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Endophytic bacteria of desert cactus (*Euphorbia trigonas* Mill) confer drought tolerance and induce growth promotion in tomato (*Solanum lycopersicum* L.)

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ABSTRACT

Endophytic bacteria isolated from cactus were characterized and assessed for their capability to induce drought tolerance and growth promotion in tomato. A total of 191-bacteria representing 13-genera and 18-species were isolated from wild cactus, *Euphorbia trigonas*. *Bacillus* (58), *Lysinibacillus* (36), *Enterobacter* (29), *Stenotrophomonas* (18), *Lelliottia* (12) and *Pseudomonas* (12) were the most represented genera. 16S rDNA sequence (> 1400-bp) comparison placed the bacterial isolates with *Bacillus xiamenensis; Bacillus megaterium; Bacillus cereus; Bacillus amyloliquefaciens; Bacillus velezensis; Brevibacillus brevis; Lysinibacillus fusiformis; Enterobacter cloacae; Lelliottia nimipressuralis; Proteus penneri; Sphingobacterium multivorum; Klebsiella pneumoniae; Pseudomonas putida; Pseudomonas aeruginosa; Stenotrophomonas maltophilia; Citrobacter freundii; Chryseobacterium indologenes* and *Paracoccus* sp. *Bacillus xiamenensis* was identified for the first time as plant endophyte. Upon bacterization, the endophytes triggered germination and growth promotion in tomato as indicated by 118 % and 52 % more root-biomass under drought-free and drought-induced conditions, respectively. *Bacillus amyloliquefaciens* CBa_RA37 and *B. megaterium* RR10 displayed broad spectrum endophytism in tomato. Bacterization of tomato with cactus endophyte showed altered oxidative status, stomatal and photosystem II functioning, internal leaf temperature and relative water content suggestive of physiological de-stressing from moisture stress. Activity of oxidative stress enzymes such as guaiacol peroxidase and catalase was also indicative of endophyte assisted de-stressing of tomato. Re-irrigation on 20-days of drought infliction showed 86.9% recovery of *B. amyloliquefaciens* CBa_RA37 primed tomato when non-primed plantlets succumbed. The cactus endophytic bacterial strain *B. amyloliquefaciens* CBa_RA37 showed promise for low-cost, efficient and environmentally friendly bio-inoculant technology to mitigate drought in arid zones of Asian and African continents.

1. Introduction

Agrarian societies in the world are vulnerable for crop failures and livelihood risks owing to climate-change driven undesirable factors especially drought. Drought can be defined as a deficiency of precipitation over an extended period of time relative to the preceding year's average for the region that is expected to worsen in the near future especially in Africa and Asia (Flexas et al., 2013; Kissinger, 2016). In fact, water scarcity is actually hampering crop yields in more than 70% of the earth's arable lands (Kissinger, 2016). Low moisture stress inflicts re-adjustment of crop physiological and as well as biochemical performance with cascade of phenotypes such as physiological wilting and retarded plant growth, quality and the yield performance (Yang et al., 2009; Timmusk et al., 2014). A drought may result in cascade of interlinked disasters such as crop failure in agriculture, reduced food supply and food insecurity leading to famine, malnutrition, societal unrest, epidemics and internal displacement of populations. Two-thirds of the African region is affected by frequent droughts, which have been particularly severe in recent years in the Horn of Africa and the Sahel. Drought mitigation options should ensure crop productivity under limited water availability is, therefore, of paramount importance to sustain agricultural production (Mancosu et al., 2015; Ngumbi and Kloepper, 2016).

Among agriculture products, vegetables contribute approximately

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22.9% with a monetary value of 164.16 million USD (98.5 billion CFA) (IRAD, 2010). Tomato is among the important vegetables contributing 27% of annual production (IRAD, 2010). To date, tomato production is constrained by numerous biotic and abiotic factors which include water scarcity or drought (Asongwe et al., 2014). Numerous options have been preconized to impart drought tolerance in agriculturally important crops that encompasses germplasm selection, classical breeding approaches and numerous agronomic cultural practices (Fleury et al., 2010; Timmusk et al., 2014; Niu et al., 2018). Although highly desired, the crop breeding for drought tolerance is still a challenge owing to its polygenic inheritance and vulnerability to Genotype by Environment (G x E) interactions. Besides, drought tolerance typically has low heritability in plants (Blum, 2010; Khakwani et al., 2012). New molecular breeding, transgenic-technologies and genome editing tools are still atits infancy and yet to make an impact in crop improvement especially the drought tolerance (Coleman-Derr and Tringe, 2014; Ngumbi and Kloepper, 2016). Therefore, alternative strategies are needed to impart drought tolerance in economically important crop plants. In the past efforts have been made at harnessing plant associated microbes to improve crop production (Ngumbi and Kloepper, 2016; Niu et al., 2018). Recent reports suggest that plants surviving in drought-prone areas have systematically shaped out their microbiome to retain valuable colonizers (Shahzad et al., 2018). It is, thus, presumed that microbes colonizing arid plants could, thus, be advantageous over others to adapt in the arid environment to confer beneficial effects to cultivated plants. However, attempts are very limited or at infancy to exploit endophytic microbes of arid plants like Cactus for mitigating drought in agriculturally important crops. To the best of our knowledge, cactus (*Euphorbia trigon*, Cactaceae) endophytic bacteria naturally tolerant to drought have not been explored so far. With this background, an attempt was made to isolate, characterize, identify, evaluate and exploit endophytic bacteria of cactus plants collected from northern Cameroon, a sub-Saharan arid region with high temperature and low humidity for harnessing their potential as microbial inoculant for microbe assisted drought tolerance in agriculturally important crop like, tomato.

2. Material and methods

2.1. Sampling site and sampling

Cactus (*Euphorbia trigona*) root and stem samples were collected in Kousséri, located in the Far North Region of Cameroon (Lattitude: 12° 5′ 13.4988″ N; longitude 15° 0′ 53.3952″E). This zone is situated within the tropical continental climatic conditions, under the influence of Sahara desert (Supplementary Fig.1 and Supplementary Fig. 2). It is characterized by a short wet season (3 months) with a long dry season (9 months). Rainfall patterns are extremely erratic (400–900 mm) (Catalina, 2013a,b), with a temperature range of 18–45 °C. Asan arid zone, the potential evapo-transpiration is around double compared to yearly precipitation (Njitchoua et al., 1997; Cheo et al., 2013). Healthy roots and above ground part of *Cactus* plant were collected, and kept in sterile labelled plastic bags. Collected samples were immediately transported to the laboratory at University of Yaoundé I in a Keep-Cool box loaded with ice.

2.2. Isolation of endophytic bacteria

Once in the laboratory, the samples were washed with running tap water to remove bulk soil and other loosely attached debris. The cleaned samples were then cut into small pieces and surface sterilized using NaoCl 1.25% (10 min) and 70% alcohol (5 min) respectively separated by two to three rinses with sterile distilled water. The sterilized parts were thereafter ground in NaCl (0.9%) using sterile mortar and pestle and incubated at 28 °C for 3 h to allow the release of endophytes. Isolation was done by the serial dilution protocol on Muller Hinton agar medium containing Benomyl (De Oliveira et al., 2012). The last rinsing water was plated separately as a sterilisation control for sterility check. Plates were incubated at 28 °C and representative colony was picked and streaked onto fresh agar plate. Pure cultures were stored at −20 °C and −80 °C as glycerol stock until processed.

2.3. Morphotyping of the isolates

Individual isolates were plated and characterized by streaking on Nutrient Agar (NA) medium supplemented with 2, 3, 5 triphenyl tetrazolium chloride (TTC, 50 μg mL^{-1}). The plates were incubated at 28 °C for 48 h and the developed colonies were differentiated on the basis of their morphology, colony size, colony margin and colour. Thereafter, the isolates were grouped into clusters (morphotypes) and representative samples were taken from each morphotypes for further characterization using molecular tools.

2.4. Genomic DNA extraction

The genomic DNA of selected isolates was extracted using CTAB (Cetyl Trimethyl Ammonium Bromide) method with slight modifications (Moore et al., 2004). Briefly, over-night cultures (OD 600 = 0.5–1.0) grown at 28 °C with constant agitation of 200 rpm were pelleted down at 12,000 *g* for 15 min). Pellets washed with 1000 μl of 0.9% NaCl and the supernatant was discarded. Membrane lysis was done by adding a mixture of 550 μl lysozyme (100 μg mL^{-1}) and 10 mM Tris-Cl- 10 mM EDTA followed by incubation at 37 °C for 30 min. Then, 76 μl of 10 % SDS + Proteinase K was added, mixed by flipping and re-incubated at 65 °C for 15 min. The resulting mixture was further added with 5 M NaCl (100 μl) and incubated for 5 min at 65 °C. The mixture was added with 80 μl of CTAB (10 %) + NaCl (1.4 M) and incubated at the same temperature for 10 min. Afterwards equal volume of chloroform: Isoamylalcohol (ice cold) was added and mixed by flipping. After spinning at 12,000 *g* for 10 min at 28 °C, the top aqueous phase was pipetted into new microfuge tube and precipitated with 0.6 volume of ice cold isopropanol. The mixture was further centrifuged at 12,000 *g* for 15 min and the pelleted DNA, thus obtained, was washed with 1.0 ml chilled ethanol (70 %) followed by centrifugation at 10,000 *g* for 10 min. After brief air drying, DNA was dissolved in 50 μl 10 mM Tris-1.0 mM EDTA buffer. RNA contaminants were removed by adding 6.0 μl RNase (20 μg mL^{-1}) and incubated at 37 °C for 30 min. DNA was quantitated and quality analysed using a Biophotometer (Eppendorf, Germany) and resolved on agarose gel (0.8%) in Tris-EDTA buffer containing 1 μg/ml of ethidium bromide at constant voltage of 80 V for 3 h. Finally 200 ng total DNA was used as template for PCR amplification.

