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Improvement of Sorghum (*Sorghum bicolor l. Moench*) Growth and Yield under Drought Stress by Inoculation with *Bacillus cereus* and Foliar Application of Potassium Silicate

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TEN plant growth promoting rhizobacteria (PGPRs) isolated from the rhizosphere of sorghum plants were screened for production of Indole acetic acid (IAA), exopolysaccharide (EPS), phosphate solubilizing, siderophore production and Hydrogen cyanide (HCN), in addition to the ability to withstand drought and heat stresses. The best performing isolate was identified by both biochemical and molecular methods as *Bacillus* with 99% similarity to *B. cereus* strain. The *in vivo* plant growth promoting activity of identified strain on Sorghum growth (*Sorghum bicolor l. Moench*) under drought stress was evaluated at a field experiment in combination with different levels of potassium silicate. Treatments included inoculation of seeds by *B. cereus* and three levels of K silicate (50.150 and 200 ppm) as foliar spray. Drought stress was achieved by spacing between irrigation times (20 days between each irrigation). Results showed that, supplementation of K silicate (50,150 and 200 ppm) plus PGPRs inoculation increased vegetative growth, RWC% and improved electrolyte leakage, recorded 76.2 %, 82.2 % and 80.2 % respectively. Proline content recorded 23.6%, 32.1% and 34.14% respectively under deficit water. As well as the combined treatment between inoculation and K silicate improved photosynthetic pigments, chlorophyll stability index, osmotic potential, plant K^+, Ca^{2+} and Mg^{2+} accumulation, while reduced Na^+ uptake besides that enhanced Na/K ratio compared to control under drought. Also, K silicate only or with PGPRs bacteria increased the total number of bacteria and actinomycetes in rhizosphere but reduced the number of soil fungi. Antioxidant enzymes were reduced affected by the combined action of inoculation with *B. cereus* and K silicate spraying. The results proved that the combination of PGPRs plus foliar application of K silicate is favorable treatment under drought stress.

Keywords: Sorghum plant, Photosynthetic pigments, Proline, Osmotic potential, Yield and Drought Tolerance Index.

Introduction

Sorghum (*Sorghum bicolor* L.) is considering the fourth in a space as a cereal crop, after wheat, maize and rice In Egypt, also it grows well under environmental stresses such as drought (Mekdad and Rady 2016). Most of countries depend on them to obtain grains from it, like Nigeria, USA and India, while Egypt has ranked fifteen in this respect (FAO, 2012). Sorghum (*Sorghum bicolor* L.*Moench*) is a located under family *Poaceae.* Sorghum is considered very important annual cereal crop grown for obtaining grain and palatable green forage production. Sorghum can be grown in many arid and semi-arid regions of the world, due to its advantages over (Marsalis et al. 2010). Abdel-Motagally (2010) cleared that sorghum is used in a double purpose; the first purpose to vegetative parts in feeding the animal in summer season, and the other purpose to obtain grains. Drought reduces nutrient uptake by inducing nutrient deficiency by decreasing the rate of nutrients from soil to root (Ashraf and Foolad 2007). Plants can be able to tolerance to drought conditions by accumulating with Proline. Proline(a non-protein amino acid formed in the leaf tissues of plants exposed to water stress) accumulation and increased ant oxidative enzymes such as peroxidase.

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Silicon is second most element in the Earth's (Ma et al. 2006), it plays an important role in plant tolerance to environmental stresses (Gong et al. 2005; Gunes et al. 2007a, 2008). It is reported that, Si is one of the greater tolerance of higher plants to drought stress. It increases the action of antioxidant defenses; also it can be reduced the oxidative damage to functional molecules and membranes, (Agarie et al. 1998). Gong et al. (2003) reported that Si able to decrease the specific leaf area in wheat and have also been suggested to be involved in the inhibition of leaf water deficit.

PGPRsbacteria is a large group of bacteria which able to colonizes the rhizosphere of soil plants and interact with it, helping the plant to grow in both direct and indirect ways (Kloepper 1999). It can be able to protect plants from the deleterious effects of some environmental stresses (Marulanda et al. 2008), also induced systemic tolerance (IST) and induced physical and chemical changes that result in enhanced tolerance of plants to abiotic stress (Yang et al. 2009). Under drought stress, bacterial can accumulate compatible solutes such as amino acids, quaternary amines, and sugars that may prevent degenerative processes and improves cell growth under adverse osmotic conditions (Potts 1994). In this study, the effect of PGPRs (MSR H1) inoculation plus concentration of K silicate on some characteristics of sorghum has been investigated under water deficit stress.

Materials and Methods

Isolation and characterization of bacteria

Bacteria were isolated from the rhizosphere soils of sorghum plants collected from different localities of Agricultural Research Center (ARC) farm (Giza Governorate, Egypt) using serial dilution technique on nutrient agar plats at 28±2^{0}C for 72 hrs. Morphologically different colonies were picked and subjected to further purification then stored on nutrient agar slants at 4^{0}C.

Bacterial characteristics

BGPRs bacterial isolates are checked to Morphological characteristics, like shape and gram reaction as described by Bergey's manual of determinative bacteriology (Holt et al.1994) and to motility by hanging drop methods as described by Bertand et al. (2001). The bacterial isolates were coded from (H1-H10).

Screening of isolates for plant growth-promoting potential

The bacterial isolates were screened for gram reaction and plant growth-promoting activities such as, IAA (Indole acetic acid) production, as described by Sarwar et al. (1992). Exopolysaccharides synthesis was determined using the method described by Ashraf et al. (2004). Siderophores production was tested to all isolates according to Schwyn and Neilands (1987). The solubilized phosphates were described by methods of Mehta and Nautiya (2001). Production of cyanide (HCN) was measured as described by Bakker and Schippers (1987). According to the previous tests, ten isolates were selected for drought and heat tolerance assay.

Drought Tolerance Assay for selected isolates

Test tubes containing seven ml sterilized nutrient broth supplemented with different concentration of polyethylene glycol (PEG6000) 0, 10, 20, 30 % were inoculated with 0.5 ml of 24h old culture of each isolate then incubated at 28 ± 1ºC for 96 h in three replicates, at the end of incubation period, optical density for all isolates was measured by spectrophotometer at 600 nm.

Thermo tolerance assay

Isolates were streaked on nutrient agar plates and incubated at different temperatures (30, 45 and 50^{0}C) for 48 h, at the end of incubation period; plates were investigated for positive or negative growth.

