Research article

Actinobacterium isolated from a semi-arid environment improves the drought tolerance in maize (*Zea mays* L.)

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ABSTRACT

Drought represents a major constraint for agricultural productivity and food security worldwide. Plant growth promoting actinobacteria have attracted the attention as a promising approach to enhance plant growth and yield under stressful conditions. In this regard, bioprospecting in arid and semi-arid environments could reveal uncommon bacteria with improved biological activities. In the present study, the ability of actinobacteria isolated from a semi-arid environment (Saudi Arabia) to mitigate the negative impact of drought on growth and physiology of maize, a drought-sensitive crop, has been investigated. Among the different actinobacterial isolates screened for secondary metabolites production and biological activities, isolate Ac5 showed high ability of flavonoid, phytohormones and siderophores production. Moreover, Ac5 improved the growth and photosynthesis and induced a global metabolic change in the bacterized plants under water-deficit conditions. Interestingly, Ac5 treatment significantly mitigated the detrimental effects of drought stress on maize. Reduced H_2O_2 accumulation and lipid peroxidation accompanied with higher levels of molecular antioxidants (total ascorbate, glutathione, tocopherols, phenolic acids and flavonoids) were observed in the bacterized plants. From the osmoregulation point of view, drought-stressed bacterized maize accumulated higher levels of compatible solutes, such as sucrose, total soluble sugars, proline, arginine and glycine betaine, as compared with the non-bacterized plants. Therefore, this study highlights the comprehensive impact of actinobacteria on the global plant metabolism and suggests the potential utilization of actinobacteria isolated from semi-arid environments to mitigate the negative impact of drought on crop plants.

1. Introduction

Dramatic climatic changes, such as temperature elevation and rainfall fluctuations, can lead to a drastic drought in arid and semiarid areas. Consequently, these factors represent a major constrain for agricultural productivity and threat food security of the growing world population (Sharma et al., 2014). It is estimated that by the year 2050 the area of drought affected lands will double, while the global water resources will decrease by 30% (Falkenmark, 2013). This situation renders drought one of the most threatening abiotic stressors that affect production and distribution of plants (Mancosu et al., 2015). Therefore, developing effective and promising approaches to preserve plant

productivity under the prospective drought is of immense importance. In this regard, several strategies have been established but most of them, such as molecular breeding programs and transgenic approaches, face several obstacles and objections (Cominelli et al., 2013; Fita et al., 2015).

Recently, plant growth promoting bacteria (PGPB) have attracted the attention of many researchers as a promising approach to enhance plant growth and yield under stressful conditions (Shameer and Prasad, 2018). Among PGPB, actinobacteria, a group of gram-positive bacteria, have been reported to mitigate the negative influences of drought stress in several crop species (Naveed et al., 2014; Yandigeri et al., 2012). The suggested mechanisms underlying actinobacteria-induced stress

tolerance in plants include; improvement of water and nutrients uptake (Ardakani et al., 2011; Hozzein et al., 2019), production of the phytohormones indole-3-acetic acid (IAA), gibberellins (GA), cytokinins (CK) and salicylic acid (SA) (Abdelgawad et al., 2019; Bhardwaj et al., 2014), nitrogen fixation (Marques et al., 2017), phosphate solubilisation (Rodriguez et al., 2004), production of proline, and other osmoregulatory compounds (Shameer and Prasad, 2018), and elevation of enzymatic antioxidants (Fukami et al., 2017). Bioactive actinobacteria have been isolated from diverse habitats, however bioprospecting in arid environments is a promising strategy for isolation of uncommon actinobacteria having thermotolerant, xerotolerant and halotolerant properties (Mohammadipanah and Wink, 2016).

Maize (*Zea mays* L.) is one of the most important cereals over all the world. Unfortunately, production of maize is severely affected by water deficit conditions (Dicko et al., 2018; Iqbal et al., 2018). The negative impacts of drought on maize have been investigated on physiological, biochemical and molecular levels (Barbosa et al., 2018). These include reduced carbon assimilation, cellular desiccation, formation of free radicals, membrane damage, disturbed enzymatic activity, lipid peroxidation and protein oxidation (Hussain et al., 2019; Masood et al., 2018). In fact, previous studies have investigated the impact of PGPB on growth and photosynthesis, yield or levels of osmolytes and certain metabolites in maize and other plants grown under stressful conditions (Naveed et al., 2014; Yandigeri et al., 2012). However, studies addressing the synchronous changes in growth, physiology and global metabolism of a particular bacterized plant during drought are missing. Therefore, the current study was undertaken to investigate the comprehensive impact of growth promoting actinobacteria isolated from a semi-arid environment, Saudi Arabia, on the growth and metabolism of maize suffering drought. To fulfill this aim, bacterized maize plants were grown in normal or artificially desiccated soils for an extended period and the associated changes in growth, photosynthesis, respiration, osmolytes, stress markers and primary and secondary metabolites were assessed. To our knowledge, this is the first study that affords a global view for the actinobateria-induced changes in plants enduring water stress.