2.5. Box PCR-based fingerprinting

In order to decipher the genetic diversity of endophytic bacteria, BOX-PCR based DNA finger printing was performed on selected isolates from each morphotype (Rademaker et al., 1997; Kumar et al., 2004).The reaction was carried-out in 25 μl mixture made up of, 12.45 μl molecular grade water (1X); 5 μl Gitschier buffer (1X), 2.5 μl DMSO (10 %); 0.4 μl BSA (4 μg/μl); 1.25 μl dNTPs (1.25 mM); 0.4 μl of (2 U) Taq polymerase (Promega Corporation, Madison, USA); 1 μl Box primer (10 μM) and Genomic DNA 2 μl (400 ng). Amplification was performed at initial denaturation at 95 °C/ 2 min, and 35 cycles of denaturation at 94 °C/ 3 min; annealing at 50 °C/ 1min and extension at 65 °C/ 8 min followed by final extension at 65 °C/ 8 min. PCR products were resolved on 1 % agarose gel containing 1 μg/ mL of ethidium bromide at constant voltage of 15 V for 18 h. Amplicons were visualized and images were acquired using Gel Doc XR + Imaging system (Bio-Rad Laboratories Inc., USA). Box-PCR profiles were converted into a binary matrix of presence (1)/ absence (0) of each amplicon and similarity coefficient was calculated. Identical profiles were pooled and duplicates were eliminated.

2.6. Species identity determination based on bacterial 16SrRNAsequence

The prokaryotic universal primers [27 F (5′-AGAGTTTGATCCTGG CTCAG-3′) and 1492R (5-GGTTACCTTGTTACGACTT-3)] were used to amplify 1465-bp (Stackebrandt and Liesack, 1993). PCR amplification was carried out in 50 µL reaction mixture, containing final concentrations of GoTaq Buffer-1X; $MgCl_2$ 1.5 mM dNTPs- 200 µM; Forward/Reverse primers-10 pmol each; DMSO-6 %; Taq DNA Polymerase- 1 Unit; DNA 200 ng (Promega Corporation, Madison, USA) (Sheoran et al., 2015; Munjal et al., 2016). PCR amplification was performed at initial denaturation at 95 °C /5 min; 35 cycles of denaturation at 95 °C /1 min; annealing at 58 °C /1 min and extension at 72 °C/1 min followed by final extension at 72 °C/ 10 min. Amplicons visualised on 1 % agarose gel amended with 1 µg/ml ethidium bromide and gel was run in TE buffer at a constant voltage of 100 V for 20 min. Amplicons imaged using Gel Doc XR imaging system (Bio-Rad Laboratories Inc., USA).

2.7. Sequencing and phylogenetic analysis

16S rRNA amplicon of size 1465 bp was eluted from agarose gel using an SV gel and PCR clean up system as per manufacturer's instructions (Promega Corporation, USA) and sequenced bi-directionally in order to obtain maximum length. Contigs assembled and sequences curated using BioEdit software (Version 5.0) and the resulting contigs were used for blast search in NCBI (https://www.ncbi.nlm.nih.gov/), RDP (https://rdp.cme.msu.edu/) and Silva (https://www.arb-silva.de/) and EMBL-EBI (https://www.ebi.ac.uk/) databases, and the identity was confirmed by closest match (Sheoran et al., 2015). Sequences were, thereafter, aligned in Fasta text format using Mega software (Version 7.0). Dendrogram was produced using the neighbour-joining method with Kimura parameter distances. Percentage of similarity was used to group the species (Sánchez Márquez et al., 2007).

2.8. Effect of endophytic bacterial inoculation on tomato seed germination and mean germination time

Bacterial isolates representing all morphotypes and BOX Groups were selected and grown overnight in 10 ml of Luria-Bertani broth (HiMedia, India) under constant shaking of 150 rpm at 28 ºC for tomato seed bacterization. Bacterial cells pelleted down by centrifugation at 8000 rpm for 2 min. and pellets were re-suspended in saline (NaCl) solution (0.9% v/v). Dilutions were then made in deionized water so as to get final bacterial titre of OD_{600} = 0.5 using spectrophotometer. Thereafter, tomato seeds, genotype Pusa-Ruby obtained from Division of Vegetable Science, ICAR-IARI, New Delhi surface sterilized by washing respectively with 70% ethanol and 1% sodium hypochlorite followed by three rounds of sterile distilled water. Thus, sterilized seeds were soaked in 15 ml of bacterial suspension (0.5 OD_{600} units) in a Petri plate. Control sets were dipped in equal volume of sterile distilled water. Bacterized and water control plates were transferred to climate controlled greenhouse set at 22/20 °C (day/night) temperature and 40% relative humidity for germination. The germination data was recorded and per cent germination was calculated for each treatment (Ellis and Roberts, 1981). The mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981). **MGT = Σnidi/ n**

Where: n: Total number of germinated across the experimental period;

ni: Number of germinated seed on day d;

i: Number of days from day one.

Thereafter, the germination index (GI), which indicates the percentage of germination and germination speed (MGT) was calculated using the Bench et al. (1991) procedure as given below GI = (10 × n1) + (9 × n2) + ⋯ + (1 × nx)

Where:

n1,n2…n10 = Number of days of germinated seeds on the first (n1), second (n2) till the day 10th (n10) and 10, 9…and 1 are weights given to the number of geminated seeds on the first, second and subsequent days, respectively.

2.9. Effect of endophytic bacterial inoculation on seedling growth

Seedlings emerged from bacterized tomato seeds were sown in pot mix consisting of garden soil: sand: farm yard manure (2:1:1) in planter's pot. The pots planted with seedlings (10 per treatment) were arranged in big plastic trays in a randomized block design in the climate controlled greenhouse set at 28–30 °C with relative humidity of 60 ± 10%. Plants were uprooted, washed carefully without damaging the root system and observed six weeks after transplanting. Various growth parameters such as roots and shoot length, root and shoot dry weight were recorded after oven drying. The plant vigour index (VI) was then calculated as given bellow (Abdul-Baki and Anderson, 1973).

Vigour Index (VI) = [Plant height (Root length + Shoot length)]/ Germination rate

2.10. Screening of selected isolates for in vitro drought tolerance traits

In order to test the potential of bacterial isolates to grow under water-stress environment, Luria Bertani broth was amended with varying concentrations (0, 5, 10, 20 and 40%) of Polyethylene Glycol (PEG6000) in order to obtain varying water potential levels. Thereafter, the resulting mixture was inoculated with mid log phase culture of bacterial cells set at OD_{600} = 0.2. Thus inoculated cultures were incubated at 28 °C under constant shaking of 150 rpm. Growth of bacteria was estimated by measuring the optical density at 600 nm using a spectrophotometer every three hours (Michel and Kaufmann, 1973).

2.11. Screening for exopolysaccharide production

Bacterial isolates were further assayed for production of exopolysaccharide (EPS) by adopting protocol suggested by Hong et al. (2016) with minor modification. Sterile Tryptic Soy broth (TSB) was amended with 0%, 10%, and 20% of PEG 6000 and inoculated with bacterial suspension (OD_{600} = 0.2) at log phase and incubated at 28 °C in a shaking incubator set at 150 rpm for 72 h. For EPS extraction, the cultures were centrifuged at 15,000 rpm for 25 min at 4 °C and the supernatant was collected. Pellets were rinsed twice (0.85% KCl) and centrifuged two more times to maximize extraction. For extraction of EPS, three volumes of pre-chilled ethanol (95%) were added to supernatant followed by centrifugation at 15,000 rpm for 25 min at 4 °C. The pellet thus obtained was dried at 55 °C for 24 h, and expressed as dry weight per unit volume according to Verhoef et al. (2003). Prior to extraction of EPS, total protein content of the supernatant was performed using Bradford's method (Bradford, 1976).

2.12. Assay for phosphate solubilisation

The phosphate solubilisation potential of endophytic bacteria was performed on Pikovskaya's agar (gL^{-1} Dextrose 10, Yeast extract 0.5, Glucose 13, Calcium Phosphate ($CaHPO_4$) 2.5, NaCl 0.2, $(NH_4)_2SO4$ 0.5, $FeSO_4$-$7H_2O$ 0.0002, KCl 0.2, $MnSO_4$ 0.0002, $MgSO_47H_2O$ 0.1, Agar 15, Deionized water 1 L) (Pikovskaya, 1948). Fresh cultures of endophytic bacteria were spot-inoculated on agar media and incubated at 28 ºC for two days. Transparent halo around the growing colony, an indicator of phosphate solubilisation was scored as positive result (Ramos et al., 2014).

2.13. Assay for endophytism in tomato

2.13.1. Isolation of spontaneous rifamycin resistant mutants

Spontaneous rifamycin resistant mutant of bacterial isolates was obtained by plating each isolates on rifamycin amended LBA. Briefly 40 ml of mid log phase bacterial suspension were spin down at 8000 rpm for 2 min. The thus obtained pellets were re-suspended in 5 ml saline (0.9 % NaCl) solution, and 100 μl of the suspension pour plated on LBA plates amended with rifamycin (100 μg mL^{-1}). Colonies obtained were then streaked on freshly prepared rifamycin amended LBA (Enne et al., 2004; Munjal et al., 2016). The mutants obtained were used in the assay.

2.13.2. Seed inoculation with rifamycin resistant bacteria and plant growth

Surface sterilized tomato seeds were allowed to germinate for 5 days in bacterial suspension (OD_{600} = 1) prepared using rifamycin resistant mutants. Control seeds were grown in sterile distilled water. The plantlets were then transferred in pots containing 500 g of pot mix mentioned above, and grown under climate controlled greenhouse set at 28 °C and 80% relative humidity for 28 days. Plantlets were uprooted, and root parts were excised using surface disinfected scissors. Thereafter, one gram of plant tissue (root and shoot) was surface sterilized with sodium hypochlorite (1%) for 5 min, and ethanol (70%) for 1 min, followed by three times rinsing with sterile distilled water. Then, samples were grounded using sterile mortar and pestle in 2 ml of Phosphate Buffered Saline (PBS; pH = 7.4). The mixture was allowed to settle at room temperature for 15 min and serially diluted up to 10^{-5} (Munjal et al., 2016). One millilitre of final dilution was pour plated on LBA amended with rifamycin (100 μg mL^{-1}) and incubated at 28 °C for 48 h. Colonies were counted and expressed as Colony Forming Unit (CFU) per gram of fresh weight (Hallmann et al., 2000).

