# Soil parameters drive the diversity of *Citrus sinensis* rhizosphere microbiota which exhibits a potential in plant drought stress alleviation

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**Abstract**

Plant associated microorganisms, particularly those exhibiting a plant growth promoting (PGP) effect, play an important role in plant nutrition and health and the adaptation to unfavorable climatic conditions, such as drought which threatens the productivity of agricultural crops. The selection of specific microbial populations in the soil habitats associated to plants depends upon the soil physico-chemical parameters besides the 'rhizosphere effect' played by each plant species through rhizodepositions. In this study, we investigated the community structure and PGP potential of the microbiota associated to *Citrus sinensis* plants located in different geographical regions of Tunisia. The bacteria community structure was correlated to soil physiochemical parameters and we identified potassium, carbon and organic matter content as drivers of the *C. sinensis* microbiota composition. The evaluation of the potential of selected bacteria as biofertilizer and bio-stimulator under drought stress was achieved through the phylogenetic and functional characterization of a large collection of bacterial strains isolated from the rhizosphere of *C. sinensis*. The strains were screened *in vitro* for putative plant growth promoting traits, and the six most promising isolates were tested *in vivo* on *Solanum lycopersicum* and *Capsicum annuum* model plants. The bacterized plants were cultivated under drought stress and compared with not bacterized and fully irrigated control plants. All the tested bacteria induced a significant increase in the number of leaves and in root biomass of both plant species compared to not inoculated plants. Our results highlighted that the strains *Ensifer adhaerens* S1B1.5 and *Pseudomonas resinovorans* S4R2.6 were, in particular, effective in promoting plant growth under water shortage, indicating them as promising strains for the development of sustainable biofertilizers suited for agriculture in arid and semi-arid regions characterized by water scarcity.

**Keywords**: *Citrus sinensis*; Soil parameters; Bacterial community; Plant Growth Promotion; Drought stress

**Abbreviation**

ACC: 1-aminocyclopropane carboxylic acid deaminase

B: bulk soil

*C. sinensis*: *Citrus sinensis*

DGGE: denaturing gradient gel electrophoresis

IAA: Indole-3-acetic acid

ITS: internal transcribed spacer region

MS: Murashige and Skoog medium

PBS: saline phosphate-buffer

PCA: principal components analysis

PGP: Plant Growth Promoting

PCoA: Principal coordinates analysis

PVK: Pikovskaya medium

R: rhizosphere soil

S: root surrounding soil

TSA: Tryptic Soy Agar

TSB: Tryptic Soy broth

YEM: Yeast Extract Mannitol

## 1. Introduction

The rhizosphere is a niche of intense bilateral exchanges between the plant and its environment (Curl, 1982). In this micro-ecosystem, a plant-driven selection and the enrichment of a peculiar microbiota especially toward beneficial microorganisms occur. Plant Growth Promoting (PGP) microorganisms establish a positive relationship with the plant, leading to a better performance of the holobiont plant organism, composed by the plant and its associated microbiome (Sanchez-Cañizares et al., 2017). PGP microorganisms could directly promote plant growth by various direct mechanisms such as nitrogen fixation and phosphate solubilization (Cakmaçi et al., 2006; Orhan et al., 2006), modulation of the synthesis of ethylene hormone *via* 1-aminocyclopropane carboxylic acid deaminase (ACC) and synthesis of phytohormones or vitamins (Dobbelaere et al., 2013). PGP microorganisms can also promote indirectly plant growth by conferring protection against phytopathogens (Saharan and Nehra, 2011). They have indeed a promising application as biofertilizers, biopesticides and biostimulators to improve crop yield and to enhance other plant services like phytoremediation (Berg, 2009).

In particular, PGP microorganisms have been shown to play an essential role in improving plant growth and crop yields under stressful conditions like those typically occurring in arid soils (Soussi et al., 2015). Water deficiency is one of the most serious environmental abiotic stresses (Sanaullah et al., 2011), and PGP inoculants have been shown to mitigate drought effect conferring bacterially-mediated tolerance (Ngumbi and Kloepper, 2016; Rolli et al., 2015). Plants inoculated with exopolysaccharide producing PGP strains acquired resistance to water stress through the improvement of the soil structure (Sandhya et al., 2009). In semi-arid lands like Tunisia, besides the problem of desertification and soil degradation, there has been a decline in groundwater owing to decreases in rainfall (Djedidi et al., 2011). This decline threatens both annual plants, such as wheat (Farooq et al., 2009) and tomato (Liptay and Schopfer, 1983), and arboreal genera like *Citrus*. The exploitation of tailored PGP microorganisms could therefore play an important role in these regions for the development of sustainable methods improving the management of water stress, in turn mitigating the yield losses due to water scarcity (Frascari et al., 2018).