Identification of the most potent bacterial isolate

According to drought and heat tolerance assay one bacterial isolate was identified by 16S rRNA gene sequencing, using PCR master mix (Promega, Madison, WI, USA) with bacterial universal primer sets 27F and 1492R (27F: 5′-AGA GTT TGA TCC TGG CTC AG-3′ and 1492R: 5′-TACGGYTACCTT GTTACGACT T-3′). Resolved 16S rRNA gene sequences were BLAST searched against the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) database (Altschul et al., 1997). Multiple alignments of the nucleotide sequences were performed with the program MUSCLE (Edgar 2004). The phylogenetic tree was constructed by the Neighbor-Joining method (Saitou and Nei 1987), based on the Kimura 2-parameter model (Kimura 1980), with bootstrap analysis (1,000 replications) using the software MEGA (version 7) (Kumar et al. 2016).

Evaluation of Bacillus cereus strain MSR-H1 for growth of sorghum in field trail under drought stress

During 2016 /2017 at Giza Research

Experimental Station, ARC, Giza Govern., Egypt (latitude of 29°26′N and longitude of 31°13′E) a field experiment was designed to evaluate the potential of *B. cereus* in alleviation of drought stress on sorghum plant. The treatments included combinations between inoculation with *B. cereus* and spraying with different concentrations of potassium silicate (K_2SiO_3, 99% purity) as follow:

T1: control under normal irrigation,
T2: control under drought stress,
T3: plants under drought sprayed with 50ppm K-Si solution,
T4: plants under drought sprayed with 150ppm K-Si solution
T5: plants under drought sprayed with 200ppm K-Si solution,
T6: plants under drought inoculated with *B. cereus*,
T7: plants under drought inoculated with *B. cereus* and sprayed with 50ppm K-Si solution,
T8: plants under drought inoculated with *B. cereus* and sprayed with 150ppm K-Si solution,
T9: plants under drought inoculated with *B. cereus* and sprayed with 200ppm K-Si solution. Drought stress was achieved by irrigating the plants every 20 days. The recommended doses of the chemical fertilizer (NPK) were used according to the recommendation of Agriculture and Land Reclamation, Egypt. Experimental treatments were laid out in a randomized complete block design (RCBD) with four replications, the plot area was 6 m^2 (sub-plot sizes 2× 3 m) = 1/400 feddan (feddan = 4200 m^2). Each plot included 3 rows with 30 cm row spacing.

Soil physicochemical properties were analysis according to Blake (1982).

Inoculation technique

drought – sensitive sorghum seeds (Shandaweel-1) (*Sorghum bicolor* L. *Moench*) obtained from field Crops Research Inst., (ARC), Giza, Egypt were inoculated with *B. cereus* (10^9 CFU/ml) carried on vermiculite carrier using Arabic gum as adhesive agent.

Data collection

Vegetative growth characteristics: Sample of 10 plants were randomly taken at physiological maturity from two internal rows after 45 and 75 days to measure, Leaf dry weight (LDW), shoot dry weight (SDW), root dry weight (RDW), total dry weight (TDW) and Shoot/Root ratio (R/S).

Relative water content (RWC%)

To evaluate the water status during the stress period, relative water content RWC was used, according to the equation described by Kaya and Higgs (2003) Cut leaves were weighed (fresh weight, FW), then left saturated in water for three hours and their turgid weights (TW) were calculated. The samples were then dried in an oven at 80°C for 24 hours and weighed (ODW). The RWC is determined as follows:

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

Electrolyte leakage (%)

Electrolyte leakage was determined according to protocol as described by Awad et al. (2006).

Photosynthetic pigments and Proline determination

Photosynthetic pigments(chlorophyll a,b, Total chlorophyll and carotenoid’s) were measured and calculated as described by Nornai (1982) as well as the proline content was determined according to Bates et al. (1973)

Chlorophyll stability index

The (CSI) was determined according to protocol as described by Sairam et al. (1997):

$$CSI = (\text{Total Chl under stress} / \text{Total Chl under control}) \times 100$$

Microbial populations

The total bacteria and fungi count in rhizosphere soil after 45 and 75 days from planting were determined using nutrient agar media (Difco 1985) and Martin (1951) for fungi count respectively. Total actinomycetes were determined as described by Rolf and Bakken (1987). Silicate bacteria were also determined according to Zahra (1969).

Antioxidant Enzymes Assays

Antioxidant Enzymes like: Catalase (CAT) enzyme was determined according to the method of Aebi (1983). Ascorbate Peroxidase (APX) was determined according to Nakano and Asada (1981). Super Oxide Dismutase (SOD) was determined according to Donahue et al (1997).

Osmotic potential in leaves of plants

The osmotic potential leaves were measured from flag leaves according to the method of Capell and Doerffling (1993).

Biochemical constituents

Total nitrogen and total phosphorus in shoots were determined according to Black (1982). Ions like K^+, $Na,^+Mg^{2+}$ and Ca^{2+}determantion according to Wolf, (1982). The initial pH of the soil was measured after adding potassium silicate using benchtop pH meter (Orion 2-Star; Thermo Scientific, USA), as described by (Margesin and Schinner 2005).

Yield parameters

Five representative guarded plants were taken from each Plot to determine, plant height (cm), grain panicle, grain yield, 100 grains weight and Biological yield were also determined.

Drought Tolerance Index

Drought or stress tolerance index of the sorghum was calculated as protocol described by (Ahmad, 2011). Tolerance (TOL) and Yield stability index (YSI) were calculated using the following relationships Farshadfar and Sutka (2002)

$$TOL=Y_s -Y_p : YSI=Y_s/Y^p$$

In the above formulas, Ys, Yp, represent yield under stress, yield non-stress for cultivar.

Statistical analysis

Data were statistically analyzed by analysis of variance (ANOVA) using MSTATC program version 2.10 (1991). The least significance difference (LSD) test (0.05) was used to find out the significance of mean difference of various treatments (Gomez and Gomez, 1984).

Results

Soil physiochemical properties

Soil used was clay loam type with 33.4% Clay, silt 35.6%, fine sand 19.6 %, and coarse sand11.2%, with a pH 7.3 and EC dS m^{-1} 2.6. Soluble elements as cat ions and anions (mg L^{-1}) as follows: for cations: K^+, Na^+, Mg^{++} andCa^{++} were1.52, 8.16, 6.67 and 9.20, respectively. Anions were SO_4^-13.07, Cl^-11.13, HCO^{3-} 1.35, CO_3^- 0.00.

