was improved by 18.34% with Si, 22.80% with PGPR and 30.44% with Si+PGPR at drought-1 while 31.13% with Si, 35.32% with PGPR and 42.36% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment.

Physiological characteristics

There was a significant ($P \leq 0.05$) effect of Si and PGPR on physiological characteristics including RWC, electrolyte leakage and proline content of tomato grown under both levels of drought stress (Table 3). Results revealed that maximum RWC of 88.40% were found in control which were reduced by 20.13% with drought-1 and 24.43% with drought-2 compared to control. However, RWC were improved by 5.09% with Si, 8.92% with PGPR and 12.60% with Si+PGPR at drought-1 while 3.44% with Si, 8.98% with PGPR and 12.60% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment. In contrast, minimum electrolyte leakage of 69.40% was observed in case of control that was increased by 26.22% with drought-1 and 32.27% with drought-2 compared to control. Electrolyte leakage was reduced by 12.21% with Si, 13.81% with PGPR and 17.35% with Si+PGPR at drought-1 while 9.15% with Si, 8.71% with PGPR and 16.55% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment. Minimum proline content of 4.4 µmoles g^{-1} FW were found in control which were increased by 61.36 and 102.27% under drought-1 and drought-2, respectively compared to control. The proline content were reduced by 18.30% with Si, 21.12% with PGPR and 28.16% with Si+PGPR at drought-1 while 24.71% with Si, 30.33% with PGPR and 28.08% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment.

Ionic characteristics

Results presented in Figure 1 indicated the individual and combined effects of Si or/ and PGPR on K^+ concentration in shoots and roots of tomato plants grown under drought stress conditions. It was found that maximum shoot K^+ concentration of 39.16 mg g^{-1} was recorded in control treatment which was decreased by 34.8 and 49.72% under drought-1 and drought-2, respectively, compared to control. Application of Si or/ and PGPR improved shoot K^+ concentration by 31.95% with Si, 34.52% with PGPR and 43.07% with Si+PGPR at drought-1 while 33.16% with Si, 29.53% with PGPR and 37.09% with Si+PGPR at drought-

Table 3: Physiological characteristics of tomato cultivar "Aroma" grown at different levels of drought stress by supplying Si and PGPR (values are the mean of five replications)

Treatment	Relative water content (%)	Electrolyte leakage (%)	Proline content (µmoles g^{-1} FW)
Control	88.4a	69.4f	4.4e
Drought-1	70.6de	87.6b	7.1bc
Drought-2	66.8f	91.8a	8.9a
Drought-1+ Si	74.2cd	76.9de	5.8d
Drought-2 + Si	69.1ef	83.4c	6.7c
Drought-1+ PGPR	76.9c	75.5e	5.6d
Drought-2 + PGPR	72.8de	83.8c	6.2cd
Drought-1 + Si + PGPR	79.5bc	72.4ef	5.1de
Drought-2 + Si + PGPR	76.4c	76.6de	6.4cd

Values in a column followed by the same letter are not significantly different at $P \leq 0.05$. (Control, irrigation at 60% of field capacity; drought-1, irrigation at 45% of field capacity; drought-2, irrigation at 35% of field capacity; drought-1+Si, irrigation at 45% of field capacity+50 ppm Si; drought-2+Si, irrigation at 35% of field capacity+50 ppm Si; drought-1+PGPR, irrigation at 45% of field capacity+PGPR; drought-2+PGPR, irrigation at 35% of field capacity+PGPR; drought-1+Si+PGPR, irrigation at 45% of field capacity+50 ppm Si+PGPR; drought-2+Si+ PGPR, irrigation at 35% of field capacity+50 ppm Si+PGPR