Dynamic interactions between soil, water and plant roots induce changes in the physicochemical and structural properties of the soil (Haynes and Swift 1990). All of these factors could affect the diversity and function of bacterial communities colonizing the rhizosphere of *Citrus* plants. The microbiota associated to this genus is poorly investigated (Trivedi et al., 2012), and the study of the factors driving the selection of rhizospheric

communities and their potential beneficial role on plant health could assist us to explain the resistance of some *Citrus* plants to biotic and abiotic stress and exploit this information to improve *Citrus* cultivation.

*Citrus* tree cultivation has a high economic relevance for the whole Mediterranean basin and it occupies a very extensive place in the Tunisian agricultural sector, where citrus fruits production reached 22,000ha. Many rural families derive their main income from citrus farms. The citrus sector has, moreover, a socio-economic importance since it covers the local needs and it provides a quantity intended for export which is estimated to be 14% of total agricultural exports (CTA, 2016). *Citrus sinensis* in particular is the most Tunisian sought exported variety (Laajimi and Zekri, 2001; Benfradj et al., 2016).

In this context, our study aimed to assess i) the influence of edaphic factors in shaping the bacterial diversity in citrus (*Citrus sinensis*) rhizosphere and ii) the potential of bacterial strains isolated from the rhizosphere of *C. sinensis* to colonize a model plant and support the growth of plants of agronomic interest under drought conditions.

## 2. Materials and methods

### 2.1. Studied sites and soil sampling

Sampling was performed from six sites located in the north and central of Tunisia, namely, Manzel bouzalfa (MB), Bni Khaled (BK), El kef (EK), Bizerte (BZ), Beja (BJ) and Sidi bouzid (SB) which were classified as sandy soil, excepting EK site which was sandy-clay (Fig. 1A, Supplementary Table 1) according to Moustarhfer et al. (2017). From each site three representative *C. sinensis* trees were selected. Using sterile equipment, from each tree specimen rhizosphere soil (R) and the less tightly adherent root surrounding soil(S) were sampled at a depth of 10 to 20 cm. In proximity, but out of the influence of the root exudates, free soil not in direct contact with any plant root was also sampled in each site after removing the upper 3 cm surface soil (bulk soil, B). Sampling and sample manipulation was done according to Ferjani et al. (2015).

### 2.2. Physico-chemical analysis

Physico-chemical analysis was performed on bulk (B) soil samples in the laboratory of soil analysis of El kef, Tunisia. An aliquot of the collected samples was dried at room temperature and subjected to the following physicochemical analyses as previously described by Moustarhfer et al. (2017): organic matter (OM), pH, carbon

(C), inorganic carbon ($CaCO_3$), potassium (K), nitrogen (N), phosphorus (P), assimilable phosphorus ($P_2O_5$) and exchangeable potassium ($K_2O$). Salinity was analyzed by measuring electrical conductivity (EC) (Supplementary Table 1).

2.3. Total DNA extraction and denaturing gradient gel electrophoresis (DGGE) analysis

Total DNA was extracted from soil samples by using the commercial kit FastDNA SPIN KIT for soil (Qbiogene, Carlsbad, USA) according to the manufacturer's procedure and stored at -20°C until use. PCR amplification of the 16S rRNA gene was performed using primers 907R and 357F-GC (Muyzer et al., 1993). Denaturing Gradient Gel Electrophoresis (DGGE) fingerprinting analysis of the PCR products was performed as previously described (Barbato et al., 2016) on 7% (w/v) polyacrylamide gel in 1X TAE pH7.4 with a 40–60% denaturing gradient. Gels were run at 90V for 17 h at 60°C and then stained in 1% ethidium bromide for 30 minutes. Gel images were acquired and analyzed using Gel Doc 2000 system (Bio-Rad, Tunis, Tunisia).

2.4. Isolation and identification of bacterial strains

To isolate bacteria from the different soil fractions, composite samples were prepared by homogenizing 1 gram of each triplicate sample. From each composite sample (n=18, S, R, B fractions from each of the 6 sites) serial dilutions were performed on saline phosphate-buffer (PBS) and plated in triplicate onto YEM (Yeast Extract Mannitol) and TSA (Tryptic Soy Agar) culture media. Plates were incubated for 3 days at 30°C. Colonies that presented different morphologies were randomly selected and purified through three subsequent streakings on the same medium. A total of 374 pure cultures were obtained and stored at -80° C in the medium supplemented by 15% glycerol.