2.14. Effects of seed bacterization on growth under induced drought stress

Tomato seeds (Pusa Ruby) were surface sterilized and pre-germinated on sterile filter paper in Petri plates (as described above). Five days later, uniform sized seedlings were selected and inoculated with bacterial suspension calibrated at OD_{600} = 1 to permit colonisation of fine roots. The mock set was treated with sterile distilled water. Both bacterized and un-inoculated seedlings were sown in plastic pot with mix, and grown under climate controlled greenhouse conditions at 28 °C and 80% relative humidity. For each treatment twenty replications were maintained with one plant per pot. Soil moisture was adjusted at 75% of water holding capacity and maintained constant throughout the experiment by daily irrigation with sterile distilled water (Sandhya et al., 2011). Two weeks later, a booster-dose was applied by pouring 10 ml of each bacterial suspension around the collar region. Five weeks post transplantation, drought stress was induced in 10 out of 20 replicates by withholding watering. Water-stressed seedlings and non-stressed controls were observed daily for signs of water stress (Naveed et al., 2014). Two weeks later, plants were re-watered and the survival rate was recorded as the number of plants recovered from drought stress.

2.15. Drought-related data recording

2.15.1. Leaf gas exchanges recording

Leaf transpiration (??), stomatal conductance, and net photosynthesis (??) were continuously measured at 1-day interval for 11 days starting from the first day of drought stress induction. Leaf gas exchange was measured using an open infrared gas analyser (IRGA; LI – COR 6400 system, LI – COR Inch, Lincoln, NE, USA), with a mixed sequence across treatments to reduce timing bias. The spike gas exchange parameters were measured under natural light conditions using the LI – COR 6400 system with a specially constructed cylindrical measuring chamber (Tanzarella and Blanco, 1979). The IRGA chamber was irradiated with a photosynthetic photon flux density (PPFD) of 1000 μmol $m^{-2}.s^{-1}$, after which ear gas exchange parameters were measured under a PPFD of approximately 1000 μmol $m^{-2}.s^{-1}$ (equivalent to natural light). Plants were also irradiated with PPFD at 1000 μmol $m^{-2}s^{-1}$ for at least 30 min prior to photosynthesis measurements. Mean values were obtained from nine replications per treatment

2.15.2. Measurement of plant temperature and relative water content

Relative water content (RWC) was determined on 9 individual leaves of three plant pots for each treatment at 12 days after drought induction. Leaves were harvested and weighed to obtain the fresh weight (FW) and then placed in distilled water (4 °C) and reweighed again after 24 h to obtain saturated weight (SW). Finally, leaf dry weight (DW) was determined after drying at 65 °C for 6 h and RWC was calculated as given by Rabiye and Asim (2006) as:

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

Where: FW, DW, and TW are fresh weight, dry weight and turgid weight, respectively.

Plant temperature was taken using automatic laser infrared apparatus.

2.15.3. Effects of bacterial priming on antioxidant enzymes activity

Leaf samples were collected at 4 days intervals starting from first day of drought induction for estimating specific oxidative enzymes activities. Leaf (1 g) from treatment and mock were crushed in liquid nitrogen and immersed in 10 ml PBS; pH = 7.0. (Knoerzer et al., 1996). The mixture was centrifuged at 14,000 rpm (Eppendorf 5810R) for 10 min at 4 °C, and the supernatant was used to determine protein content (Bradford, 1976) using Bovine Serum Albumin (BSA) as standard. The protein extract was then used to determine the specific activities of Ascorbate peroxidase (APX), Catalase (CAT), Superoxide dismutase (SOD) and Guaiacol peroxidase (GPX) at 4 times points (0, 4, 8 and 12 days post drought induction).

2.15.3.1. Superoxide dismutase (EC 1.15.1.1). Superoxide dismutase (SOD) activity relied on the principle developed by Dhindsa et al. (1980), modified by Dhindsa and Matowe (1981)based on the spectrophotometric (560 nm) recording of the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture (5 ml) contained 4 μM riboflavin, 13 mM methionine, 0.1 μM EDTA, 75 μM NBT, and required amount of enzyme extract, all in potassium phosphate buffer (pH 7.8). The desired reaction began upon addition of riboflavin and exposition to fluorescent lamps (15 min). Another set following the same treatment except the enzymatic solution served as control while non-irradiated set was used as blank.

2.15.3.2. Catalase activity (EC 1.11.1.6). The catalase (CAT) activity was determined by monitoring the disappearance of H_2O_2 at 240 nm according to the method described by Aebi (1984). The reaction mixture contained 50 mM K-phosphate buffer (pH7.0), 33 mM H_2O_2 and enzyme extract.

2.15.3.3. Ascorbate Peroxidase (EC 1.11.1.11). The Ascorbate Peroxidase (APX) activity was determined as per the Nakano and Asada (1981) method based on the decrease in the original amount of Ascorbate in the reaction mixture monitored spectrophotometrically at 290 nm. The reaction mixture (5.0 mL) contained 0.2 mM EDTA, 0.5 mM ascorbic acid, 0.25 mM H_2O_2, all in 0.05 M potassium phosphate buffer (pH 7.0).The reaction started upon introduction 0.5 ml enzyme extract at room temperature.

2.15.3.4. Guaiacol Peroxidase (EC 1.11.17). The Guaiacol Peroxidase (GPX) activity was measured spectrophotometrically as described by

Tatiana et al. (1999). The assay principle is based on the inhibition of guaiacol oxidation into tetraguaiacol mediated by H_2O_2. Briefly, the reaction mixture (5.0 mL) consisted of 2.7 mM guaiacol, 2 mMH_2O, in 0.05 M potassium phosphate buffer (pH 7.0). The reaction was started by the addition of 0.1 ml enzyme extract. The rate of formed tetra guaiacol was measured at 470 nm. The overall enzymes activities were expressed as variation in absorbance per time unit per mg total protein content. Ultimately, the enzyme activities were normalized with respect to the activities in unstressed plants.

2.16. Statistical analysis

The analysis of variance (ANOVA) of the obtained numerical data was performed using Sigma plot (version 11) statistical software. Mean values from treatments were separated using the Duncan's multiple range test at $P \leq 0.05$.The Pearson correlation testwas utilized to study the relationship among the tested parameters when needed. The results were then expressed in terms of mean ± SD on mean.

3. Results

3.1. Isolation and characterization of culturable endophytic bacteria from Cactus

A total of 191endophytic bacterial isolates obtained from Cactus were morphologically characterized on 2, 3, 5 triphenyl tetrazolium chloride amended medium that displayed characteristic morphological phenotypes. The morphology based clustering of the isolates by colony characteristics such as colour, shape, margin colour and shape yielded 19 morphotypes (Fig. 1). The morphotypes were subjected to BOX-PCR based DNA fingerprinting that resolved the isolates into 19 Box-Groups (Fig. 2).

3.2. 16S rRNA-based species determination

The 16S ribosomal RNA sequences of the 19morphotypes with minimum sequence length of 1400 bp were end trimmed, contig assembled and blast analysed in NCBI, EMBL-EBI, RDP and Silva database (Supplementary Table 1). The isolates were then identified at species level by closest match. As many as thirteen genera could be found which includes *Bacillus*, *Stenotrophomonas*, *Lynsinibacillus*, *Enterobacter*, *Pseudomonas*, *Sphingobacterium*, *Proteus*, *Klebsiella*, *Lelliottia*, *Chryseobacterium*, *Paracocus*, *Brevibacillus* and *Citrobacter* which represented 19 species of bacteria (Fig. 1). The most represented bacteria among the 191 isolates were *Bacillus* (58), *Lysinibacillus* (36), *Enterobacter* (29), *Stenotrophomonas* (18), *Lelliottia* (12) and *Pseudomonas* (12) (Table 1).

3.3. Effect of cactus endophytic bacteria on tomato germination

Having identified the endophytic bacteria associated with arid plant, Cactus, series of other experiments were performed to decipher their effect on germination of agriculturally important vegetable, tomato. While significant number of cactus endophytes (47.3%) showed positive effect on germination of tomato seeds few others (5.2%) inhibited the germination (Fig. 3). For the MGT, the isolates *Bacillus velezensis* (6.1 days), and *Stenotrophomonas maltophilia* (8.5 days) significantly reduced the germination time compared to the control (11 days) and other isolates. Ultimately, considering the Germination Index, which considers both the rate and the speed of germination, Cactus isolates *Bacillus amyloliquefaciens* CBa_RA37 (363), *Bacillus megaterium* CBm_RR10 (457), and *Enterobacter cloacae* CEc_LGR7 (550) were found to be 'germination enhancers' by shortening seeds dormancy duration and final germination counts compared to the mock (357) and other isolates (Fig. 4). Nearly 53% of bacterial species tested did not significantly affect the germination (Table 2).

3.4. Effect of cactus endophyte on growth and development of tomato

Cactus endophytic bacterial isolates promoted the growth of shoot, root and overall biomass in a significant manner upon bacterization (Table 3; Supplementary Fig. 3).While shoot development was found enhanced by 48% of the bacterial isolates tested, significant root growth promotion was observed with 22% of endophytic isolates. Whereas *Bacillus amyloliquefaciens* CBa_RA37 and *Bacillus cereus* CBc_LPR8 were found to be shoot promoters, the *Bacillus megaterium* CBm_RR10 and *Enterobacter cloacae* CEc_LGR7 was roots growth promoter (Fig. 4).

3.5. Growth potential of the cactus endophytic bacterial strains under osmotic pressure

All four shortlisted cactus endophytic bacteria such as *B. megaterium* CBm_RR10, *B. amyloliquefaciens* CBa_RA37, *K. pneumonia* CBp_RR19, and *Enterobacter cloacae* CEc_LGR7 could tolerate drought stress caused by PEG6000 in general but the growth was inversely related to PEG concentration with Pearson correlation coefficient ranging from 0.73 to 0.96 at p = 0.05 (Fig. 5). No significant alteration in growth was recorded for *B. amyloliquefaciens* CBa_RA37; *B. megaterium* CBm_RR10 and *B. xiamenensis* CBx_LGR21 at 40% PEG6000 concentration indicating its inherent capacity to tolerate water stress.

3.6. Exopolysaccharide and phosphate solubilisation by cactus endophytic bacteria

In general, EPS production increased with increasing concentrations of PEG that clearly indicated readjustment of the bacterial physiological functions to withstand osmotic stress. *B. amyloliquefaciens* CBa_RA37 (6.2 mg/ml) produced more EPS as compared with other isolates followed by *B. xiamenensis* CBx_LGR7 (5.4 mg/ml) and *B. megaterium* CBm_RR10 (4.97 mg/ml) (Fig. 6). All four bacterial isolates could also solubilise phosphate on Pikovskaya's medium as indicated by clear halo zone around the colonies (Data not shown). Based on their respective vigour index and their capability of producing exo-polysaccharides, the strains *B. amyloliquefaciens* CBa_RA37 (EPS, 6.2 mg/ml; Vigour index 1878.5) and *B. megaterium* CBm_RR10 (EPS, 4.97 mg/ml; Vigour index, 3039.1) were selected.