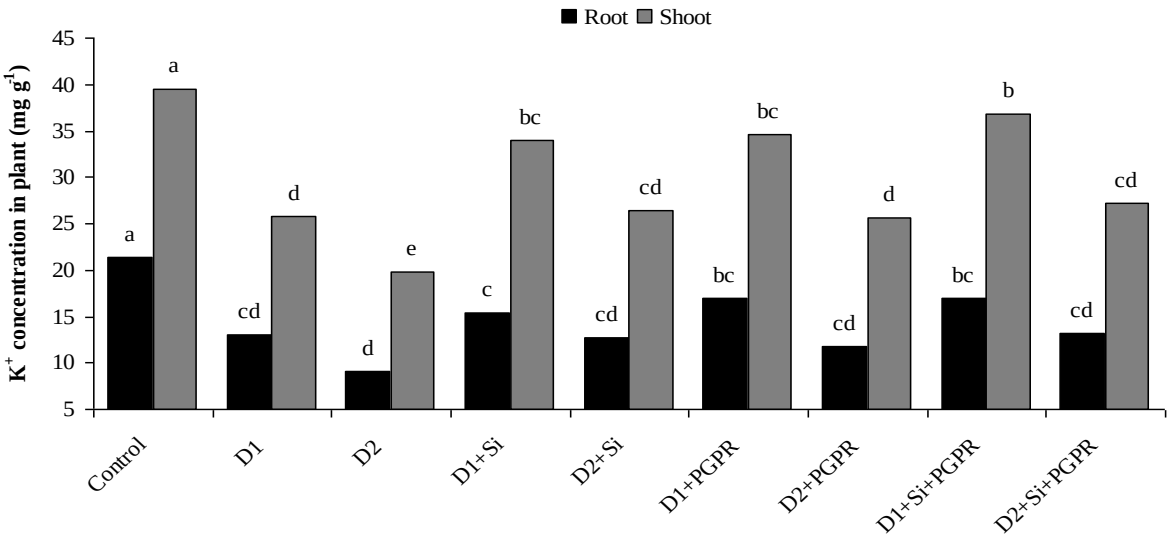

Figure 1: Root and shoot K^+ concentration (mg g^{-1}) of tomato (*Solanum lycopersicum* L.) plants grown under drought stress conditions by supplying Si or/ and PGPR. (Control, irrigation at 60% of field capacity; D1 (drought-1), irrigation at 45% of field capacity; D2 (drought-2), irrigation at 35% of field capacity, D1+Si, drought-1+50 ppm Si; D2+Si, drought-2+50 ppm Si; D1+PGPR; drought-1+PGPR; D2+PGPR, drought-2+PGPR; D1+Si+PGPR, drought-1+50 ppm Si+PGPR; D2+Si+PGPR, drought-2+50 ppm Si+ PGPR)

2 compared to drought stressed plants without any amendment. Likewise, maximum root K^+ concentration of 21.40 mg g^{-1} was found in control treatment which was reduced by 38.78% with drought-1 and 57.24% with drought-2 compared to control. However, root K^+ concentration of tomato plants was improved by 17.17% with Si, 29.0% with PGPR and 29.46% with Si+PGPR at drought-1 while by 38.25% with Si, 29.39% with PGPR and 44.26% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment. Data regarding Na^+ concentration in shoots and roots of tomato plants were presented in Figure 2. It indicated that shoot Na^+ concentration in control treatment was 2.16 mg g^{-1} which was increased by 50.92% with drought-1 and

123.14% with drought-2 compared to control. However, shoot Na^+ concentration was decreased by 32.51% with Si, 12.88 with PGPR and 57.66% with Si+PGPR at drought-1 while by 26.97% with Si, 21.99% with PGPR and 41.90% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment.