Isolation of rhizobacteria

A total of ten bacterial isolates obtained from the rhizosphere soil of sorghum plant using plate count technique on nutrient agar were screened for plant growth promoting activity (indole acetic acid, E.P.S production, phosphate solubilizing and siderophore production (Table 1). Higher IAA and exopolysaccharides production were noted for isolates H1 (16.4 mgL^{-1}) and (6.8 g/100ml) respectively, while isolate H6 gave lower IAA production and EPS production (11.5 mgL^1) and (3.2 g/100ml). Isolates No (2.3.4 and 9) were not able to phosphate solubilizing in media while isolates No. (1,4,5,6,7 and 10) can able to phosphate solubilizing in media. All tested isolates were able to produce siderophores while in case of HCN, isolates No (H6, H7, H10) gave negative results for the test.

TABLE 1. Characteristics of selected PGPRs bacteria for plant growth promoting activity.

Isolates	Gram reaction	Indole acetic acid (IAA) (mgL^{-1})	Exo polysaccharides (E.P.S) (g/100 ml	phosphate solubilizing	Siderophore production	HCN production
H 1	+	16.4	6.8	+	+	++
H 2	-	13.2	5.9	-	++	++
H 3	+	14.7	4.6	-	+	+
H 4	-	12.3	5.3	+	+	+
H 5	+	13.9	4.1	+	++	+
H 6	-	11.5	3.2	+	+	-
H 7	-	13.1	4.2	+	++	-
H 8	+	13.5	3.9	-	+	+
H 9	-	12.2	4.8	-	+	+
H 10	-	13.9	3.5	+	+	-
L.S.D at 0.05		n.s	n.s			

-- Negative growth, + positive growth, n.s non significant

Screening isolates for drought tolerant stress and thermo tolerance

Ten bacterial isolates screened for drought and heat tolerance (Table 2). All isolates were able to grow at different concentration of (PEG %). Isolates H1 showed the highest optical density followed by isolate H5 at PEG 10%, while at 20% PEGH 1, H 2 and H 8 recorded the highest growth. At concentration 30% from (PEG) H1, H3 and H5 recorded highest growth while H4 recorded lowest growth.H1 was the most tolerant isolate among the others. Besides that, isolate H1can grow at 45^0C followed by isolates H2 and H5, whereas isolates H4, H6, H8, H9, and H10 cannot grow at 40^0C and 45^0C.

Identification of the most potent isolate

According to previous results, isolate H1 was selected to complete the study and identified by16S rRNA sequences technique. Based on the 16S phylogenetic classification, the isolate MSR_

TABLE 2. Screening isolates for drought and heat stresses tolerance.

Isolates	Drought tolerant at (OD) 600 nm				Heat tolerance		
	PEG %				Temperature		
	0	10	20	30	40^0C	45^0C	50^0C
H1	1.02	0.45	0.22	0.10	+	+	-
H2	0.80	0.39	0.20	0.08	+	+	-
H3	0.83	0.40	0.17	0.09	+	-	
H4	0.66	0.30	0.12	0.05	-	-	-
H5	0.70	0.42	0.19	0.09	+	+	
H6	0.44	0.25	0.17	0.08	-	-	-
H7	0.33	0.20	0.11	0.06	+	-	
H8	0.36	0.19	0.20	0.08	-	-	-
H9	0.44	0.30	0.12	0.07	-	-	-
H10	0.40	0.29	0.18	0.08	-	-	-

+ Positive growth,- negative growth.

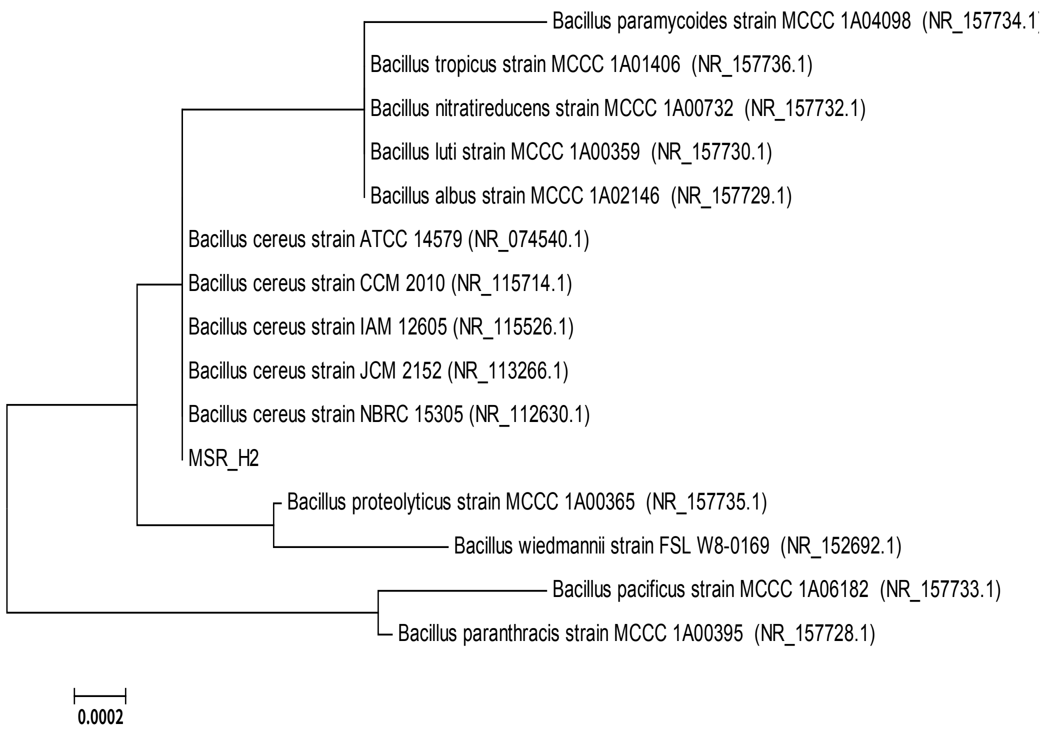

Fig. 1. Phylogenetic tree of rhizosphere bacteria based on 16S rRNA gene sequence comparison. Evolutionary relationships of the bacterial strains inferred using the Neighbor-Joining method and the evolutionary distances were computed using the Kimura 2-parameter method. GenBank accession numbers of strains are presented in parentheses. Bootstrap test (1,000 replicates).