2. Materials and methods

2.1. Isolation of actinobacteria colonies

Isolation of actinobacteria was carried out following the soil dilution method adopted by Waksman (1961). Soil samples were collected from the rhizosphere of *Panicum turgidum*, a drought resistant desert plant, from different sites in Jouf region, Saudi Arabia. Glycerol-Yeast agar amended with nystatin (50 μg/l) was used as isolation medium. One g of soil was vigorously shaken in 10 ml distilled H_2O and heated for 30 min at 50 °C. The prepared soil suspension was added with different serial dilutions into sterilized petri-plates followed by pouring 20 ml of isolation medium. The poured plates were shaken gently and then kept for one to two weeks at 27 °C. After appearance of well-defined colonies, purification of actinobacteria colonies was performed by sub-culturing the selected colonies on the same isolation medium, and incubation for one week at 27 °C. The purified isolates were kept in starch casein agar as slants at 4 °C and in glycerol (20%) as suspensions at −20 °C (Haefner, 2003).

2.2. Morphological and biochemical characterization of the actinobacterial isolates

For identification of the isolated actinobacteria, morphological and biochemical screenings were performed. The morphological identification was performed by examining cover-slips of the isolates culture with a light microscope (Shirling and Gottlieb, 1966). Physiological and biochemical features including nitrogen and carbon utilization as well as some enzymatic activities were done according to the method of

Williams et al. (1989). The bioactive isolates were morphologically characterized using Bergey's manual keys (Williams et al., 1989). We measured IAA and siderophore production according to Gordon and Weber (1951) and Schwyn and Neilands (1987), to investigate growth promoting capacity of actinomycetes isolates.

2.3. Experimental setup, plant materials and growth conditions

The spores of the selected actinobacteria were collected and their concentration was adjusted to 10^7 spores/ml using a Haemocytometer and a light microscope. Healthy maize seeds were surface disinfested as described in Hosseini et al. (2017); then seeds were germinated on sterile filter paper for five days at 25 °C to obtain uniform seedlings. Maize seedlings with or without the living actinobacteria isolates were then planted into the autoclaved soil under aseptic conditions. Actinobacteria treated and non-treated plants were exposed to severe drought stress. Controlled pots were rewatered daily to 60% (soil water content (SWC)). For drought treatments, water contents were allowed to drop after sowing to 30% SWC (severe stress, leaves wilting during the day). Plants were maintained in a controlled greenhouse at 21/18 °C, 16/8 h day/night, and 60% humidity; and they were regularly irrigated. After 6 weeks, control and treated plants were collected for morphological and biochemical analyses.

2.4. Determination of photosynthetic rate

Photosynthetic rate (μmol $CO_2 m^{-2} s^{-1}$) of fully matured leaves was determined (LI-COR LI-6400, LI-COR Inc., Lincoln, NE, USA) (Al Jaouni et al., 2018). Leaf equilibration was set at every step at least 5 min prior data were recorded.

2.5. Metabolite profiling

Extraction and quantification of the contents of individual sugars, organic acids, amino acids, phenolic acids and flavonoids using HPLC or GC/MS analyses were performed according to the protocols mentioned in our previous study (Abdelgawad et al., 2019). Individual compounds were identified and measured depending on peak area and a calibration curve of the corresponding standards. Extraction and estimation of the total content of phenolics, flavonoids and sugars were performed following Folin–Ciocalteu, aluminium chloride colorimetric and Nelson's assays, respectively, as described previously (Mohamed et al., 2017; Saleh et al., 2015).

For determination of mineral concentrations, a known weight of dried materials was digested in 13 M nitric acid in an oven according to Agusa et al. (2005). Standard minerals were prepared in 0.23 M nitric acid. The levels of macro- and micro-elements were quantified (ICP-MS, Finnigan Element XR, Scientific, Bremen, Germany).

2.6. Total antioxidant activity

The free radical scavenging capacity was evaluated in the alcoholic (80% ice-cold ethanol) leaf extracts by FRAP and DPPH methods (Abdelgawad et al., 2019). For the DPPH assay, a known volume of each plant extract was shaken together with equal volume of DPPH solution (0.25 mM in 95% ethanol) and then allowed to stand at room temperature for 30 min. Afterwards the absorbance was measured at 517 nm and the inhibition percentage was calculated. FRAP assay was performed by mixing 20 μl of each ethanol extract with 180 μl of freshly prepared pre-warmed FRAP reagent in a micro-titration plate. The absorbance was measured at 593 nm after 30 min of incubation at 37 °C. The antioxidant capacity of the extracts was calculated using Trolox calibration curve.

Table 1

Morphological and biochemical identifications of the actinobacterial isolates. The + and – signs indicate the presence and absence, respectively.