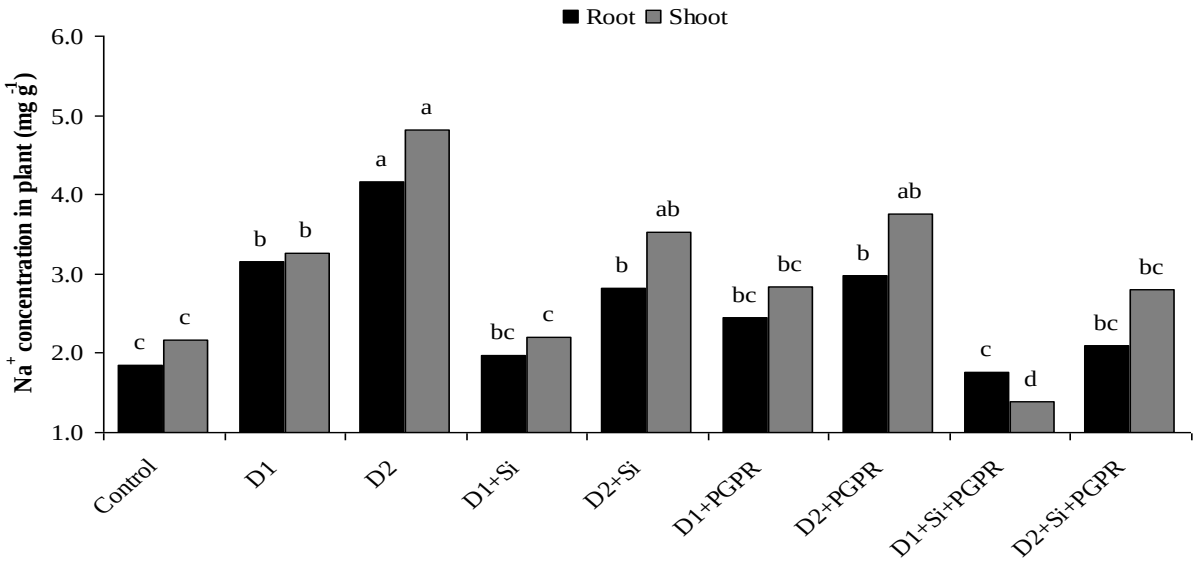

Figure 2: Root and shoot Na^+ concentration (mg g^{-1}) of tomato (*Solanum lycopersicum* L.) plants grown under drought stress conditions by supplying Si or/ and PGPR. (Control, irrigation at 60% of field capacity; D1 (drought-1), irrigation at 45% of field capacity; D2 (drought-2), irrigation at 35% of field capacity, D1+Si, drought-1+50 ppm Si; D2+Si, drought-2+50 ppm Si; D1+PGPR; drought-1+PGPR; D2+PGPR, drought-2+PGPR; D1+Si+PGPR, drought-1+50 ppm Si+PGPR; D2+Si+PGPR, drought-2+50 ppm Si+ PGPR)

Similarly, in control treatment root Na^+ concentration was found 1.84 mg g^{-1}. However, when tomato plants were exposed to drought stress, root Na^+ concentration was increased by 71.19% with drought-1 and 126.63% with drought-2 compared to control. When Si and PGPR were applied, root Na^+ concentration was decreased by 37.14% with Si, 22.53% with PGPR and 44.12% with Si+PGPR at drought-1 while 32.37% with Si, 28.53% with PGPR and 49.64% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment. Maximum shoot Ca^{2+} concentration of 7.65 mg g^{-1} was found in control treatment which was reduced by 44.31 and 49.80% under drought-1 and drought-2, respectively compared to control. Application of Si or/ and PGPR were significantly ($P \leq 0.05$) effective to alleviate drought stress and improve shoot Ca^{2+} concentration under both drought stress levels. The shoot Ca^{2+} concentration was improved by 19.72% with Si, 25.59% with PGPR and 39.67% with Si+PGPR at drought-1 while 10.67% with Si, 21.09% with PGPR and 28.12% with drought-1 and drought-2, respectively compared to control. The root Ca^{2+} concentration was improved by 11.54% with Si, 15.44% with PGPR and 24.55% with Si+PGPR at drought-1 while 19.23% with Si, 20.19% with PGPR and 32.30% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment (Figure 3). Results of Mg^{2+} concentration in shoots and roots of tomato plants were presented in Figure 4. It revealed that maximum shoot Mg^{2+} concentration of 4.26 mg g^{-1} was found in control treatment which was reduced by 30.51% with drought-1 and 50.70% with drought-2 compared to control. However, shoot Mg^{2+} concentration was improved by 29.72% with Si, 11.48% with PGPR and 32.43% with Si+PGPR at drought-1 while 39.04% with Si, 14.28% with PGPR and 64.28% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment. Likewise, maximum root Mg^{2+} concentration of 3.50 mg g^{-1} was found in control treatment when tomato plants were irrigated at 60% of field capacity which was reduced by 31.42% with drought-1 and 38.57% with drought-2 compared to control. However, root Mg^{2+} concentration of tomato plants was improved by 16.25% with Si, 9.16% with PGPR and 31.25% with Si+PGPR at

drought-1 while 12.56% with Si, 4.18% with PGPR and 30.23% with Si+PGPR at drought-2 compared to drought stressed plants without any amendment.