H1 was found to belong to phyla Firmicutes and were highly aligned with the genera *Bacillus* with close relation to *Bacillus cereus* (99%) sequence similarity. We proposed the name of our isolate as *Bacillus cereus* strain MSR_H1 (Fig. 1).

Field experiment

Vegetative growth

Results of vegetative growth parameters (Leaf dry weight (LDW), shoot dry weight (SDW), root dry weight (RDW), total dry weight (TDW) and (Shoot/Root ratio) as affected by inoculation with MSR-H1plus different rates of K silicates under drought stress are presented in (Table 3). There is a significant variation as a result of application of K silicate only or when combined with inoculation with bacteria in all growth parameters compared to control. Drought reduced all growth parameters while the highest growth rate was associated with 150 ppm application plus bacteria, on the other hand, the small concentrations of K silicates (50 ppm) only was less pronounced. The highest growth parameters (11.17gLDW, 35.84 gSDW and 27.01gRDW) were obtained at T8 treatment (inoculation with bacteria and spraying by 150 ppm K silicates) after 45 days after sowing).

TABLE 3. Effect of bacterial inoculation and foliar application of Potassium silicate rates on Leaf dry weight (LDW), shoot dry weight (SDW), root dry weight RDW), total dry weight (TDW) and Shoot/Root ratio (S/R) ratio under drought stress conditions on sorghum plants during season 2017.

Treatments	LDW (g)		SDW (g)		RDW (g)		TDW (g)		Shoot /Root ratio	
	45th d	75th d	45th d	75th d	45th d	75th d	45th d	75th d	45th d	75th d
T1=Control	16.97	18.61	36.14	38.71	29.28	34.11	66.17	72.81	1.23	1.13
T2=Drought	5.44	9.28	20.51	26.14	16.04	19.81	36.55	45.95	1.28	1.32
T3=K-Si 50	7.18	12.04	26.07	28.00	22.61	24.21	48.68	52.11	1.15	1.16
T4=K-Si 150	8.04	13.08	24.07	28.21	20.44	24.68	44.48	52.99	1.18	1.14
T5=K-Si 200	6.41	10.40	22.07	26.77	20.74	21.71	44.81	48.48	1.07	1.23
T6=*B. cereus*	9.10	14.64	29.28	31.58	23.06	27.14	52.34	57.32	1.27	1.16
T7=*B. cereus* + K-Si 50	9.91	14.31	29.21	31.54	23.11	27.31	52.32	72.26	1.26	1.15
T8=*B. cereus* + K-Si 150	11.17	16.28	35.84	37.00	27.01	29.81	62.85	66.82	1.34	1.24
T9=*B. cereus* + K-Si 200	9.24	14.84	31.04	37.04	26.71	26.57	57.72	61.81	1.14	1.39
L.S.D at 0.05	0.336	0.472	0.550	0.959	0.496	0.440	0.601	0.687	------	------

Physiological characteristics

There was a significant effect of MSR-H1and Si application on Physiological characteristics including relative water content RWC, electrolyte leakage and Proline content of sorghum grown under drought stress (Table 4). In general, PGPRs inoculation plus K-Si significantly increased RWC under drought treatment decreases. The lowest RWC was noted 61.6% at T3 (Si 50 ppm). Conversely, RWC improved by inoculation with bacteria plus applied K silicate 50, 150 and 200 ppm recorded 77.1, 81.2 and 81.1 respectively. Electrolyte leakage (%), reduction in electrolyte leakage was 22.47%in case of T3 (K-Si 50 ppm) compared with control. Conversely, inoculation with PGPRs plus Si (50, 150,200ppm) improved electrolyte leakage, recorded 76.2 %, 82.2 % and 80.2 % respectively, compared to control under drought. Under water deficit conditions, sorghum plants received Si concentration showed lower Proline concentration in leaves (Table 4). Minimum proline content was (6.7 mg/g d.w) found in T1 treatment (control under normal conditions) which increased by 58.65% in case of T2 (control under drought conditions), conversely, the proline content was reduced by applied Si concentrations 50, 150, 200 ppm

TABLE 4. Effect of bacterial inoculation and foliar application of Potassium silicate rates on relative water content (R. W.C %), Electrolyte leakage (%) and Proline content on sorghum plant under drought stress conditions during season 2017.

Treatments	R.W.C (%)	Electrolyte leakage (%)	Proline (mg /gd. w)
T1=Control	98.9	51.1	6.7
T2=Drought	72.0	91.2	10.63
T3=K-Si 50	61.6	70.7	9.5
T4=K-Si 150	64.5	71.8	8.8
T5=K-Si 200	61.3	70.9	8.4
T6=*B. cereus*	76.5	73.6	8.6
T7=*B. cereus* + K-Si 50	77.1	76.2	8.1
T8=*B. cereus* + K-Si 150	81.2	82.2	7.2
T9=*B. cereus* + K-Si 200	81.1	80.2	7.0
L.S.D at 0.05	0.927	0.916	0.270

recorded 10.63%, 17.21%, 20.97% respectively. With $PGPR_S$ inoculation plus concentration of Si application (50,150 and 200 ppm) the reduction in of proline content were 23.6%, 32.1% and 34.14% respectively.

Photosynthetic pigments

Water deficit caused a significant decrease in photosynthetic pigments, the reduction in Chl.a, Chl.b, Carotenoids and total chlorophyll were 50%, 68%, 61% and 41% respectively after 45 DAS. Application of Si had significant effects on photosynthesis; it improved chlorophyll a, b and carotenoids as well as total chlorophyll in all treatments compared to control under drought (T2). Spraying with K-Si 50 (T3) and 150 (T4) ppm only led to increase in the Chla (20%, 25 %) over control (T2) after 45 days. Generally, applied to Si 200 ppm had a little effect on photosynthesis. Inoculation with PGPRs only (T6) or with K-Si

TABLE 5. Effect of bacterial inoculation and foliar application of Potassium silicate rates on chlorophyll a, chlorophyll b, carotenoids and total chlorophyll under drought stress conditions on sorghum plants during season 2017.