	Isolates	Ac1	Ac2	Ac3	Ac4	Ac5
Spore chain	Aerial mycelium	+	+	+	+	+
	Pigmentation	+	–	+	–	–
	Spiral	–	+	+	–	–
	Rectiflexibiles	+	–	–	+	+
	Verticillat	–	–	–	–	–
Substrate	Yellow	–	–	+	–	+
	Orange	+	–	–	+	–
	Gry	–	–	–	–	–
	Red	–	+	–	–	–
N source utilization	L-Cysteine	–	+	+	+	–
	L-Phenylalanine	+	+	–	+	+
	L-Histidine	+	–	+	+	–
	L-Lysine	–	+	–	+	+
	L-Asparagine	+	–	+	–	+
	L-Arginine	–	+	+	+	+
	L-proline	+	–	–	–	–
	L-Valine	–	+	–	+	+
	Tyrosine	+	+	+	+	–
C source utilization	D-fructose	–	+	+	+	–
	D-glucose	+	–	+	–	+
	Sucrose	+	+	+	+	+
	Maltose	–	–	+	–	–
	Raffinose	+	–	–	+	+
	Lactose	+	–	–	+	–
	Galactose	–	+	–	+	+
	Meso-Inositol	–	–	+	–	+
	Celullose	+	+	+	+	+
	Xylose	–	–	–	+	–
	Dextran	+	+	+	–	–
Enzymes activity	Catalase	–	+	–	+	+
	Peroxidase	+	–	+	+	–
	Starch hydrolysis	+	–	–	–	+
	Gelatin liquefiction	–	+	+	–	–
	Casein hydrolysis	–	–	+	+	+
	Lipolysis	–	+	+	+	+
	Citrate utilization	+	–	+	–	+
	Nitrate reduction	–	+	–	+	–
	Urease	–	+	–	–	+
	H₂S Production	+	–	–	–	+
	DNase	+	–	+	+	–

2.7. Statistical analyses

Statistical Analyses were performed using the SPSS statistical package (SPSS Inc., Chicago, IL, USA). One-way Analysis of Variance (ANOVA) was applied to all data. Tukey's Test ($P \leq 0.05$) was carried out as the post-hoc test for mean separations. Each experiment was done in three replicates (n = 3).

3. Results and discussion

3.1. Identification, characterization and selection of the biologically active actinobacteria

Five different actinobacteria (Ac1 to Ac5) were isolated from

rhizospheric soil in Jouf region, Saudi Arabia, and identified at the morphological and biochemical levels (Table 1). The five isolates were identified to belong to the genus *Streptomyces* and its morphologically related genera with extensively branched hyphae and coiled spore chains (Abdelgawad et al., 2019; Hozzein et al., 2019). The tested isolates showed different substrate color and aerial mycelia and only isolates Ac1 and Ac3 produced diffusible pigments. Aerial hyphae of isolates Ac1, Ac4 and Ac5 have long rectiflexible spore chains, while long spiral spore chains were observed for isolates Ac2 and Ac3 (Table 1). The different isolates vary in their ability to utilize several kinds of nitrogen and carbon sources and in the enzymes they produced. Besides, a positive correlation was observed between the production of phenolic compounds and the antioxidant, antibacterial (*Streptococcus sp and Escherichia coli*) and antiprotozoal (*Trypanosom acruzi*) activities of the tested isolates (Table 2). However, isolate Ac5 showed the highest contents of total flavonoids and phenolics and levels of biological activities. Similarly, morphological and biochemical variations were reported among nine actinobacteria isolated from palm tree rhizosphere (Abdelgawad et al., 2019). Moreover, high TAC and antimicrobial properties were reported for the extracts and secretions of a novel marine actinobacterium (*Streptomyces variabilis* RD-5) (Dholakiya et al., 2017).

Based on its richness in biologically active compounds and their vigorous biological activities, isolate Ac5 was selected as the candidate bacterium to test its plant growth promotory action. The flavonoids profile and production of phytohormones and siderophore were assayed in the selected isolate (Table 3). Twelve flavonoids were identified in isolate Ac5, whereas fisetin was the most dominant followed by daidzein, genistein and quercetrin. Moreover, Ac5 was able to produce considerable amounts of IAA, IBA and GA, and both catechol and salicylate-types of siderophores. In accordance, we have previously reported that actinobacteria isolated from Jouf region, Saudi Arabia, were able to produce phytohormones and siderophores and promote plant growth (Abdelgawad et al., 2019; Hozzein et al., 2019).

3.2. Actinobacteria treatment recovered the negative impact of drought on photosynthesis and growth of maize

It well known that drought threats all vital processes in plants especially photosynthesis and, therefore, reduces the plant growth (Hasanuzzaman et al., 2013). Similarly, the current results revealed a severe reduction in biomass production of maize in response to drought alone, whereas fresh mass (FW) and dry mass (DW) decreased by about 49% and 58% respectively, relative to the control plant (Table 4). On the other hand, inoculation with actinobacteria caused significant increases in FW and DW by 61% and 67%, respectively, under water deficit conditions. Moreover, about 68.7% reduction in photosynthesis was observer under drought alone treatment. Such reduction was significantly recovered by inoculation with actinobacteria, whereas significant increase in photosynthetic C assimilation (92.6%) was recorded in bacterized plants, relative to the non-inoculated ones (Table 4). Similar reductions in growth and photosynthesis were recorded in maize and other crop plants suffering drought (Dicko et al., 2018; Efeoğlu et al., 2009; Iqbal et al., 2018). This inhibitory effect of drought on

Table 2

Screening of the contents of flavonoids and phenolics (mg g^{-1} extract) as well as antioxidant (FRAP, µmole trolox g^{-1} extract; DPPH % inhibition), antimicrobial (diameter of inhibition zone, mm) and antiprotozoal (% reduction in parasite) activities of the actionobacterial isolates. Values are the average of 3 replicates (mean ± S.D.).