affected by various biotic and abiotic environmental factors. Among these, drought stress is considered the most critical growth limiting factor for agricultural crops all around the

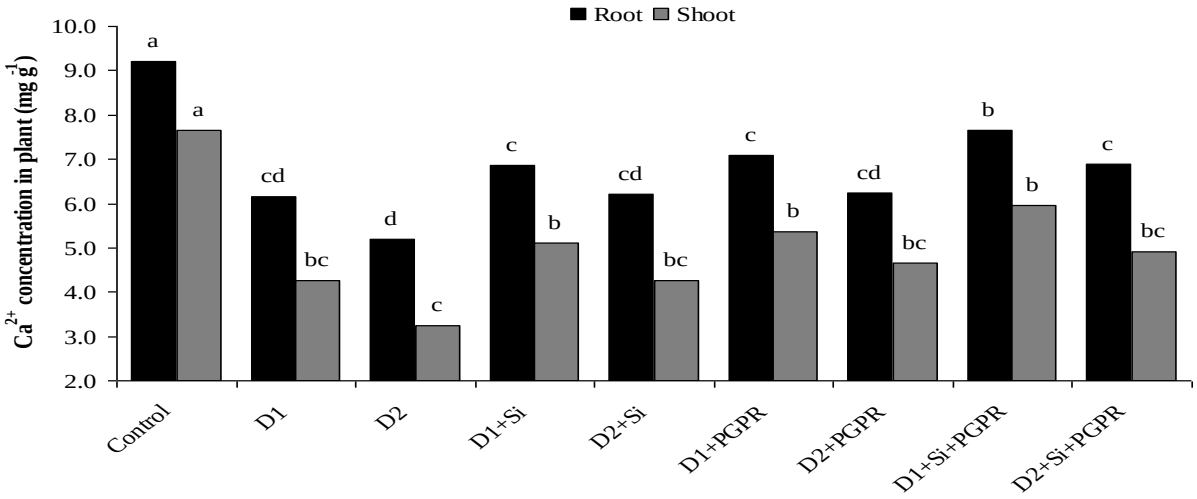

Figure 3: Root and shoot Ca^{2+} concentration (mg g^{-1}) of tomato (*Solanum lycopersicum* L.) plants grown under drought stress conditions by supplying Si or/ and PGPR. (Control, irrigation at 60% of field capacity; D1 (drought-1), irrigation at 45% of field capacity; D2 (drought-2), irrigation at 35% of field capacity, D1+Si, drought-1+50 ppm Si; D2+Si, drought-2+50 ppm Si; D1+PGPR; drought-1+PGPR; D2+PGPR, drought-2+PGPR; D1+Si+PGPR, drought-1+50 ppm Si+PGPR; D2+Si+PGPR, drought-2+50 ppm Si+ PGPR)