3.7. Endophytism of cactus endophytic bacteria in tomato

The ability of the growth enhancing-drought-tolerant bacterial candidates to colonize the tomato endosphere was confirmed using rifamycin resistant spontaneous mutants of selected bacterial endophytes. All bacterial endophytes could be recovered from the endosphere of 30 days old axenically grown bacterized tomato. *Bacillus amyloliquefaciens* CBa_RA37 and *B. megaterium* CBm_RR10 recorded 1.89 and 1.38 Log CFU/g fresh weight, respectively (Supplementary Table 2).

3.8. Effects of bacterial priming on growth and development of tomato under drought stress

Upon inoculation, Cactus endophytic *B. amyloliquefaciens* CBa_RA37 and *B. megaterium* CBm_RR10 triggered growth promotion in tomato as indicated by significantly more plant height (38.1% more height) and dry biomass (61.0% more biomass) as compared to drought inflicted non-primed plants. Growth promotion was found to be on par with irrigated and unstressed tomato indicating a nullifying effect of endophytic bacterial colonization on drought and a consequent growth and development of tomato (Fig. 7).

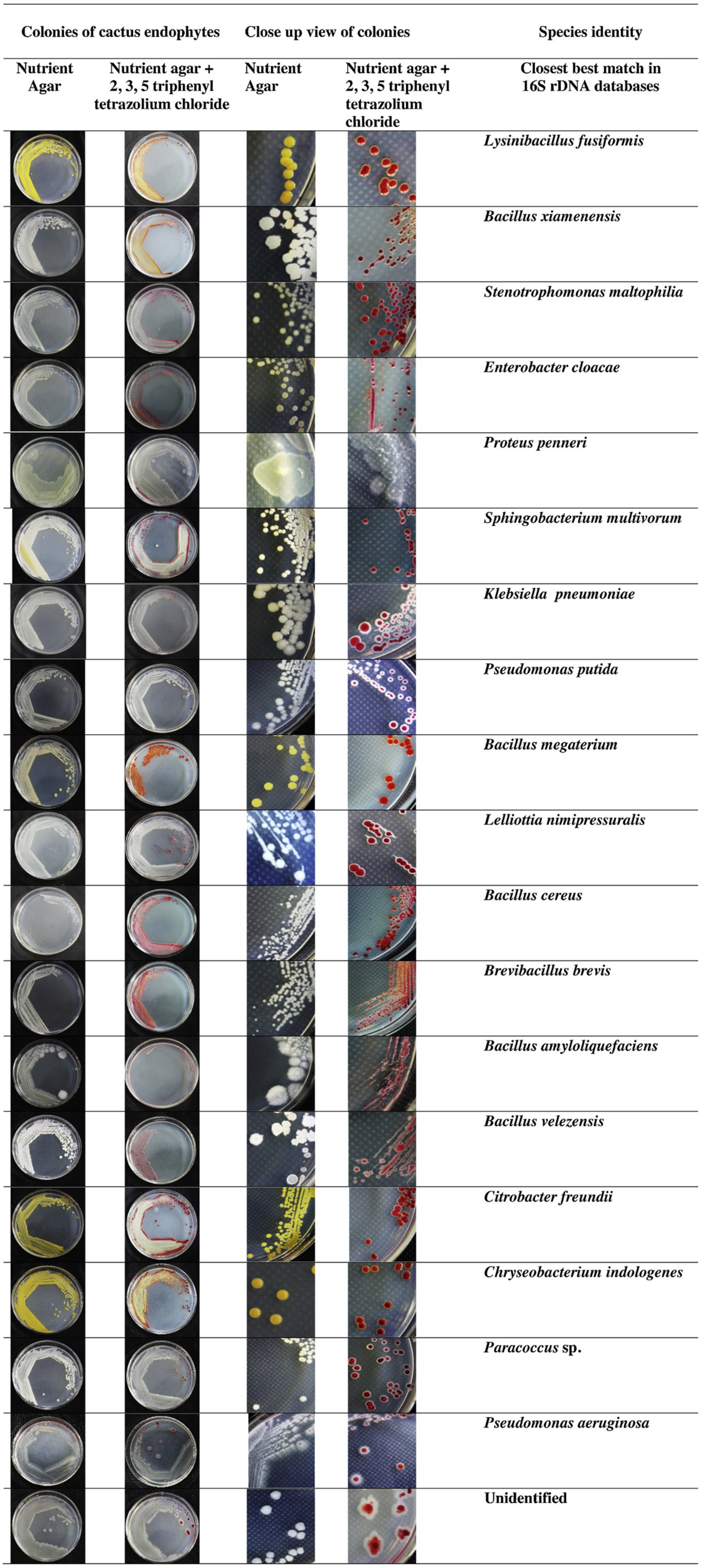

Fig. 1. Colony morphology of various morphotypes of cactus endophytic bacteria. From the left to the right.
i. Column 1: Colony morphology on Nutrient Agar medium.
ii. Column 3: Close up view of colonies on Nutrient Agar medium.
iii. Column 2: Colonies on Nutrient Agar medium supplemented with 2, 3, 5 triphenyl tetrazolium chloride (TTC).
iv. Column 4: Close up view of colonies on TTC amended medium.

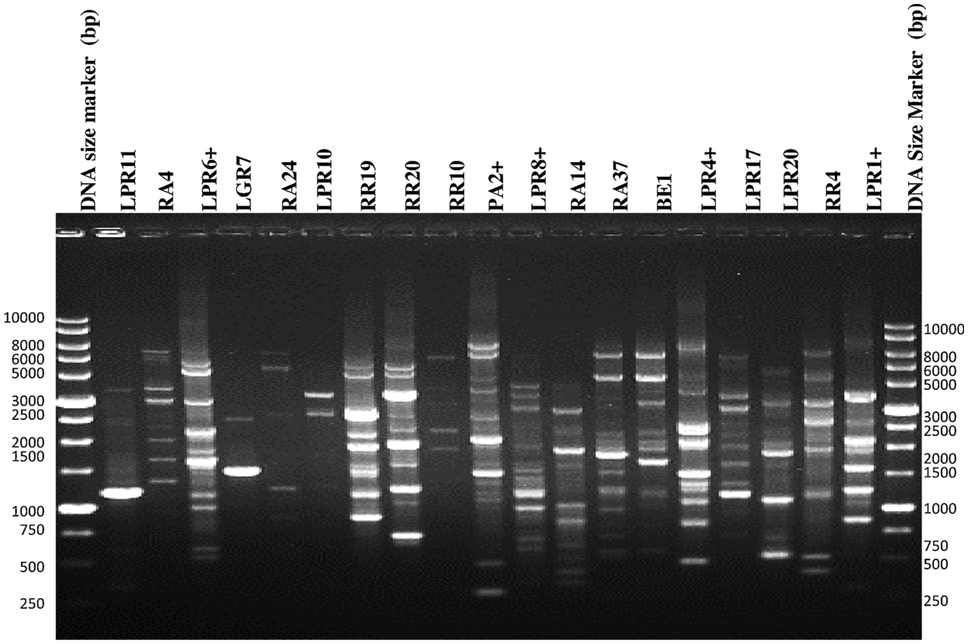

Fig. 2. BOX-PCR based DNA fingerprinting of cactus bacterial isolates.
1. LPR11 : *Lysinibacillus fusiformis*.
2. RR4: *Bacillus xiamenensis*.
3. LPR6 + : *Stenotrophomonas maltophilia*.
4. LGR7 : *Enterobacter cloacae*.
5. RA24 : *Proteus penneri*.
6. LPR10: *Sphingobacterium multivorum*.
7. RR19: *Klebsiella pneumoniae*.
8. RR20 : *Pseudomonas putida*.
9. RR10: *Bacillus megaterium*.
10. PA2 + : *Lelliottia nimipressuralis*.
11. LPR8 + : *Bacillus cereus*.
12. RA14 : *Brevibacillus brevis*.
13. RA37: *Bacillus amyloliquefaciens*.
14. BE1: *Bacillus velezensis*.
15. LPR4 + : *Citrobacter freundii*.
16. LPR17: *Chryseobacterium indologenes*.
17. LPR 20: *Paracoccus* sp.
18. RR4 : *Pseudomonas aeruginosa*.
19. LPR1 + : Unidentified.

3.9. *Effect of bacterization by cactus endophytic bacteria on physiology of tomato under drought*

3.9.1. *Relative water content and stomatal conductance*

Water stress triggers physiological changes and causes cascade of plant responses such as water contents in foliage coupled with stomatal conductance. Foliage water retention was 32% or more in tomato plants bacterized with *Bacillus amyloliquefaciens* CBa_RA37 and *B. megaterium* CBm_RR10 as compared to non-bacterized seedlings. Whereas stomatal conductance was found drastically reduced in untreated tomato plants upon drought induction. Bacterization with *Bacillus megaterium* CBm_RR10 or *Bacillus amyloliquefaciens* CBa_RA37 could sustain stomatal conductance during water stress condition (Fig. 8A and B).

3.9.2. *Plant temperature and photosystem II efficiency*

Colonization of tomato by selected cactus endophytic bacteria recorded reduced leaf temperature and enhanced photosynthetic potential (PS II) (Fig. 9A and B). The well irrigated unstressed tomato plants showed leaf temperature in the range of 28.3–28.9 °C throughout the experimental period whereas the water stressed plants recorded 27.8 °C on Day-1 of drought induction to 31.3 °C on 14th Day of drought stress. Endophytic bacteria treated plants recorded temperature in the range of 27.6 °C on Day-1 to 30.4 °C on Day 14 for *Bacillus amyloliquefaciens* CBa_RA37. *Bacillus megaterium* CBa_RR10-primed plants showed 27.4–30.8 °C. No significant change in PS-II was observed for all treatments during the initial 8 days of drought stress induction. However, photosynthetic performances decreased gradually in the drought stressed tomato as compared to unstressed plants which recorded normal range of 0.75-0.85 (Björkman and Demmig-Admas, 1987). Cactus endophytic bacteria were found to improve the photosynthetic activities in tomato upon bacterization. *B. amyloliquefaciens* CBa_RA37 *and B. megaterium* CBm_RR10 primed plants showed nearly identical photosynthetic activity superior to mock indicating drought mitigation by endophytic bacteria.