Isolate	Total flavonoids	Total Phenols	Antioxidant Activity (FRAP)	Antioxidant Activity DPPH (%)	Antiprotozoal (*Trypanosom acruzi*)	Anti-bacterial (*Streptococcus* sp)	Anti-bacterial (*Escherichia coli*)
Isolate Ac1	8.11 ± 0.50	36.28 ± 1.11	53.66 ± 4.98	65.35 ± 9.71	3.83 ± 0.38	17.18 ± 0.77	21.07 ± 3.08
Isolate Ac2	7.69 ± 0.28	36.80 ± 0.05	47.46 ± 3.56	49.76 ± 7.68	3.33 ± 0.28	16.89 ± 0.29	16.38 ± 2.52
Isolate Ac3	7.14 ± 0.28	40.51 ± 5.37	43.03 ± 3.35	50.38 ± 6.39	2.71 ± 0.20	16.43 ± 0.48	10.65 ± 1.60
Isolate Ac4	8.45 ± 0.20	39.08 ± 0.57	54.08 ± 2.08	61.79 ± 2.65	3.82 ± 0.16	18.22 ± 0.31	20.03 ± 1.39
Isolate Ac5	12.93 ± 0.52	52.21 ± 4.46	67.50 ± 12.84	63.41 ± 9.66	5.98 ± 0.40	27.65 ± 0.83	28.78 ± 2.99

Table 3
The contents of flavonoids, hormones and siderophores (mg g^{-1} extract) produced by the selected actinobacteria (isolate Ac5). Values are the average of 3 replicates (mean ± S.D.).

Parameter	Ac5
Quercetin	0.95 ± 0.07
Quercetrin	1.59 ± 0.12
Luteolin	0.72 ± 0.08
Apigenin	6.20 ± 0.47
Isoquercetrin	6.92 ± 0.52
Rutin	0.70 ± 0.05
Ellagic acid	0.36 ± 0.02
Velutin	0.30 ± 0.02
Naringenin	1.02 ± 0.08
Genistein	1.56 ± 0.12
Daidzein	1.72 ± 0.13
Fisetin	3.18 ± 0.24
O-hydroxydaidzein	0.86 ± 0.06
IAA-Me	2.99
13C6-IAA-Me	
ABA	0.18
d6-ABA	
GA	0.17
Catechol siderophore	2.20
Salicylate siderophore	4.17

growth and photosynthesis was ascribed primarily to the oxidative damage resulted from generation of reactive oxygen species (ROS). Moreover, drought was reported to reduce photosynthesis through inhibition of enzyme activities, membranes dysfunction and stomatal closure (Ghannoum et al., 2003; Kaushal and Wani, 2016). On the other hand, the positive impact of actinobacteria on growth and photosynthesis has been shown in several plant species under both normal and drought stress conditions. For instance, Hozzein et al. (2019) reported that inoculation of barley, wheat, maize, sorghum and oat plants with actinobacteria caused significant improvements in growth and photosynthesis. Dicko et al. (2018) found that inoculation of maize seeds with actinobacteria (sp.H7) resulted in a greater biomass production compared to a non-inoculated control. Also, significantly higher shoot length and dry biomass in drought-stressed maize treated with PGPR were recorded (Govindappa, 2012). The positive role of actinobacteria in enhancing plant growth and photosynthesis has been ascribed to increments in chlorophyll and nitrogen contents (Abdelgawad et al., 2019; Hozzein et al., 2019) and elevation of the availability of specific elements that are required for enzymes catalyzing chlorophyll biosynthesis and other metabolic pathways (Vafadar et al., 2014).

3.3. Global metabolic changes in maize as affected by actinobacteria and drought treatments

Photosynthesis is a key process in plant metabolism through which plants synthesize sugars which are utilized as substrates and sources of metabolic energy needed for all other metabolic processes. Therefore, regarding its notable positive impact on photosynthesis, we hypothesized that inoculation with actinobacteria could alter the chemical composition of maize, which in turn affect its behavior under drought. To test this hypothesis, we have analyzed the accumulation of several classes of primary and secondary metabolites in both inoculated and non-inoculated maize under drought as well as normal conditions.