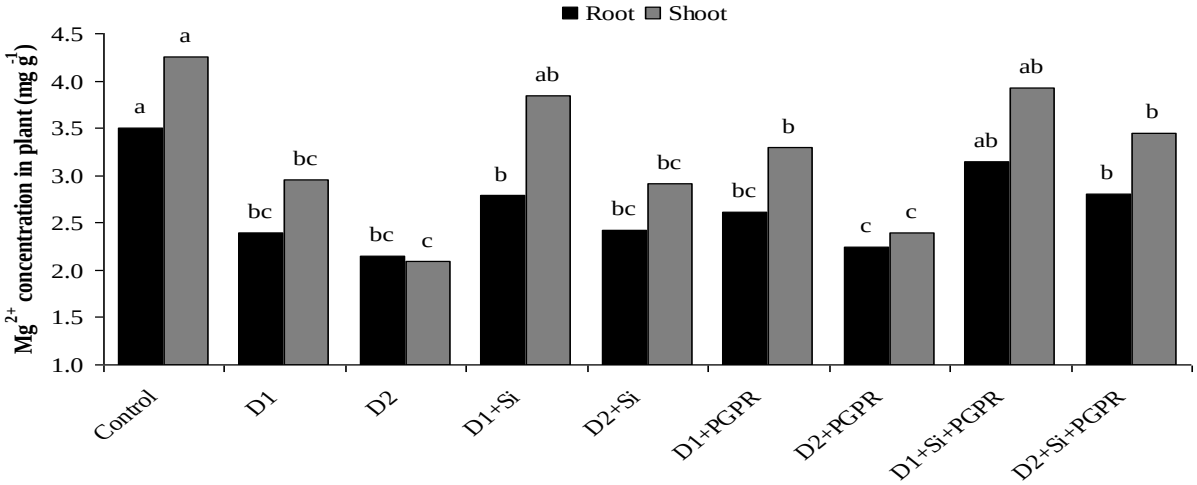

Figure 4: Root and Shoot Mg^{2+} concentration (mg g^{-1}) of tomato (*Solanum lycopersicum* L.) plants grown under drought stress conditions by supplying Si or/ and PGPR. (Control, irrigation at 60% of field capacity; D1 (drought-1), irrigation at 45% of field capacity; D2 (drought-2), irrigation at 35% of field capacity, D1+Si, drought-1+50 ppm Si; D2+Si, drought-2+50 ppm Si; D1+PGPR; drought-1+PGPR; D2+PGPR, drought-2+PGPR; D1+Si+PGPR, drought-1+50 ppm Si+PGPR; D2+Si+PGPR, drought-2+50 ppm Si+ PGPR)

Discussion

Plant growth is generally the outcome of cell division, cell enlargement and differentiation, and involves various genetic, physiological, ecological as well as morphological processes and their interactions. Plant growth might be

world, particularly in arid and semiarid regions. It has been reported that global temperature will rise by 4 ºC by 2050 that causes the melting of glaciers, resulting in floods for the first few years and then acute shortage of water. Drought was considered a major catalyst of the great

famines of the past. In present study, both levels of drought stress caused a significant ($P \leq 0.05$) reduction in plant height, plant girth, biomass accumulation and fruit yield. However, drought-induced reduction in plant growth and yield was much higher at drought-2 compared to drought-1. Showemimo and Olarewaju (2007) demonstrated that drought is one of the major abiotic stress factors because it decreased rate of plant growth and development, caused flower aborting, reduced fruit set and eventually decreased crop yields. Earl and Davis (2003) also indicated that drought stress could reduce leaf area, stem elongation and root proliferation, upset plant water relations and subsequently reduced plant growth and yield. According to Taiz and Zeiger (2006), drought-induced reduction in plant growth was ascribed to the reduction in turgor pressure under drought stress conditions. Application of Si and PGPR under both levels of drought stress alleviated the detrimental effects of drought and improved plant growth and yield. The ameliorative effects of Si and PGPR were relatively higher at drought-2 compared to drought-1. Furthermore, the efficiency of Si and PGPR to enhance plant tolerance to drought stress was higher when applied in combination compared to their sole application. Gong *et al*., 2005 also reported that Si could ameliorate the deleterious effects of drought by reducing water loss through transpiration and increasing internal water status of plants. Moreover, Gunes *et al*., 2008 demonstrated that Si could also help the plants in osmotic adjustment by depositing in the cytoplasm and complexation in the vacuole and thus improve plant tolerance to drought stress. Likewise, PGPR inoculation under drought stress conditions could improve plant water status and thus increased biomass accumulation (Nadeem *et al*., 2007). According to Lucy *et al*. (2004), major role of PGPR in influencing plant growth and development under drought stress were ascribed to increased synthesis of phytohormones and vitamins, inhibiting plant ethylene synthesis, improving nutrient accumulation, solubilizing inorganic phosphate, and mineralizing organic phosphate.