3.9.3. *Cactus endophytic bacteria mediated drought tolerance in tomato*

Tomato plantlets subjected to water stress showed gradual decline in growth and development from 4th day of drought induction. Plants showed physiological wilting symptoms as the water potential of the soil dropped. Nearly all plantlets showed wilting syndromes due to 20 days of water stress mimicking drought situation. None of the untreated plantlets survived the artificially induced drought. Interestingly, significant number of drought survivors could be observed among the endophytic bacteria primed plantlets. *Bacillus megaterium* CBm_RR10 and *B. amyloliquefaciens* CBa_RA37 inoculation enabled 37.8 and 86.9 % of tomato plants recover from drought upon re-watering (Figs.10 and 11).

3.9.4. *Effect of cactus endophytic bacteria on drought linked biochemical parameters*

Oxidative stress associated enzymes were assayed during the drought period between (0–16 days of water stress) on tomato plantlets inoculated with cactus endophytic bacteria. The results indicated that the specific activity of all assayed enzyme dramatically increased in water stressed plantlets whereas the irrigated plantlets showed nearly identical activity. Significant increase in specific enzyme activity was observed for APX, GPX, CAT and SOD 16 days post drought induction. Activity of CAT and GPX was found significantly dropped in *B. amyloliquefaciens* CBa_RA37 and *B. megaterium* CBm_RR10 primed tomato seedlings as compared to non-bacterized seedlings indicating the role of endophytic bacteria to de-stress the plantlets under water stress. On 16th day of drought, 24–25 % reduction in GPX enzyme activity was found in bacterized tomato plantlets. For CAT enzyme, *B. megaterium* CBm_RR10 and *B. amyloliquefaciens* CBa_RA37 priming caused reduction of 31.5% and 22.6% enzyme respectively. No clear cut trend could be found for APX or SOD in tomato plantlets bacterized with cactus endophyte (Fig. 12).

4. Discussion

Water, one of the most important drivers of crop production, is among the limiting factors that impact global agricultural production in all major continents especially Africa. It is perceived that global climate change, especially alterations in precipitation patterns and enhanced air temperature are responsible for severe drought conditions (Lau and Lennon, 2012). Located at the vicinity of Sahara desert, the north region of Cameroon is facing frequent drought with substantial impact on economy, livelihood and food security. In addition, no control strategy has been envisaged neither by public policy nor research attempts to mitigate the impacts of water scarcity on nutritious crops in northern Cameroon. Imparting drought tolerance in crops through classical breeding techniques and natural resource management by good

Table 1
Morphological characterization and identification of cactus endophytic bacteria.

Morpho-type	Colony characteristics	Isolates	Total	Closest match in GenBank	GenBank Accession (16S rDNA)
M1	Large, light red with irregular margin	RA1, RA7,RA10, RA1, RA15, RA18, RA20, RA21, RA25, RA29, RA34, RA35, RA36, LPR31, PA3, **LPR11, LPR2,**LGR24, RR7, LGR8,LGR25, RR3, RR5, LGR15, RR8, LGR17, RR14, LGR28, PR8, LGR20, LGR23, PA5, LGR9, LGR10, LGR18, LGR5	36	*Lysinibacillus fusiformis*	**MH788986 MH788987**
M2	Small, pink with metallic shine, entire	RA4, RA5, RA27, RA3, RA32, **LGR21**, LGR29, RR1	8	*Bacillus xiamenensis*	**MH788976**
M3	Medium, round, dark red with white and smooth margin	RA2, RA8, RA16, RA19, RA23, RA26, RA28, **RA33,** RR6, RR16, RR17, RR21, LGR11, LGR14, PR1, **LPR6+,** LPR10^{+}, LPR13, **LPR5**	18	*Stenotrophomonas maltophilia*	**MH788993 MH788994 MH788995**
M4	Medium, round, light red with white and smooth margin	PA1, PA4^{+}, PA6, PA11, PA13, PA14, PA15, PA17, RR23, LGR2, LGR4, **LGR7**, LGR13, **LGR19,** LGR26, LGR27, RR1, RR2, RR15, PLR5, **LPR2**$^{+}$, LPR5+, PLR6, **LPR7**, LPR9, LGR3, LGR6, LGR27, LGR30, LGR31, PR1	30	*Enterobacter cloacae*	**MH788980 MH788981 MH788982**
M5	Large, irregularly shaped, entirely pinkish	**RA24**, RA9, RA6, RR25	4	*Proteus penneri*	**MH788991**
M6	Medium, circular, dark pink centre with light pink regular margin	**LPR10**	1	*Sphingobacterium multivorum*	**MH788992**
M7	Medium, round, dark red with white and wavy margin	**RR19**, PA1^{+}	2	*Klebsiella pneumoniae*	**MH788983**
M8	Medium, circular, yellow with smooth opaque white margin	RA13, RA17, RA30, RA31, RA38, **RR20**, PA2, PA3^{+}, PA4, PA9, PA12, PA13	12	*Pseudomonas putida*	**MH788990**
M9	Small circular dark red to black with convex surface	RR13, **RR10**	2	*Bacillus megaterium*	**MH788974**
M10	Punctiform, light red with white hallow	PA7, BE2, LGR1, LGR4^{+}, LGR12, RR11, RR12, RR18, RR22, RR24, PA8, **PA2**$^{+}$, LPR15, PA16, BFR4, LGR16	16	*Lelliottia nimipressuralis*	**MH788984**
M11	Medium, circular to irregular shaped, Pink with regular white margin	RA12, PR4, PR5, PR6, PR7, PR9, PR10, PR12, PR13, PR14, PR15, PR16, LPR4^{+}, RA11, LGR22,, LGR32, LGR33, LGR34, LPR1, PLR3, LPR11, BE6, BFR5 BFR6, BFR7, PR17 BFL3, LPR12, LPR14, LPR16, LPR19, **LPR8**	32	*Bacillus cereus*	**MH788973**
M12	Small circular, brown, translucent margin	RA14	1	***Brevibacillus brevis***	**MH788977**
M13	Small, irregularly shaped, red centre and light pinkish border	BFR1, BFR2, BFR3, BFL1, **BFL2**, **RA37**, PR18, **RA22**, RA39	9	*Bacillus amyloliquefaciens*	**MH788970 MH788971 MH788972**
M14	Medium, irregularly shaped, red centre and light pinkish border	PA18, **BE1,** BE4, BE5, BE7, LGR35	6	*Bacillus velezensis*	**MH788975**
M15	Medium, circular, dark pink centre with light pink regular margin	**LPR4**	1	*Citrobacter freundii*	**MH788979**
M16	Small, circular, red to brown with yellowish smooth margin	LPR11^{+}, LPR12^{+},**LPR17**	3	***Chryseobacterium indologenes***	**MH788978**
M17	Small, conical, irregularly shaped, pink, white wavy margin	PR2, PR3, RR9, **LPR20**, LPR6, RR26	6	*Paracoccus* sp.	**MH788988**
M18	Small, flat, irregularly shaped, metallic pink margin	**RR4**	1	*Pseudomonas aeruginosa*	**MH788989**
M19	Medium, circular, white milky margin	**LPR1**$^{+}$**,** LPR3^{+}	2	Unidentified	–

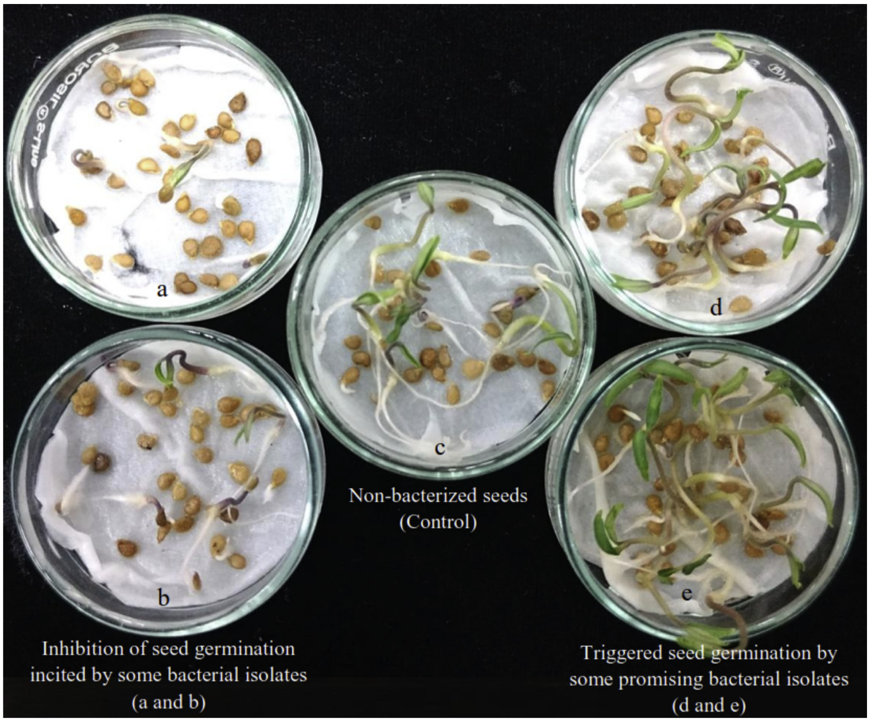

Fig. 3. Effect of selected cactus endophytic bacteria on germination of tomato seeds.