3.3.1. Primary metabolites

The impact of drought and/or actinobacteria on the accumulation of sugars, organic acids and amino acids in leaves of maize plants was assessed (Table 4). Regarding carbohydrates, results showed that drought alone significantly increased the contents of sucrose, total soluble sugars, starch and total carbohydrates. Inoculation with

actinobacteria caused a further increase in all sugar fractions, especially sucrose, relative to the non-inoculated plants, under water deficit. The positive impact of actinobacteria on accumulation of carbohydrates could be ascribed to the observed enhancement in photosynthetic rate. In this context, the positive correlation between improved photosynthesis and accumulation of carbohydrate is well recognized (Al Jaouni et al., 2018). Moreover, the accumulation of non-structural carbohydrates such as sucrose, glucose and fructose could up-regulate the tricarboxylic acid cycle (TCA) which in turn leads to accumulation of TCA intermediate such as some organic acid (Saleh et al., 2018; Watanabe et al., 2014). Supporting this explanation, the present results revealed that drought-stressed bacterized plants accumulated significantly higher levels of oxalic, malic, succinic, citric, isobutyric and fumaric acids, as compared with non-inoculated plants (Table 4). Similarly, significant accumulation of carbohydrates was recorded in drought-stressed chickpea bacterized with PGPR (Khan et al., 2018). Moreover, the decline in photosynthesis, alterations in biosynthesis of carbohydrates and organic acids are characteristic behaviors of plants suffering drought (Dicko et al., 2018; Efeoğlu et al., 2009; Iqbal et al., 2018).

In addition to sugars, amino acids are accumulated to improve plant fitness, as they are the substrate for protein biosynthesis and some of them act as antioxidants or osmoprotectants under stress environments (Al-Alawi et al., 2017). Generally, data obtained herein revealed that drought treatment alone caused elevation in the levels of some amino acids (glutamine, proline and asparagine), while the majority of amino acids were significantly accumulated in the bacterized plants suffering water deficit (Table 4). The enhanced accumulation of amino acids in bacterized maize could be ascribed to the well-recognized role of actinobacteria in enhancing availability of soil nutrient, particularly N and P. In this context, inoculation with actinobacteria was found to increase the availability of N and P in soil and enhance the accumulation of amino acids in barley, wheat, maize, sorghum, oat and date palm (Abdelgawad et al., 2019; Hozzein et al., 2019). Moreover, similar to the present results, increased levels of some amino acids in response to drought has been reported (Bacic et al., 2011).

3.3.2. Secondary metabolites

Accumulation of secondary metabolites, such as phenolic acids and flavonoids, in response to drought stress was investigated in several plants (Ashraf et al., 2018). Similarly, the present results revealed that ferulic and p-coumaric acids and most of the detected flavonoids were significantly increased under drought stress (Table 4). On the other hand, inoculation of maize with actinobacteria resulted in increments in the levels of most the detected phenolic acids and flavonoids in normal and to more extent in drought stressed plants. Generally, the accumulation of phenolics and flavonoids under drought may help in drought tolerance as these compounds regarded as a general defense strategy of plants under stress (Verma and Shukla, 2015). However, the further accumulation of secondary metabolites in presence of actinobacteria treatment could be ascribed to the stimulated photosynthesis, which in turn provide the precursors and energy requited for the biosynthesis of these metabolites (Al Jaouni et al., 2018; Saleh et al., 2018). From another point of view, accumulation of phenolics and flavonoids may be ascribed to the secreted secondary metabolites by actinobacteria which could be absorbed and accumulated in the treated plant (Solecka et al., 2012). Supporting this explanation, the present results revealed the ability of the used actinobacteria to secrete considerable amounts of phenolic compounds and flavonoids (Tables 2 and 3). In this regard, it was reported that exogenous application of phenolic compound, either in crude or pure form, improved the contents of total flavonoids and phenolics in the target plant species (Madany and Saleh, 2015; Saleh et al., 2015). Similar to our results, treatment with plant growth promoting actinobacteria was found to promote the accumulation of phenolic compounds in several plant species (Abdelgawad et al., 2019; Hozzein et al., 2019).

Table 4

Effect of drought on the biomass (g/plant), photosynthesis (μmoles CO_2 m^{-2} leaf area S^{-1}) and the contents of amino acids (μg g^{-1} dry weight), carbohydrates, organic acids, minerals, phenolic acids and flavonoids (mg g^{-1} dry weight) of maize inoculated and not inoculated with actinobacteria. Values are the average of 3 replicates (mean ± S.D.). Different letters represent significant differences between the treatments in each group (Tukey's test P < 0.05; n = 3).