Physiological attributes, particularly RWC and electrolyte leakage are the indicatives of metabolic activities within plants and used for the evaluation of plant tolerance to different abiotic stresses including drought. It was found that both levels of drought stress caused marked decrease in RWC of tomato, with higher reduction at drought-2 compared to drought-1. Egilla *et al.* (2005) also reported that RWC, turgor potential and stomatal conductance were greatly reduced under drought stress conditions. Drought-induced reduction in RWC was mainly ascribed to reduced water uptake and increased transpiration. The maintenance of plant water status by applying Si and PGPR in terms of high RWC under drought stress could be ascribed to their role in stomatal resistance, water use efficiency and lowering transpiration rate. Romero-Aranda *et al*. (2006) reported that Si supplementation (2.5 mM) could improve water storage within plant tissues in tomato by reducing transpiration. Likewise, Sarig *et al*. (1992) found an increase in root hydraulic conductance in sorghum plants by inoculation with *Azospirillum brasilense* under control and osmotic stress conditions which could help to maintain plant water status under drought. In contrast to RWC, electrolyte leakage was increased under drought stress conditions, with greater increase at drought-2 compared to drought-1. Showemimo and Olarewaju (2007) also reported that drought stress increased electrolyte leakage and caused membrane instability in plants. Application of Si and PGPR alleviated drought stress effects and reduced electrolyte leakage, greater reduction with combined application of Si and PGPR compared to their individual application. Past studies indicated that Si could reduce electrolyte leakage, stabilize the membrane structure and integrity by affecting the stress-dependent peroxidation of membrane lipids (Cakmak, 2005). Proline could contribute to membrane stability (Hanson and Burnet, 1994) by ameliorating the deleterious impacts of drought stress on cell membrane disruption and also serving as a free radical scavenger (Maneesuwannarat *et al.,* 2013). In present study, proline contents were significantly increased as drought stress level increased but decreased after the application of Si and PGPR. It seemed possible that Si and PGPR could provide a protective cover in tomato plants under drought stress, preventing them from being severely affected by drought stress. Therefore, the level of proline accumulated in Si- and PGPR-treated plants under drought stress was not as high as in drought-stressed plants without Si and PGPR supplementation.

Under drought stress conditions, decreasing water availability caused a marked reduction in the uptake and accumulation of K^+, Ca^{2+} and Mg^{2+} while increase Na^+ in tomato grown at both levels of drought stress. Samarah *et al.* (2004) also reported that water deficit could affect nutrient uptake by decreasing nutrient transport from soil to root surface as well as by reducing root growth and extension. McWilliams (2003) indicated that drought-induced reduction in the absorption of essential nutrients might be attributed to drought interference in nutrient uptake and unloading mechanism as well as reduced transpirational flow, depending upon plant species and genotypes. Application of Si and PGPR interacted with Na^+, reduced its uptake while increased the concentration of K^+, Ca^{2+} and Mg^{2+} in plant tissues. Gunes *et al*. (2008) demonstrated that Si application under drought stress significantly improved the uptake of K, S, Mg and Fe. Gao

et al. (2006) also found that Si addition to drought stressed medium markedly improved Ca^{2+} concentration in plant tissues which could help to maintain membrane stability and permeability under drought stress. Liang (1999) found that under drought stress, Si supplementation could improve plant K^+ concentration probably because of activation of H+-ATPase in membranes. Lucy *et al*. (2004) reported that PGPR could influence plant growth and development under drought stress by improving nutrient uptake and accumulation.

Conclusion

Plant growth, yield, physiological characteristics and ionic relations were significantly influenced by drought stress, with greater effect of drought-2 compared to drought-1. However, Si and PGPR had great potential to alleviate drought stress and influenced tomato growth and yield, in addition to their effects on physiological characteristics and ionic relations. Combined applications of Si and PGPR hold a lot of promise as an efficient tool to enhance plant tolerance to drought stress. Furthermore, ameliorative effects of Si and PGPR were mostly higher in case of drought-2 compared to drought-1. The results need to be confirmed under field conditions and the economic feasibility of Si and PGPR application under drought stress conditions should be worked out.

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