Treatments	Chl.a (mg /g. fresh weight)		Chl.b (mg /g. fresh weight)		Carotenoids (mg /g. fresh weight)		Total chlorophyll (mg/g. fresh weight)	
	45th d	75th d	45th d	75th d	45th d	75th d	45th d	75th d
T1=Control	6.64	7.94	3.34	6.01	2.01	2.92	6.77	7.31
T2=Drought	3.28	3.86	1.04	2.78	0.77	0.90	3.95	4.21
T3=K-Si 50	3.96	4.07	2.58	3.02	0.59	1.00	4.01	5.55
T4=K-Si 150	4.11	4.11	2.64	3.21	1.00	1.20	4.87	5.85
T5=K-Si 200	4.01	4.11	2.00	3.07	0.89	1.00	4.15	5.82
T6=*B. cereus*	4.68	5.65	2.24	4.71	1.04	1.00	5.88	6.57
T7=*B. cereus* + K-Si 50	5.01	6.08	2.96	4.75	1.72	1.80	5.92	6.89
T8=*B. cereus* + K-Si 150	5.21	6.80	3.06	5.11	1.83	1.93	5.89	7.00
T9=*B. cereus* + K-Si 200	5.00	6.00	2.89	5.00	1.73	1.88	6.00	6.37
L.S.D at 0.05	0.390	0.391	0.197	0.1 97	0.059	0.0 59	0.223	0. 223

application (50, 150ppm) mitigated the adverse drought effects, the highest increase was recorded with PGPRs plus 150 ppm recorded 76% increase in Chl a, 83% in Chl b, 114% in carotenoids and 66% in total chlorophyll after 75 DAS over control (T2) (Table 5).

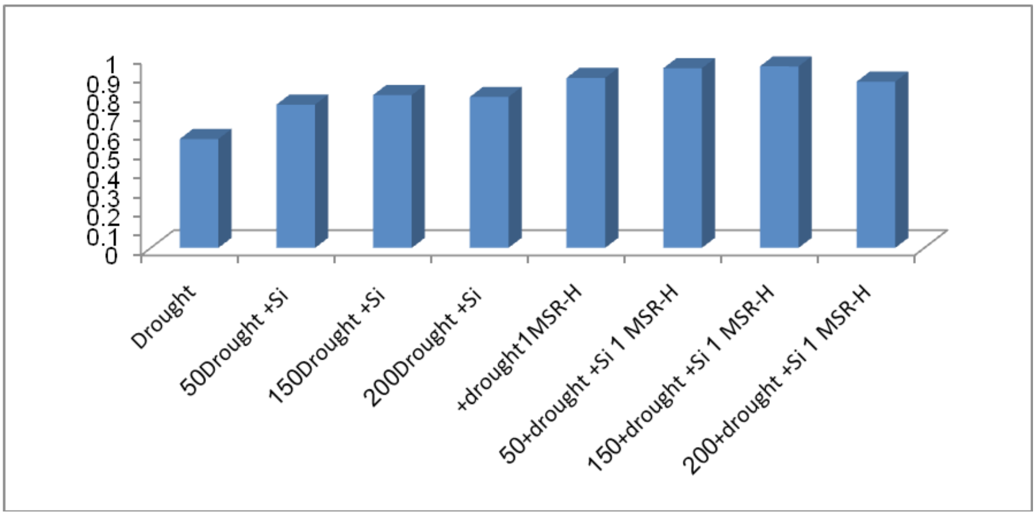

Fig. 2. Effect of bacterial inoculation and Si application on chlorophyll stability index (CSI) of sorghum plant under drought stress conditions.

Chlorophyll stability index

The chlorophyll stability index decreased under water deficit conditions (Fig. 2), conversely, it increased with application with K-Si alone or in combination with bacterial inoculation. The highest chlorophyll stability index noted with inoculation by MSR-H1 (T6) followed by application of Si 150 ppm plus MSR-H1 (T8).

Soil Biological activities

Microbial population

Control under drought (T2) recorded the lowest total bacterial count, total fungi, actinomycetes and silicate bacteria 6.37×10^6,10.05 × 10^4,3.27×10^4 and 15.47×10^{4}CFU·g^{-1} soil respectively after 45 days. The highest total bacteria count was noted with inoculation with PGPR plus 150 ppm K-Si (T8) application 39.77×10^6 after 75 days, while at the same treatment fungal population recorded

TABLE 6. Effect of bacterial inoculation and foliar application of Potassium silicate rates on total bacterial, fungal and Actinomycetes count (CFU) under drought stress conditions on sorghum plants during season 2017

Treatments	Bacteria (x10^6 CFU g^{-1} soil)		Fungi (x10^4 CFU g^{-1} soil)		Actinomycetes (x10^4 CFU g^{-1} soil)		Silicate bacteria (x10^4 CFU g^{-1} soil)	
	45th d	75th d	45th d	75th d	45th d	75th d	45th d	75th d
T1=Control	30.6	55.66	27.3	33.68	7.25	9.3	42.47	50.13
T2=Drought	6.37	10.25	10.05	12.07	3.27	4.88	15.47	19.16
T3=K-Si 50	14.89	20.54	13.11	13.11	1.60	2.56	22.67	27.14
T4=K-Si 150	17.56	25.77	13.15	13.58	1.8	2.68	24.45	26.34
T5=K-Si 200	17.25	23.36	12.83	13.28	1.66	2.3	23.85	31.93
T6=*B. cereus*	23.48	33.40	11.22	11.25	3.47	4.04	33.07	38.04
T7=*B. cereus* + K-Si 50	22.44	39.91	9.53	10.14	4.36	5.62	36.38	40.04
T8=*B. cereus* + K-Si 150	28.92	39.77	8.06	10.88	5.42	5.51	34.79	42.05
T9=*B. cereus* + K-Si 200	27.55	38.10	9.78	10.19	5.47	5.24	36.77	44.04
L.S.D at 0.05	1.114	3.104	0.447	0.291	0.668	0.696	4.097	1.266

the lowest value among other treatments 9.78 and 10.19×10^4 after 45 and 75 days respectively. Actinomycetes recorded the highest number in case of MSR-H1plus K silicates 50 ppm. The highest population of silicate bacteria was recorded at 75 days with inoculation PGPRs plus 200ppm K silicates 44.04×10^4CFU g^{-1} soil (Table 6).