Parameter	Control	Actinobacteria	Drought	Drought + Actinobacteria
Photosynthesis	6.51 ± 1.09a	7.04 ± 0.88a	2.04 ± 0.81c	3.92 ± 1.32b
Fresh mass	4.07 ± 0.08a	4.94 ± 0.97 ab	2.08 ± 1.03d	3.35 ± 1.67c
Dry mass	0.69 ± 0a	0.78 ± 0.13 ab	0.29 ± 0.07d	0.48 ± 0.09c
Carbohydrates				
Glucose	0.77 ± 0.08c	0.98 ± 0.07 ab	1.05 ± 0.13a	1.11 ± 0.1a
Fructose	0.94 ± 0.12b	1.20 ± 0.15b	1.28 ± 0.01b	1.79 ± 0.09a
Sucrose	1.08 ± 0.15c	1.24 ± 0.18c	1.80 ± 0.23b	2.90 ± 0.40a
Soluble sugars	2.79 ± 0.46c	3.42 ± 0.32bc	4.13 ± 0.25b	5.50 ± 0.37a
Starch	14.45 ± 1.20d	20.26 ± 0.20c	23.93 ± 1.79b	27.60 ± 1.50a
Total Carbohydrates	34.82 ± 1.83d	44.68 ± 3.72c	49.71 ± 0.50b	54.30 ± 1.80a
Organic acids				
Oxalic acid	2.38 ± 0.26b	3.84 ± 0.77 ab	2.88 ± 0.41b	5.42 ± 1.08a
Malic acid	15.19 ± 1.27c	11.88 ± 0.12c	19.93 ± 1.99b	25.89 ± 1.36a
Succinic acid	1.72 ± 0.09c	2.11 ± 0.18c	2.66 ± 0.22b	3.64 ± 0.46a
Citric acid	3.16 ± 0.45cd	4.96 ± 0.45c	9.64 ± 1.21b	12.09 ± 1.73a
Isobutyric acid	3.26 ± 0.36 ab	2.02 ± 0.40b	2.90 ± 0.41b	4.15 ± 0.83a
Fumaric acid	5.25 ± 1.05c	6.21 ± 0.56c	8.52 ± 1.07b	12.75 ± 1.82a
Amino acids				
Glutamic acid	18.06 ± 1.81c	20.6 ± 1.37c	25.01 ± 3.13b	34.06 ± 4.30a
Glutamine	72.8 ± 9.10c	78.92 ± 9.86c	90.92 ± 0.91b	133.23 ± 16.70a
Lysine	4.52 ± 0.65b	3.66 ± 0.33bc	4.55 ± 0.57b	6.16 ± 0.90a
Alph-keto glutaric acid	0.03 ± 0b	0.03 ± 0.01b	0.04 ± 0.01 ab	0.05 ± 0a
Histidine	0.69 ± 0.14a	0.57 ± 0.11 ab	0.78 ± 0.16a	0.85 ± 0.20a
Alanine	20.76 ± 1.09c	29.95 ± 2.5b	27.73 ± 1.13b	43.94 ± 6.30a
Arginie	0.84 ± 0.08bc	1.02 ± 0.07b	1.07 ± 0.03b	1.73 ± 0.02a
Ornithine	0.06 ± 0.03a	0.06 ± 0.03a	0.06 ± 0.02a	0.10 ± 0.10a
Proline	0.48 ± 0.07b	0.46 ± 0.04b	0.55 ± 0.07b	0.77 ± 0.10a
Asparagine	0.72 ± 0.08c	0.96 ± 0.19b	0.88 ± 0.13b	1.35 ± 0.30a
Isoleucine	0.14 ± 0.01b	0.19 ± 0.00b	0.19 ± 0.04b	0.28 ± 0.02a
Leucine	0.11 ± 0.00bc	0.14 ± 0.01b	0.14 ± 0.01b	0.23 ± 0.01a
Methionine	0.10 ± 0.01b	0.12 ± 0.01b	0.12 ± 0.02b	0.20 ± 0.01a
Threonine	0.073 ± 0.00b	0.06 ± 0.00b	0.10 ± 0.00b	0.18 ± 0.01a
Valine	0.90 ± 0.13b	1.15 ± 0.10b	1.08 ± 0.13 ab	1.74 ± 0.20a
Serine	0.17 ± 0.02b	0.21 ± 0.04b	0.23 ± 0.03b	0.41 ± 0.10a
Phenylalanine	0.43 ± 0.04c	0.37 ± 0.01c	0.45 ± 0.06b	0.68 ± 0.04a
Tyrosine	0.60 ± 0.06 ab	0.47 ± 0.03b	0.66 ± 0.08 ab	0.8 ± 0.10a
Aspartate	0.04 ± 0.01b	0.04 ± 0.0b	0.05 ± 0.01b	0.06 ± 0.01a
Cystine	0.11 ± 0.012b	0.13 ± 0.03b	0.14 ± 0.02b	0.23 ± 0.05a
Glycine	0.60 ± 0.03b	0.42 ± 0.04b	0.59 ± 0.045b	0.79 ± 0.11a
Glycine betaine	3.96 ± 0.22c	3.34 ± 0.32c	5.8 ± 0.79b	7.93 ± 0.50a
Minerals				
K	11.71 ± 1.3a	9.11 ± 1.82a	8.83 ± 1.26 ab	10.19 ± 2.04a
Ca	17.18 ± 0.03b	20.88 ± 0.04 ab	15.92 ± 0.03b	22.24 ± 0.04a
Mg	1.46 ± 0.03a	1.3 ± 0.038a	1.23 ± 0.029a	1.45 ± 0.04a
P	8.79 ± 1.26a	8.61 ± 0.33a	6.06 ± 0.88b	7.04 ± 0.9 ab
Na	0.52 ± 0.03a	0.16 ± 0.04b	0.17 ± 0.03b	0.18 ± 0.04b
Cu	8.27 ± 1.18a	8.05 ± 0.73a	4.97 ± 0.62b	8.59 ± 1.23a
Fe	0.17 ± 0.03 ab	0.27 ± 0.04a	0.19 ± 0.03 ab	0.29 ± 0.04a
Mn	0.05 ± 0.03a	0.04 ± 0.04a	0.05 ± 0.03a	0.05 ± 0.04a
Zn	0.29 ± 0.03a	0.31 ± 0.04a	0.19 ± 0.03b	0.33 ± 0.04a
Phenolic acids				
Caffeic acid	0.03 ± 0.00a	0.03 ± 0.00a	0.03 ± 0.00a	0.03 ± 0.00a
Ferulic acid	2.86 ± 0.36 ab	3.03 ± 0.38a	3.18 ± 0.03a	3.23 ± 0.40a
Protocatechuic acid	0.23 ± 0.03 ab	0.3 ± 0.03a	0.26 ± 0.03 ab	0.32 ± 0.00a
Catechin	0.85 ± 0.09 ab	0.91 ± 0.18a	0.94 ± 0.13a	0.97 ± 0.2a
Gallic acid	22.48 ± 1.87a	19.6 ± 0.20b	24.55 ± 4.91a	20.88 ± 1.1b
p-Coumaric acid	4.19 ± 0.25b	4.84 ± 0.36 ab	4.57 ± 0.41 ab	5.29 ± 0.7a
Resorcinol	0.05 ± 0.00a	0.04 ± 0.00a	0.05 ± 0.01a	0.04 ± 0.00a
Chlorogenic acid	0.30 ± 0.04a	0.24 ± 0.06 ab	0.32 ± 0.00a	0.26 ± 0.00 ab
Syringic acid	1.33 ± 0.19a	1.34 ± 0.34a	1.47 ± 0.73a	1.43 ± 0.20a
Flavonoids				
Quercetin	1.99 ± 0.22c	2.34 ± 0.47bc	3.55 ± 0.51b	4.76 ± 0.39a
Quercetrin	0.22 ± 0.02b	0.88 ± 0.01a	0.31 ± 0.06b	0.94 ± 0.00a
Luteolin	0.07 ± 0.01b	0.07 ± 0.02b	0.11 ± 0.01a	0.14 ± 0.00a
Apigenin	0.43 ± 0.04b	0.43 ± 0.03b	0.72 ± 0.09a	0.88 ± 0.10a
Isoquercetrin	1.07 ± 0.13c	1.23 ± 0.15b	1.89 ± 0.02 ab	2.49 ± 0.30a
Rutin	1.39 ± 0.20cd	1.62 ± 0.15c	2.48 ± 0.31b	3.30 ± 0.50a
Ellagic acid	0.31 ± 0.03c	0.37 ± 0.07c	0.56 ± 0.08b	0.76 ± 0.20a
Velutin	0.37 ± 0.03c	0.45 ± 0.00c	0.67 ± 0.13b	0.92 ± 0.00a
Naringenin	0.01 ± 0.00a	0.01 ± 0.00b	0.01 ± 0.00a	0.01 ± 0.00a