Surface sterilized tomato seeds (Cultivar: Pusa-Ruby) were soaked in each test-bacterial suspensions ($OD_{600} = 0.5$) and allowed to germinate on filter paper in Petri plates. Seed soaked in autoclaved distilled water served mock. Seeds were incubated in climate controlled greenhouse [22/20 °C (day/night) temperature and 40% relative humidity] for germination.

i. a & b: Plate showing inhibition of seed germination by endophytic bacterial species.

ii. c. Mock.

iii. d & e: Plate showing enhanced seed germination by endophytic bacterial species.

agronomic practices is among the strategies that met with limited success in few food crops (Niu et al., 2018).Therefore, alternative approaches are needed for the sustainable and eco-friendly drought management in major crops such as cereals, pulses and vegetables. In the recent years, environment compatible agro-technologies are emphasized for crop management. Among them, microbe assisted crop production options have attracted the attention of farmers, researchers and all stakeholders of farming owing to multitude of ecological benefits. Among the microbes, endophytic microbes that colonize plant interiors are believed to benefit crops in a holistic way by conferring probiotic effect on crop growth and antibiotic effect on disease causing agents including nematodes (Zahir et al., 2008; Sandhya et al., 2009; Nascimento et al., 2015; Toghueo et al., 2016; Vurukonda et al., 2016; Strobel and Daisy, 2003).Natural biological functions played by the

Fig. 4. Shoot and Root growth triggered by cactus endophytic bacteria.

a. Mock; b–e. Shoot emerged from tomato seed bacterized with *Bacillus megaterium* RR10 **(b)**; *Bacillus amyloliquefaciens* RA37 **(c)**; *Bacillus cereus* LPR8 **(d)** and *Klebsiella pneumoniae* RR19 **(e)**.

a. Mock; b–d. Root emerged from seeds treated with *Bacillus megaterium* RR10 **(b)**; *Bacillus amyloliquefaciens* RA37 **(c)** and *Klebsiella pneumoniae* RR19 **(d)**.

Table 2
Effect of cactus endophytic bacterial inoculation on germination of tomato seeds.

Cactus endophytic bacteria	MGT (Day)	Germination Rate (%)	Germination Index (GI)
1 *Lysinibacillus fusiformis*	10.6	88.0	239
2 *Bacillus xiamenensis*	10.8	82.7	220
3 *Stenotrophomonas maltophilia*	8.5*	88.6	186
4 *Enterobacter cloacae*	9.9	90.0*	236
5 *Proteus penneri*	13.3	100*	303
6 *Sphingobacterium multivorum*	12.5	87.3	354
7 *Klebsiella pneumoniae*	10.8	93.1*	193
8 *Pseudomonas putida*	11.1	97.4*	336
9 *Bacillus megaterium*	13.2	86.6	457*
10 *Lelliottia nimipressuralis*	13.2	87.8	305
11 *Bacillus cereus*	11.6	86.1	350
12 *Brevibacillus brevis*	10.5	86.8	225
13 *Bacillus amyloliquefaciens*	9.1	98.0*	363*
14 *Bacillus velezensis*	6.1*	66.3	158
15 *Citrobacter freundii*	15.7	97.0*	354
16 *Chryseobacterium indologenes*	10.6	91.3*	203
17 *Paracoccus* sp.	10.0	85.1	252
18 *Pseudomonas aeruginosa*	9.5	84.2	188
19 Control	11.0	84.2	357

Within each column, mean values superscripted by a star.
* are significantly higher (p = 0.05) relative to their respective control.

plant associated endophytic microorganisms on plant provide the best clues for harnessing them for crop management. Microbes play a vital role for plant metabolism to enable the plant communities to adapt biotic and abiotic factors in natural environment. Bacteria associated with plants naturally adapted in water deficit arid regions are believed to be drought resilient, and such microbial communities are not yet exploited for drought management in agriculture (Niu et al., 2018). Here, we report for the first time a diverse bacterial communities naturally adapted in arid plant Cactus (*Euphorbia trigona*) wildly grown in northern Cameroon in the peripheral areas of Sahara desert. We have also demonstrated their potential to promote growth and alleviate drought stress effects in tomato as well.

A total of 19 morphotypes representing 191 bacterial isolates were isolated and characterized by adopting polyphasic taxonomic tools including highly discriminatory bacterial repetitive sequences based DNA fingerprinting. Nineteen bacterial species belong to 13genera such as *Bacillus, Stenotrophomonas*, *Lynsinibacillus, Enterobacter*, *Pseudomonas*, *Sphingobacterium*, *Proteus*, *Klebsiella*, *Lelliottia*, *Chryseobacterium*, *Paracocus*, *Brevibacillus* and *Citrobacter* were identified. *Bacillus* and *Lynsinibacillus* genera were the most highly represented followed by *Enterobacter*. *Bacillus* species are among the most dominant and highly adapted prokaryote in diverse ecological niches including plant roots and endogenous regions (Aravind et al., 2009; Laguerre et al., 1994; Lambert et al., 1987; Lalande et al., 1989). *Bacillus amyloliquefaciens*, *Bacillus cereus, Bacillus megaterium, Bacillus velezensis, Bacillus xiamenensis,* and *Brevibacillus brevis* are the major species of *Bacillus* found in the endosphere of cactus. Other species of bacteria found among the collections are *Chryseobacterium indologenes, Citrobacter freundii, Enterobacter cloacae, Klebsiella pneumoniae, Lelliottia nimipressuralis, Lysinibacillus fusiformis, Paracoccus* sp., *Pseudomonas aeruginosa, Pseudomonas putida, Proteus penneri, Sphingobacterium multivorum,* and *Stenotrophomonas maltophilia.*

In planta assays for seed germination and vigour revealed that 47.3% of the cactus endophytic bacteria shortened seed dormancy by accelerated germination. Among them *Bacillus megaterium* RR10, *B. amyloliquefaciens* CBa_RA37, *Enterobacter cloacae* CEc_LGR7 and *Klebsiella pneumoniae* CKp-RR19 significantly enhanced the germination and the subsequent root and shoot elongation of tomato in greenhouse conditions. Growth promotion may be attributed to production of growth hormones like gibberellic acid, abscisic acid, cytokinins, and auxin (Foley et al., 2011; Smol 2012). The plant growth-promoting cactus endophytic *Bacillus megaterium* CBm_RR10, *B. amyloliquefaciens*CBa_RA37, *Enterobacter cloacae* CEc_LPR7 and *Klebsiella pneumoniae* CKn_RR19 were found to grow under osmotic pressure at 40% PEG 6000*in vitro*. Besides, these bacteria were found to produce significantly more extra cellular polysaccharide (EPS) in the medium under water stress condition.In fact, Naseem and Bano (2014) asserted that maize primed with EPS-producing bacterial strains *Alcaligenes faecalis* AF3, *Proteus penneri* Pp1 or *Pseudomonas aeruginosa* Pa2 improved plant agromorphological parameters as well as soil moisture contents under drought stress. Being hygroscopic in nature, hydrogen molecule of EPS binds water particles, which provides the highly congenial microenvironment for bacteria and host roots to survive desiccation triggered by deficit moisture (Hepper, 1975). One of the well-studied bacterial EPS is alginate; this sugar polymer possess high water-holding and low water-losses properties, thereby keeping root cells hydrated long enough to allow for cellular metabolic adjustments necessary to enhance drought stress tolerance (Halverson, 2009; Timmusk et al., 2014).

The drought mitigating potential of cactus endophytic *Bacillus*

Table 3
Effect of cactus endophytic bacterial inoculation on growth parameters and vigour index in tomato.

Cactus endophytic bacteria	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	Vigour Index
1 *Lysinibacillus fusiformis*	17.2 ± 1.7	13.0 ± 1.4*	0.5 ± 0.1	0.29 ± 0.07*	2658.9
2 *Bacillus xiamenensis*	15.1 ± 0.7	11.5 ± 2.3	0.4 ± 0.1	0.16 ± 0.09	2201.3
3 *Stenotrophomonas maltophilia*	15.3 ± 1.2	6.3 ± 0.4	0.4 ± 0.04	0.12 ± 0.01	1913.8
4 *Enterobacter cloacae*	16.8 ± 1.7	13.0 ± 0.9*	0.6 ± 0.04	0.17 ± 0.07*	2757.7
5 *Proteus penneri*	15.8 ± 2.1	8.6 ± 1.7	0.4 ± 0.06	0.21 ± 0.04	2440.0
6 *Sphingobacterium multivorum*	19.0 ± 1.4*	10.5 ± 2.0	0.6 ± 0.1*	0.25 ± 0.03	2576.1
7 *Klebsiella pneumoniae*	19.6 ± 1.8*	12.7 ± 0.9*	0.7 ± 0.1*	0.30 ± 0.03*	3039.1
8 *Pseudomonas putida*	16.5 ± 1.2	11.6 ± 1.2	0.5 ± 0.1	0.14 ± 0.01*	2739.1
9 *Bacillus megaterium*	19.3 ± 1.1*	15.7 ± 2.0**	0.8 ± 0.2**	0.48 ± 0.02*	3039.1
10 *Lelliottia nimipressuralis*	15.2 ± 2.2	9.6 ± 1.1	0.5 ± 0.1	0.11 ± 0.13*	2177.6
11 *Bacillus cereus*	20.7 ± 2.3**	10.6 ± 3.7	0.6 ± 0.1	0.28 ± 0.03*	2695.4
12 *Brevibacillus brevis*	18.2 ± 0.9*	10.0 ± 1.8	0.6 ± 0.2	0.31 ± 0.03*	2442.6
13 *Bacillus velezensis*	17.2 ± 2.3	10.0 ± 0.8	0.6 ± 0.2	0.43 ± 0.16*	2120.6
14 *Bacillus amyloliquefaciens*	19.8 ± 0.6**	8.5 ± 0.5	0.8 ± 0.2**	0.31 ± 0.03*	1878.5
15 *Citrobacter freundii*	18.2 ± 1.2*	10.2 ± 1.2	0.6 ± 0.2	0.24 ± 0.08	2752.9
16 *Chryseobacterium indologenes*	16.1 ± 0.2	10.5 ± 1.8	0.4 ± 0.2	0.14 ± 0.05	2430.6
17 *Paracoccus* sp.	16.8 ± 0.8	8.3 ± 1.2	0.4 ± 0.1	0.10 ± 0.02	2136.7
18 *Pseudomonas aeruginosa*	18.7 ± 0.9*	9.8 ± 1.6	0.4 ± 0.1	0.10 ± 0.02	2400.7
19 Control	15.5 ± 2.3	9.0 ± 3.3	0.4 ± 0.2	0.22 ± 0.14	2063.8

*, ** indicate significant difference at $p \leq 0.05$ and $p \leq 0.01$ respectively referring to uninoculated control.