Soil pH

Changes in the pH of soil due to K silicates application are presented in Fig. 3. Data illustrated that there is no significantly increased in soil pH during the experiment trite after 45 and 75 DAS. However, K silicate slightly influences in soil pH and low it by up to 7.2 equivalents making the soil more natural. Microbial populations are affected with pH of soil (alkaline or acidic). Under drought conditions the pH of soil was decreased after 45 and 75 DAS recorded 6.8 and 6.7 respectively. In this study, the concentration of silicon application had a little effect in the soil pH during the state of growth sorghum plants whereas compared with under drought stress the pH of soil decreased.

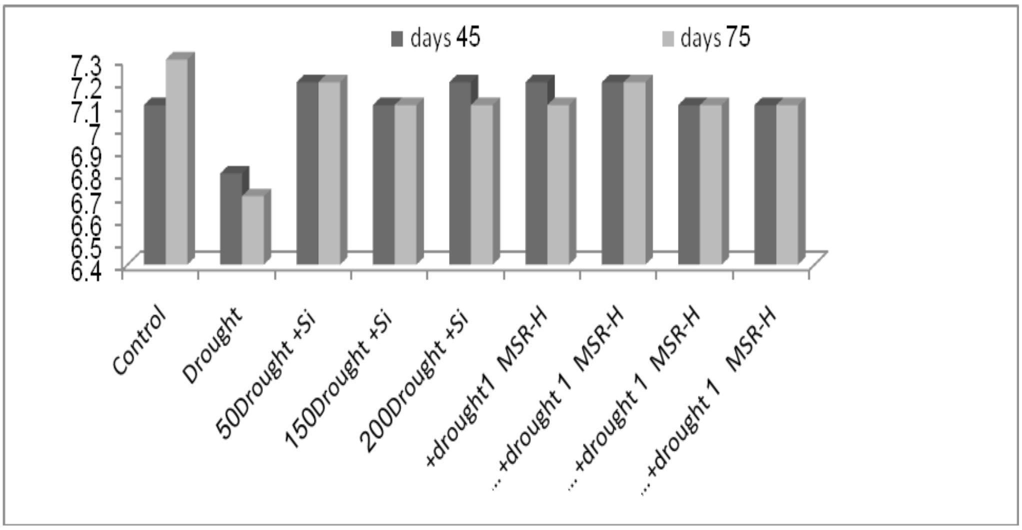

Fig. 3. Effect of bacterial inoculation and Si application on soil pH of sorghum plants under drought stress conditions.

Antioxidant activity

Under drought stress T2, Catalase (CAT) recorded the highest activity 24.3 (µmol H_2O_2 mg^{-1} protein min^{-1}), Ascorbate Peroxidase (APX), 19.9 (µmol ASA mg^{-1} protein min^{-1}) and 15.7 (µmol /100g FW) for Super Oxide Dismutase (SOD). Conversely application of K silicates only 50,150 and 200 ppm led to reduction in the

TABLE 7. Effect of bacterial inoculation and foliar application of Potassium silicate rates on ant oxidative enzymes under drought stress conditions on sorghum plants during season 2017.

Treatments	CAT µmol H_2O_2 mg^{-1} protein min^{-1}	APX µmol ASA mg^{-1} protein min^{-1}	SOD µ /100g FW
T1=Control	8.8	6.8	8.8
T2=Drought	24.3	19.9	15.7
T3=K-Si 50	13.2	14.4	13.7
T4=K-Si 150	12.8	15.1	13.9
T5=K-Si 200	10.9	14.13	11.4
T6=*B. cereus*	11.7	14.4	11.8
T7=*B. cereus* + K-Si 50	14.3	12.2	11.9
T8=*B. cereus* + K-Si 150	10.7	12.3	10.9
T9=*B. cereus* + K-Si 200	10.0	11.7	7.0
L.S.D at 0.05	0.569	0 .859	0.412

activity of CAT enzyme 45.67 %, 47.3% and 55.14% respectively compared with control under drought. Also, inoculation with $PGPR_S$ bacteria plus application of K silicates significantly reduced the activity of CAT 41.15%, 55.96% and 58.84% respectively, and APX14.4, 12.2 and 11.7 µmol and SOD 11.9, 10.9 and 7.0 µ /100g FW

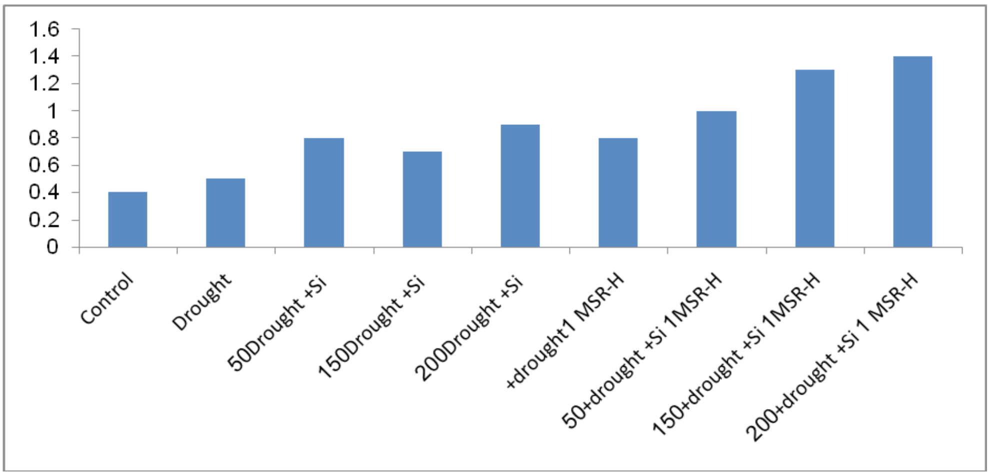

Fig. 4. Effect of bacterial inoculation and Si application on osmotic potential of sorghum plants under drought stress conditions.

compared to control (T2) (Table 7).

Osmotic potential

Data showed that application with K silicates recorded an increase in osmotic potential compared to control. Inoculation with $PGPR_S$ plus application K silicates recorded higher osmotic potential, inoculation with PGPRs plus 200 ppm from K silicates under drought stress gave highest osmotic potential compared with control (Fig. 4).