Table 5

Lipids peroxidation products (MDA), proteins oxidation (as carbonyl), total peroxides (H_2O_2), ascorbate (ASC), glutathione (GSH) and tocopherols, as well as antioxidant capacity (FRAP) in maize inoculated or not inoculated with actinobacteria under normal or water deficit conditions. Values are the average of 3 replicates (mean ± S.D.). Different letters represent significant differences between the treatments in each group (Tukey's test $P < 0.05$; $n = 3$).

Parameter	Control	Actinobacteria	Drought	Drought + Actinobacteria
MDA (mg g^{-1} FW)	6.49 ± 0.08c	6.98 ± 0.97c	13.88 ± 1.03a	8.06 ± 1.67b
H_2O_2 (µmol g^{-1} FW)	344.5 ± 5.84c	359.67 ± 11.10c	459.56 ± 7.13a	398.35 ± 9.01b
Protein oxidation (mg g^{-1} FW)	1.10 ± 1.09b	1.11 ± 0.88b	2.43 ± 0.81a	1.18 ± 1.32b
ASC (µmol g^{-1} FW)	2.62 ± 0.09c	3.06 ± 0.08c	3.64 ± 0.10b	4.90 ± 0.13a
GSH (µmol g^{-1} FW)	0.2 ± 0.01c	0.21 ± 0.02c	0.49 ± 0.02b	0.60 ± 0.04a
Tocopherols (mg g^{-1} FW)	2.02 ± 0.25b	1.9 ± 0.27b	3.29 ± 0.33a	3.90 ± 0.30a
FRAP (µmole trolox g^{-1} DW)	17.85 ± 1.13c	19.74 ± 1.02c	25.10 ± 0.92b	32.90 ± 0.80a