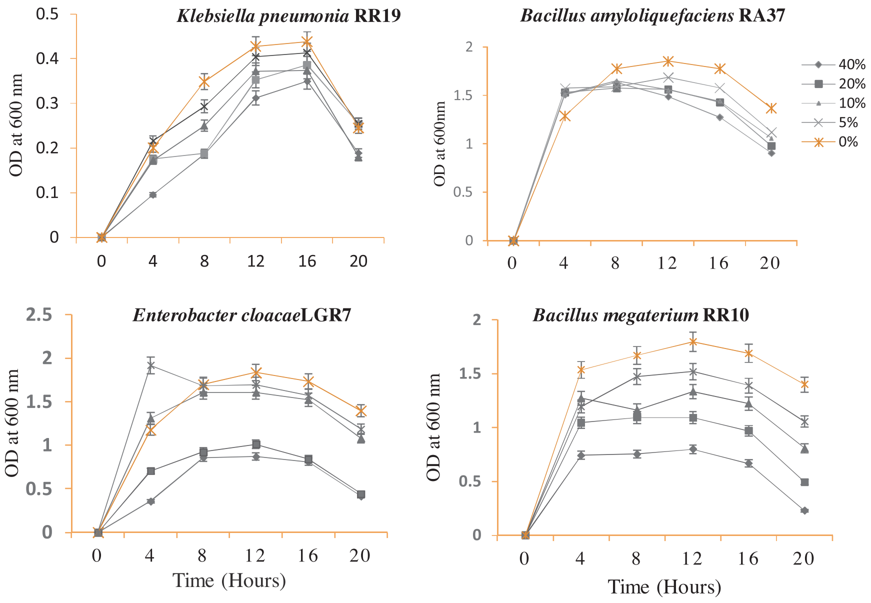

Fig. 5. Growth dynamics of plant growth promoting cactus endophytic bacteria in PEG600 mediated osmotic stress.
Mid log phase bacteria cells (OD_{600} = 0.2) were grown in Luria Bertani broth amended with varying concentrations of Polyethylene Glycol (PEG6000) under constant stirring at 150 rpm.
The growth of the bacteria was monitored steadily at 600 nm using a spectrophotometer every three hours. The experiment was repeated twice. Error bars indicate standard deviations of mean values.

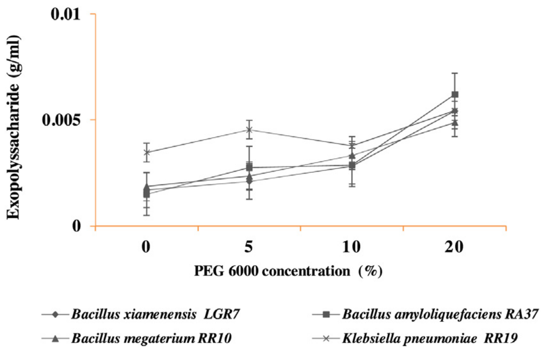

Fig. 6. Production of exopolysaccharide by cactus endophytic bacteria under PEG mediated osmotic stress.
Mid-log phase bacteria cells (OD_{600} = 0.2) were grown in Luria Bertani broth amended with varying concentrations of Polyethylene Glycol (PEG6000) under constant shaking (150 rpm).
The EPS obtained was expressed as dry weight per unit volume.
Error bars indicate standard deviations of mean values.

amyloliquefaciens CBa_RA37 and *B. megaterium* CBm_RR10 is further exemplified in enhanced biomass during water stress coupled with significant drought recovery upon re-irrigation. Similar observation is reported in wide range of crops including rice (Lafitte et al., 2007), common bean (Figueiredo et al., 2008), maize (Naveed et al., 2014) and wheat by various prokaryotic microorganisms (Timmusk et al., 2014; Chen et al., 2017). The microbe induced drought tolerance in plants has alsobeen ascribed to up-regulation of 1-aminocyclopropane-1-carboxylate (ACC) deaminase gene (acdS) by the bacterium which cleaves the precursor of ethylene (ACC). As such, reduced ACC levels lead to a reduction of endogenous ethylene content and its effects on plants during stress conditions (Ngumbi and Kloepper, 2016; Niu et al., 2018).

Drought remains a multi-dimensional stressor affecting plants at sub-cellular, tissue, organs and whole plant level. The exposition to drought at sub-cellular niches leads to the accumulation of reactive oxygen species (ROS) that are deployed as plant stress signal which further activates plant redox system. Overproduction of these chemically unstable and reactive metabolites breaks the pro-oxidant/anti-oxidant equilibrium rendering the inherent plant ROS scavenging machinery inefficient. With cascade of cellular perturbations like lipids per-oxidation, enzymes inactivation and DNA damages which seriously hindered plant normal functions or even cell dead (Miller et al., 2010; Vurukonda et al., 2016).

Activation of anti-oxidant enzymes is one of the biochemical responses of drought subjected plants. Higher activity of anti-oxidant enzymes such as SOD, APX, CAT and GPX recorded in drought inflicted plantlets as compared to irrigated plantlets is suggestive of plant's ability to withstand water stress. Among them, activity of CAT and GPX was found to be on par with irrigated tomato that was primed by cactus

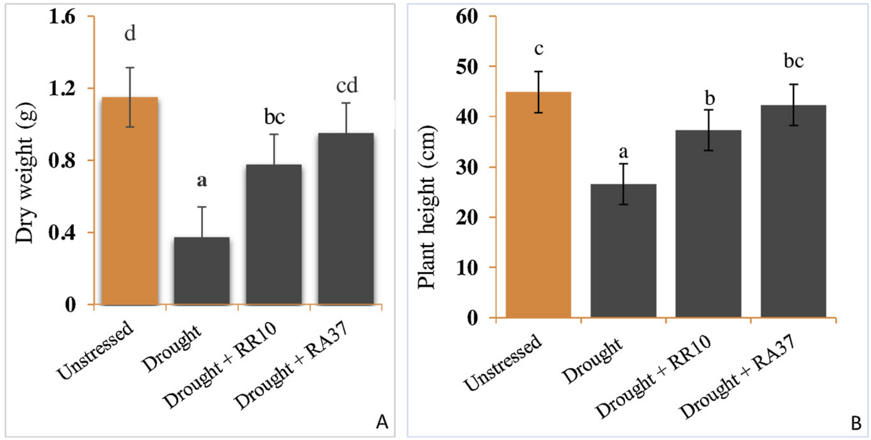

Fig. 7. Effect of cactus endophytic bacteria mediated priming on growth of tomato grown under drought stress. Surface sterilized tomato seeds soaked in bacterial suspension (OD600 = 1) were grown under climate controlled greenhouse.
i. Unstressed: Non-bacterized and well-watered plants.
ii. Drought: Drought stressed and non-bacterized plantlets.
iii. Drought + RR10: *B. megaterium* RR10 inoculated plants subjected to drought.
iv. Drought + RA37: *B. amyloliquefaciens* RA37 inoculated plants subjected to drought.
Error bars indicate standard deviations (SD) of mean values.
Error bar labelled with alphabet indicates statistical significance according to Duncan's multiple range test at P = 0.05.

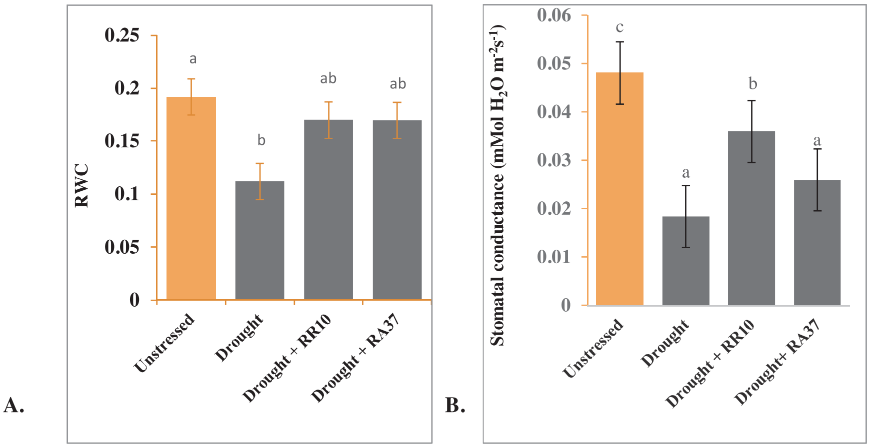

Fig. 8. Effect of bacterization by cactus endophytic bacteria on relative water content and stomatal conductance of drought stressed tomato.
i. Unstressed: Non-bacterized and well-watered plants.
ii. Drought: Drought stressed and non-bacterized plantlets.
iii. Drought + RR10: *B. megaterium* RR10 inoculated plants subjected to drought.
iv. Drought + RA37: *B. amyloliquefaciens* RA37 inoculated plants subjected to drought.
Error bars indicate standard deviations (SD) of mean values.
Error bar labelled with alphabet indicates statistical significance according to Duncan's multiple range test at P = 0.05.

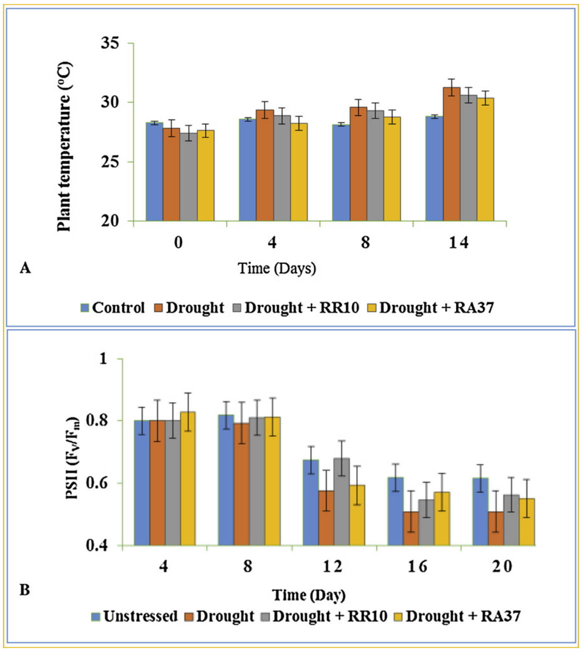

Fig. 9. Effects of cactus endophytic bacterial priming of tomato on internal leaf temperature and photosynthetic fluorescence.
i. Unstressed: Non-bacterized and well-watered plants.
ii. Drought: Drought stressed and non-bacterized plantlets.
iii. Drought + RR10: *B. megaterium* RR10 inoculated plants subjected to drought.
iv. Drought + RA37: *B. amyloliquefaciens* RA37 inoculated plants subjected to drought.
Error bars indicate standard deviations (SD) of mean values.
Bar labelled with alphabet indicates statistical significance according to Duncan's multiple range test at P = 0.05.

endophytic bacteria prior to drought induction. *Bacillus megaterium* CBm_RR10 and *B. amyloliquefaciens s*CBa_RA37 priming showed by 31.5% and 22.6% less activity of CAT enzyme. The reduced CAT and GPX enzymes activity is attributed to bacteria mediated drought destressing. Similar observation is earlier reported by Vardharajula et al. (2011) and Heidari and Golpayegani (2011) in *Bacillus* primed plants subjected to drought stress. It is also pertinent to note that stress enzymes activity are strongly influenced by the length and severity of water stress as well as the plant age and developmental stage (Bray, 1997).