Yield components

Data presented in Table 8 show that, generally, drought stress significantly reduced plant height, grain panicle, grain yield, 100 grains weight and biological yield. There were significant differences among various treatments. The plant height reduced about 20%, in control under drought compared to control under normal irrigation. Spraying with K silicates 50, 150 and 200ppm improved plant height about 5%,11% and 5 % respectively compared to control under drought while the increase percent in case of inoculation with PGPRs plus concentration from K silicate 50, 150 and 200 ppm were 10%, 15% and 11% respectively. Inoculation with PGPR plus 150 ppm of K silicate under drought stress recorded the highest grain panicle and grain yield recorded 49.3 g^{-1} and 2.00 (ton/fed) respectively. The reduction caused in control under drought in case of 100 grains weight and biological yield was 48% and 58% respectively compared to control under normal irrigation. The maximum 100 grains weight 26.2 (g) was achieved by the treatment by PGPRs plus 150 K silicate. It was a significant difference by drought stress on biological yield, the reduction in biological yield under drought was 58.42 % while inoculation PGPRs plus 150 ppm K silicate recorded 99.9 (g $plant^{-1}$) respectively.

Drought tolerance index (DTI), Tolerance (TOL) and Yield stability index (YSI)

The DTI was significantly affected by drought, Si and PGPR (Table 8). Bacterial inoculation plus K silicate helped sorghum plants to grow under water deficit stress. Data showed that the highest DTI (0.92) noted for inoculation with PGPR plus 150ppm K silicate and the lowest DTI (0.84) noted for treatment with K silicate 50 ppm. The DTI in case of treatment with K silicate 50, 100 and 200 ppm recorded 0.84, 0.88 and 0.84 respectively, while inoculation with PGPRs bacteria only under drought stress recorded 0.90 DTI.

Mineral Contents

Under water deficit stress, result (Table 9) showed that foliar application of 50, 150 and 200 ppm K silicate increased all ionic concentrations. K silicates 150 ppm recorded highest N, P, K, Ca and Mg recorded 27.6, 2.7, 24.5,4.01 and 2.43 (mg g^{-1}) respectively. While Ksili cate 50 ppm reduced Na uptake and enhanced Na/K selectivity ratio recorded 4.81 and 0.21. (mg g^{-1}) respectively. Application of Si and PGPR improved shoot N concentration by 42%, 46% and 40% respectively. The improvement in shoot P was 80%, 85% and 50% with Si (50,150,200 ppm) application and PGPRs respectively. Na^+ concentration in control

TABLE 8. Effect of bacterial inoculation and foliar application of Potassium silicate rates on yield component of sorghum plant under drought stress conditions on sorghum plants during season 2017.

Treatments	Plant height (cm)	Grain panicle ^{1}(g)	Grain yield (Ton fed^{-1})	100 grains weight (g)	Biological yield (g) Plant^{-1}	Drought tolerance index	Tolerance (TOL)	Yield stability index (YSI)
T1=Control	142.1	52.3	2.65	31.6	112.1	---------	------------	----------
T2=Drought	113.1	30.7	1.22	16.2	64.6	0.79	47.5	0.57
T3=K-Si 50	119.7	38.9	1.52	23.2	81.2	0.84	30.5	0.72
T4=K-Si 150	125.7	40.3	1.52	24.0	85.5	0.88	26.6	0.76
T5=K-Si 200	119.6	38.9	1.58	23.1	81.2	0.84	30.9	0.72
T6=*B. cereus*	128.3	44.8	1.80	25.0	98.1	0.90	14	0.88
T7=*B. cereus* + K-Si 50	125.0	46.3	1.96	25.4	96.4	0.88	15.7	0.86
T8=*B. cereus* + K-Si 150	130.9	49.3	2.00	26.2	99.9	0.92	12.2	0.89
T9=*B. cereus* + K-Si 200	125.6	46.9	1.94	24.2	94.3	0.88	17.7	0.84
L.S.D at 0.05	1.225	1.01 2	0.171	0.477	3.259	-----------	-----------	-----------

TABLE 9. Effect of bacterial inoculation and foliar application of Potassium silicate rates on macronutrient (N, P, K, Na, Ca, Mg and Na/K ratio) in shoots of sorghum plants under drought stress conditions during season 2017.

Treatments	Nutrients (mg g^{-1} plant)						
	N^{-3}	P^{-3}	K^{+}	Na^{+}	Ca^{+2}	Mg^{+2}	Na^{+}/K^{+} ratios
T1=Control	30.6	3.8	36.93	2.31	7.51	4.58	0.06
T2=Drought	18.8	2.0	19.8	5.24	3.68	2.31	0.26
T3=K-Si 50	25.8	2.7	23.4	4.81	3.97	2.34	0.21
T4=K-Si 150	26.6	2.7	24.5	4.11	4.01	2.43	0.17
T5=K-Si 200	24.4	2.6	22.9	3.98	3.90	2.27	0.17
T6=*B. cereus*	25.7	3.7	25.2	3.92	4.96	2.76	0.16
T7=*B. cereus* + K-Si 50	26.7	3.6	31.61	3.68	5.01	3.01	1.01
T8=*B. cereus* + K-Si 150	27.6	3.7	52.4	3.10	5.88	3.57	0.06
T9=*B. cereus* + K-Si 200	26.4	3.0	31.13	3.03	5.87	3.48	0.27
L.S.D at 0.05	0.651	0.093	9.950	0.083	0.282	0.187	----------

treatment was 2.31 mg g^{-1} which was increased to 5.24 mg g^{-1} compared to control. Application of K silicate plus PGPRs led to decreased shoot Na in all treatments. Application K silicate 50,150 and 200 ppm recorded 4.81, 4.11 and 3.98 (mg g^{-1}) respectively. The highest reduction in Na recorded with PGPRs plus 200ppm followed to PGPRs plus K silicate 150 ppm recorded 3.10 (mg g^{-1}) compared to drought stressed plants without any amendment.

Discussion

In this study, ten bacterial isolates were obtained from the rhizosphere of sorghum plants grown in Giza Govern., Egypt. The isolates are characterized to morphologically & biochemically tested according to the Burges manual. These bacteria can produce phytohormones to stimulate plant growth under drought stress. IAA may play an important role in the promoting ability Radwan et al., (2002) and Torres et al. (2000) they found that PGPRs are able to produce IAA, gibberellins and cytokines in vitro and these phytohormones can help plants to grow and increase the germination rate.