3.3.3. Minerals

The present results revealed that drought treatment alone reduced the levels of the majority of the measured minerals. However, inoculation with actinobacteria induced elevations in almost all the detected minerals, as compared with non-inoculated plants, under water deficit conditions (Table 4). The observed decline in minerals content in maize under drought could be attributed to inhibition in their uptake due to reduced root growth and decrease rate of mineralization (Matías et al., 2011). This explanation agrees with the finding of Schimel et al. (2007), who reported that drought may slow down the availability of soil nutrients via lower mineralization and decreased diffusion, which in turn negatively influence uptake of nutrients by plant roots. On contrary, enhancement of minerals accumulation in response to actinobacteria could be attributed to the positive role of actinobacteria in supporting the availability of soil minerals via improved solubility, increased soil pH, and enhanced cation exchange capacity (Ahmad et al., 2016; Uroz et al., 2009). Moreover, actinobacteria may improve minerals accumulation in plants by enhancing root growth, which in turn enhance the uptake of water and nutrient (Naveed et al., 2014). Similar to our results, inoculation with actinobacteria was reported to enhance the accumulation of minerals in several plant species including maize (Abdelgawad et al., 2019; Hozzein et al., 2019).

3.4. Bacterized maize effectively adjusts its osmotic potential via accumulation of compatible solutes

Synthesis and accumulation of osmolytes or compatible solutes is one of the principal mechanisms whereby plants can maintain proper water uptake and cell turgidity to tolerate water stress (Rhodes et al., 2002). Compatible solutes are low molecular mass biomolecules, such as proline, arginine, glycine betaine sugars and sugar alcohols, which could be highly accumulated in the cytosol without hurting the cell (Shao et al., 2009). Beside its role in osmotic adjustment, osmolytes are believed to protect the cell compartments from oxidative damage (Sekmen et al., 2014). In the current study, significantly higher accumulation of sucrose, total soluble sugars, proline, arginine and glycine betaine were recorded in bacterized maize grown under water deficit conditions, relative to the corresponding non-bacterized plants (Table 4). This result suggests a beneficial role for actinobacteria on the process of osmotic adjustment in plants suffering water stress. In accordance, Singh and Jha (2016) found that *Serratia marcescens*-induced tolerance against salinity in chickpea was related to accumulation of osmolytes like soluble sugars.

3.5. Actinobacteria treatment improved the redox homeostasis and reduced the cell damage in maize under drought

Drought is known to induce oxidative stress in plants as indicated by accumulation of ROS and cell damage products (Hasanuzzaman et al., 2013). Such accumulation of ROS, in absence of effective protective mechanisms to maintain redox homeostasis, is known to cause cell damage via oxidation of functional components such as proteins and membrane lipids (Apel and Hirt, 2004). Similarly, the present results revealed a marked elevation in the levels of H_2O_2 and lipid peroxidation products (MDA) in non-bacterized maize in response to drought (Table 5).

To cope with oxidative stress, tolerant plants have evolved effective antioxidant systems, both molecular (ASC, GSH, tocopherols, polyphenols and some minerals such as Se, Zn, Cu and Mn) and enzymatic (superoxide dismutase, catalase and various peroxidases) antioxidants (Hasanuzzaman et al., 2013; Saleh et al., 2019). Interestingly, the present results showed that inoculation of maize plants suffering drought with actinobacteria improved redox homeostasis as indicated by lower levels of oxidative stress markers (H_2O_2 and MDA) and higher TAC, relative to the non-inoculated ones. Such improved antioxidant capacity in bacterized-maize could be ascribed to improved accumulation of molecular antioxidants. Supporting this hypothesis, significant accumulation of ASC, GSH, tocopherols, phenolic acids and flavonoids were observed in actinobacteria-inoculated maize under drought, as compared with the non-inoculated plants. Similarly, inoculation with actinobacteria was reported to reduce membrane damage in drought stressed plants (Grover et al., 2010).

4. Conclusion

Based on the results presented herein, inoculation of maize with actinobacteria represents an effective approach for enhancing growth and tolerance against drought. The positive impact of actinobacteria may be ascribed to production of IAA and siderophores which improve root architecture and enhance the uptake of water and nutrients from soil. Besides, actinobacteria seem to play a function in induction of the biosynthesis of essential metabolites that have a key role in drought tolerance. For instance, drought-stressed bacterized maize accumulated higher levels of compatible solutes, such as sucrose, total soluble sugars, proline, arginine and glycine betaine, as compared with the non-bacterized plants. Moreover, improved redox homeostasis as indicated by lower levels of H_2O_2 and lipid peroxidation products (MDA) and higher TAC was observed in the bacterized plants. The enhanced TAC is ascribed to improved levels of molecular antioxidants such as total ascorbate, glutathione, tocopherols, phenolic acids and flavonoids. Therefore, this study recommends the utilization of actinobacteria isolated from semi-arid environments as an effective approach for enhancing crop growth and productivity under water deficit conditions.

Contribution

Authors contributed equally to this manuscript.

Acknowledgments

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