Tomato pre-treated with *B.amyloliquefaciens*CBa_RA37 prior to drought induction recorded 86.9% recovery of plantlets from 18 days of water stress. None of the mock un-treated tomato plantlets survived

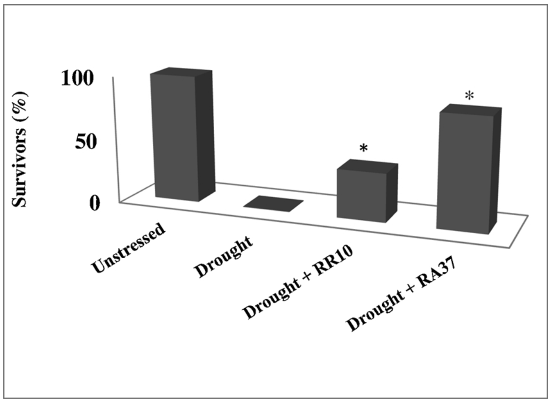

Fig. 10. Endophytic bacteria mediated recovery of drought affected tomato plants.
i. Unstressed: Non-bacterized and well-watered plants.
ii. Drought: Drought stressed and non-bacterized plantlets.
iii. Drought + RR10: *B. megaterium* RR10 inoculated plants subjected to drought.
iv. Drought + RA37: *B. amyloliquefaciens* RA37 inoculated plants subjected to drought.
Error bars indicate standard deviations (SD) of mean values.
Bar labelled with * indicates statistical significance according to Duncan's multiple range test at P = 0.05.

drought in our experiment. Lowest stomatal conductance, leaf temperature, and the highest RWC were recorded in *Bacillus amyloliquefaciens* CBa_RA37 or *Bacillus megaterium* CBm_RR10 treated plantlets. In fact, stomatal closure is a routine mechanism often activated by plant suffering from water shortage. Their closure is indicative of reduced internal water losses through evapo-transpiration. The, thus, retained water could serve in coping with drought for certain period of time since the oxygen comprises in water molecules sustains electron transfer in photosynthetic processes leading to a triggered photosynthesis. In the contrary, the lack of water (O_2) in plant cells will redirect the metabolic processes towards macromolecules oxidation, leading to temperature raises and most of all to leaves chlorosis (inhibition of the PSII system), and a gradual reduction in seedlings vigour till total dead. By slowing down the stomatal closure, the bacterial candidates altered simultaneously a huge number of metabolic pathways which could for some extend justify the observed changes in bacterial primed tomato seedlings. Therefore, it is tempting to suggest that cactus endophytic bacteria triggered physiological alterations in plants were capable of conferring drought tolerance in tomato.

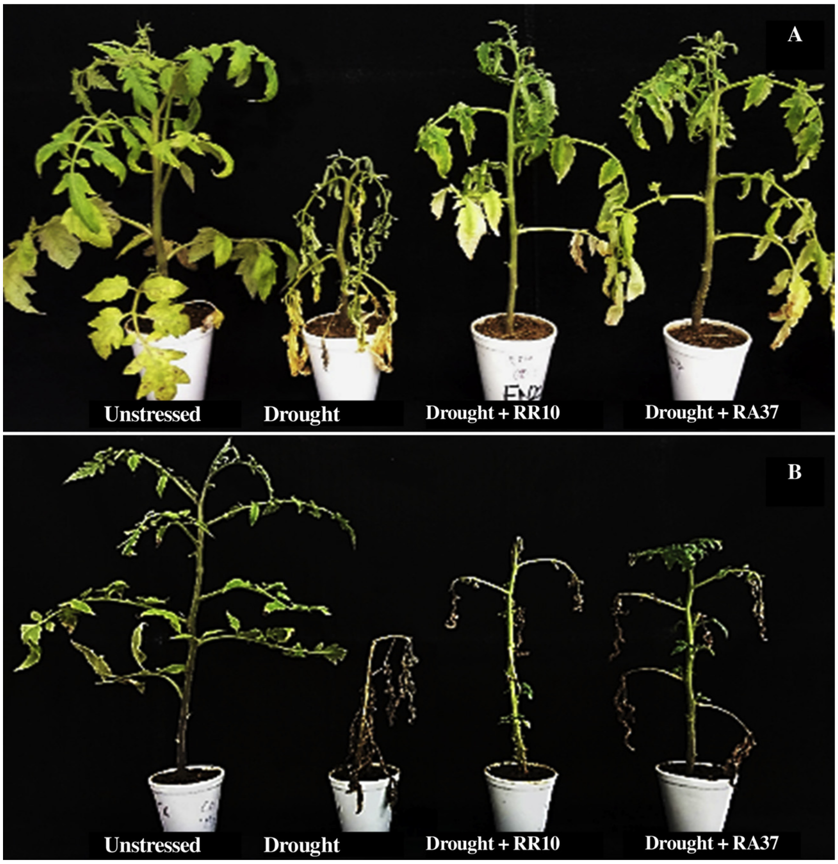

Fig. 11. Endophytic bacteria mediated recovery of drought affected tomato plants.

i. Unstressed: Non-bacterized and well-watered plants.

ii. Drought: Drought stressed and non-bacterized plantlets.

iii. Drought + RR10: *B. megaterium* RR10 inoculated plants subjected to drought.

iv. Drought + RA37: *B. amyloliquefaciens* RA37 inoculated plants subjected to drought.

5. Conclusion

Culturable endophytic microbiome of arid plant, Cactus naturally found in Cameroon harboured diverse bacterial species. Among them species belong to *Bacillus*, *Enterobacter,* and *Sphingomonas* dominated the endosphere of Cactus. Upon bacterization, the bacteria triggered varied phenotypic responses in tomato seed germination and early growth during its interaction. Seed germination ‘enhancers’ and germination ‘retarders’ were found among the bacterial endophytic species. Seed dormancy ‘breakers’ were also found among the bacterial collection. While species belong to *Bacillus* accelerated germination and reduced germination time, other bacterial genus actually reduced the

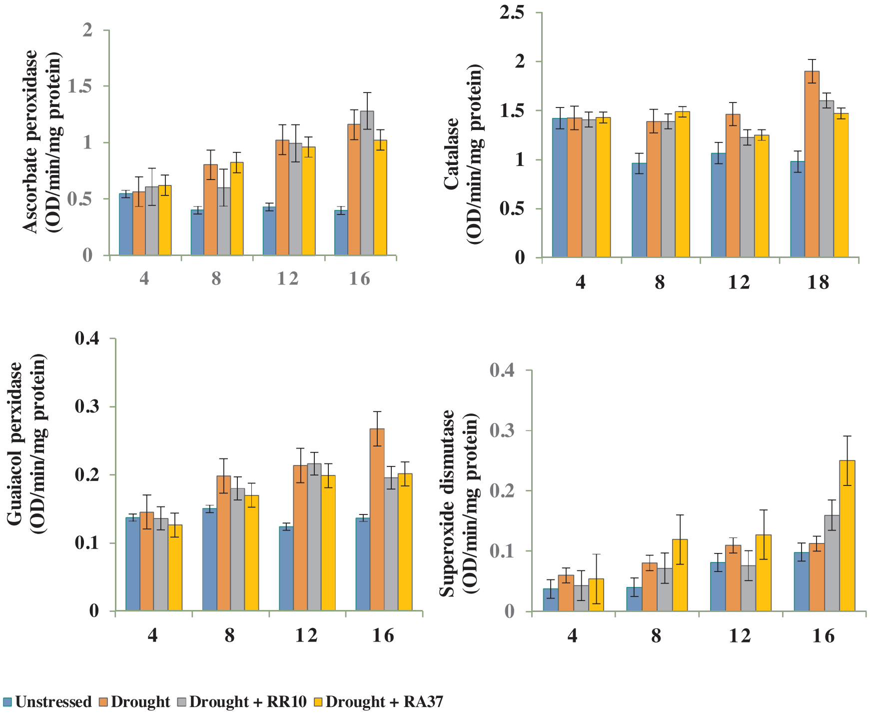

Fig. 12. Effect of bacterial priming with cactus endophytic bacteria on activity of oxidative stress related enzymes.

Labels 4, 8, 12 and 16: Specific activity of four stress responsive enzymes were measured 4, 8, 12 and 16 days post drought induction.

i. Unstressed: Non-bacterized and well-watered plants.

ii. Drought: Drought stressed and non-bacterized plantlets.

iii. Drought + RR10: *B. megaterium* RR10 inoculated plants subjected to drought

iv. Drought + RA37: *B. amyloliquefaciens* RA37 inoculated plants subjected to drought germination. *Bacillus xiamenensis* CBx_LPR21 is being reported as an endophyte for the first time. Species such as *Bacillus amyloliquefaciens* CBa_RR37 and *Bacillus megaterium* CBm_RR10 were able to impart drought tolerance as well as promote growth in tomato upon their seed and root interaction. Drought induction in tomato triggered several physiological and biochemical responses with consequent physiological wilt and eventual death of plantlets within three weeks of sustained water stress. It is further observed that cactus endophytic bacteria could colonise tomato with significant concomitant effects on diverse physiological functions and anti-oxidant enzyme activities. Endophyte-primed-drought-inflicted plants showed altered physiological parameters for stomatal conductance, relative water content, leaf temperature and photosynthetic efficiency as well as reduced activity of anti-oxidative enzymes like catalase and guaiacol peroxidase is suggestive of bacteria mediated de-stressing of plantlets from water stress. *Bacillus amyloliquefaciens* CBa_RA37 and *Bacillus megaterium* CBm_RR10 showed promise for large scale multilocation field evaluation and commercialization as a microbial agent for drought management in tomato. Our results provide new opportunities to harness the potential of unexplored 'arid plant microbiome' to mitigate abiotic stress in agricultural crops.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.micres.2019.126302.

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