Bacillus cereus MSR-H1 are able to phosphates production enzymes and these results are harmony with Cheng et al. (2017) and Agnieszka et al. (2018) they found that *Bacillus spp* is responsible role for releasing available forms of phosphorus (such as tricalcium phosphate, di calcium phosphate, and rock phosphate) to host plants in the soil through the production of organic acids and increase in activity of acid phosphatases responsible for the mineralization of organic phosphorous. PGPRs can produce exopolysac charides (E.P.S) which helps in the development of biofilm, protection from extremities and cement soil aggregates (Vanderlinde et al., 2010). *Bacillus cereus* MSR-H1 can able to siderophores production and the cyanide in vitro, Lalloo et al. (2010) mentioned the siderophore production from *Bacillus cereus* and they found that it can produce siderophore. José et al. (2017) showed that PGPRs inoculation led to increase in the tolerance of maize to drought stress and increased the growth parameters.

Isolate H1 can grow at media containing PEG (10, 20,30 %), similar results recorded by Amrani et al. (2010); Fitouri et al. (2012) they reported that some species can grow in media counting PEG up to 25% in YMB media. Similarly, 45 strains of Rhizobium can grow in 60% PEG. Drought stress caused a significant ($P \leq 0.05$) reduction in LDW, SDW and RDW. Showemimo and Olarewaju (2007) cleared that drought is biotic stress factors it, decreased the rate of growth, development and yields.

Vegetative growth parameters of sorghum plant were significantly improved by using foliar application of potassium silicate compared to the untreated ones. The foliar application of potassium silicate at 150 ppm caused significant increase in LDW, SDW, RDW; these results are in complete accordance with (Taiichiro et al., 2005) which found that, the application of silicon led to increase in shoot heights and dry matter production.

Resulted indicated that inoculation with PGPRs plus application of K-Si under drought stress improved plant growth, these results are harmony with Taiz and Zeiger (2006), they found that drought-induced reduction in plant growth due to the mitigation in turgor pressure under drought conditions but inoculation of PGPRs plus Si with stress alleviated the detrimental effects of drought and increased plant growth and yield. PGPRs not only could help the germination of plant seed and consequent root elongation (Muralikannan and Anthomiraj 1998). RWC and electrolyte leakage are the indicatives of metabolic activeties within plants, these used for to different abiotic stress like drought. Under drought stress the RWC and electrolyte leakage of sorghum plants, Showemimo and Olarewaju (2007) reported that, drought stress increased electrolyte leakage and caused membrane instability in plants. Application of Si and inoculation of PGPRs alleviated drought stress effects and reduced electrolyte leakage.

Si supplementation could improve water storage within plant tissues in tomato by reducing transpiration and can reduce the bad effect of drought by accumulation of proline and soluble protein content (Romero-Aranda et al. 2006). (Sapre and Vakharia, 2016). Mauad et al. (2016) found that, proline content was reduced under water deficiency conditions, which could be an indicator of stress tolerance. Si and PGPRs could improve chlorophyll parameters and provide useful information on photosynthetic metabolism in stressed plants (Kastori et al., 2000) Maghsoudi et al. (2015). Oliveira et al. (2016) found that foliar application of silicon improved chlorophyll pigment (Chl a, b and carotenoid's) concentration of wheat under water deficit conditions. High chlorophyll stability indices are a sign that plants withstand stress through better availability of chlorophyll(Madhan Mohan et al., 2000). K-Silicate application may cause significant decrease in soil fungi (Brecht et al., 2001).

Some bacterial species can solubilize silicates, phosphates by production of organic acids such as 2 keto-gluconic acids, alkalis and polysaccharides (Joseph et al., 2015). pH is helping microorganisms; to growth and improved the value of soil nutrient. Lauber et al. (2009) reported that the (alkaline or acidic) pH will affect microbial population, which in turn affects soil nutrient value. PH is a biomarker effect for growth, bacteria, fungi and soil nutritional value Rousk et al (2009).

Si application induced high drought tolerance by organizing antioxidant activities of CAT, SOD, and GR (Gong et al. 2005; Kohler et al. 2008). PGPRs can able to alleviate the oxidative damage produced under water shortage. Osmotic adjustment is very important mechanism of acclimation to drought (Arndt et al. 2000). High osmotic adjustment helps sorghum cope with drought (Machado and Paulsen 2001).

Sorghum under drought stress led to significant decrease in plant growth, grain and biological yields of sorghum plants (Ibrahim et al. 2013). The 100-seed weight and biological yields were decreased under water defiant conditions as a result to the reduction in the number of filled grains (Kousar et al. 2012); (Venuprasad et al.

2007). The relationship between grain yield and water is complex because the sorghum is more sensitive to water and depends on water at certain growth stages (Garrity et al. 1982).

Si may be involved in metabolic or physiological and/or structural activity in higher plants exposed to abiotic and biotic stresses (Liang et al. 2003). Spraying silicon on plant leaves led to significant increase in grain yield comparing to control (without Si application) as a result of photosynthetic area increase which led to increasing in dry matter and number of spikes (Soratto et al. 2012; Pilon et al. 2013; El-Hedek 2013). Inoculation with PGPRs improve drought tolerance index of the seedlings due to the production of osmolyte and antioxidants in the rhizosphere (Fitouri et al.,2012). Samarah et al. (2004) found 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. N, P and K uptake in sorghum were enhanced significantly by PGPRs inoculation and Silicon treatments. Silicon has played an important role in balancing the uptake, transport, of minerals in plants under water deficit (Nayer and Reza 2008). On the contrarily Soratto et al. (2012) found that the minerals concentration in plants did not affect by application of Si and the K concentration in leaves. The increased uptake of Ca and K may be attributed to a decrease in plasma membrane permeability and increase in plasma membrane H+ -ATP activity as a result of silicon addition (Kaya et al. 2006) and also PGPRs plus Si interacted with Na^+, reduced its uptake while increased the concentration of K^+, Ca^{2+} and Mg^{2+} in plant tissues.

Conclusion

Under water stress conditions, application of foliar K silicate individually or plus bacterial inoculation in sorghum plants can reduce drought stress and improve sorghum growth and yield in addition to their effects on physiological characteristics, photosynthetic pigments, chlorophyll stability index as well as improved soil biological activities, antioxidant activity which could be indicative of stress tolerance. Under water stress conditions PGPR inoculation plus K silicate succeed to alleviate drought stresses in sorghum plants.

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