Total DNA from each strain was extracted by boiling lysis (Ferjani et al., 2015). The supernatant containing DNA was used as template for the dereplication of the collection by PCR amplification of the internal transcribed spacer region (ITS) using universal primers S-DBact-0008-a-S-20 and S-D-Bact-1495-a-S-20 and visualization of ITS profiles by agarose gel electrophoresis (Daffonchio et al., 1998). By visual comparison of the ITS profiles the strains have been classified in groups exhibiting the same profile, thus belonging to the same species/subspecies (Daffonchio et al., 1998). One or two strains for each ITS group have been selected for subsequent phylogenetic identification (n=94).

Partial 16S rRNA gene was amplified from selected strains as previously described (Cherif et al., 2015). Amplicons were purified with Exonuclease-I and Shrimp Alkaline Phosphatase (Exo-Sap, Fermentas, Life Sciences) following the manufacturer's standard protocol. Sequence analysis of purified DNA was performed using a Big Dye Terminator cycle sequencing kit V3.1 (Applied Biosystems) and an Applied Biosystems 3130XL Capillary DNA Sequencer. Bacterial 16S rRNA gene sequences were analyzed by BLAST and compared with those available at the National Centre for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov) and Ribosomal Database Project (RDP) databases. The sequences were submitted to the NCBI nucleotide database under the accession numbers (MG569795-MG569888).

2.5. *In vitro* screening for PGP-related activity and Resistance to abiotic stress

The ninety-four identified strains were screened *in vitro* for PGP-related traits in triplicate assays. The quantification of the production of indole-3-acetic acid (IAA) in liquid culture was based on the colorimetric reaction between IAA and Salkowski's reagent. The color intensity was spectrophotometrically measured at 535 nm (Glickmann et al., 1998). Ammonia production was assessed using Nessler's reagent as detailed on Banerjee et al. (2010). Siderophore production was detected by the development of a clear orange halo around the bacterial colony covered with CAS medium (Neilands et al., 1986). The detection of protease activity was carried out in nutrient agar medium blended with an equal volume of skimmed milk; strains producing protease presented a clear zone around the colony (Kumar et al., 2012). Cellulase production was detected in CMC agar medium as described by Teather and Wood (1982). For qualitative estimation of phosphate solubilization, bacterial strains were tested on Pikovskaya (PVK) medium. The detection of a halo zone around bacterial colonies showed the ability of tested strains to solubilize phosphate (Nautiyal, 1999). Potential nitrogen fixation was evaluated on nitrogen free medium (Day et al., 1975).

Resistance to abiotic stresses was tested in triplicated assays by checking the growth of the isolates in medium tryptic soy agar or broth (TSA/ TSB) in the presence of each chemical and physical stress. An abiotic control, consisting in a sterile plate or tube, was also run parallel to each experiment. Resistance to salt was evaluated by growing the isolates at 30° C in solid medium supplemented by different NaCl concentrations, ranging from 0 to 15% w/v (Ferjani et al., 2015). Tolerance to osmotic stress was assessed by adding to liquid media 20–30% of Poly-Ethylene Glycol (PEG) (Ferjani et al., 2015; Mapelli et al., 2013). Temperature tolerance was verified by incubating each strain in solid medium at 40°C, 45°C or 50°C. Tolerance to acid and alkaline pH was assessed

by adjusting the solid medium with concentrated HCl (12 N) and the liquid medium with concentrated NaOH (3 M), respectively. The isolates were subsequently incubated for 48 hours at 30° C (Abolhasani et al., 2010).

2.6. Colonization of *Arabidopsis thaliana* plantlets

The strain *Pseudomonas japonica* S3R2.1 was chromosomally tagged with a gene coding for a red fluorescent protein (DsRed) using a mini-Tn7 transposon system by conjugation procedure using *E. coli Lam#5*as donor (Lambertsen et al., 2004). The transconjugant strain *P. japonica* S3R2.1_DR showed resistance to rifampicin and kanamycin conferred by resistance genes harbored by the transposon system and exhibited red fluorescence under epifluorescent microscopy. *P. japonica*S3R2.1_DR was used to bacterize *Arabidopsis thaliana* plantlets as detailed by Mapelli et al. (2013). Briefly, cells of *P. japonica* S3R2.1_DR harvested in exponential growth phase were supplemented to *A. thaliana* by dipping for a short time (1 to 4 hours) the axenic roots in Murashige and Skoog medium (MS, SIGMA, Italy) containing $10^8$ cells/ml. Plants were then transferred on sterile MS medium and incubated for 14 days in growth chamber (temperature 25°C, 55% humidity). Plant leaves were randomly sampled by cutting, washed with sterile water, crushed and re-suspended in sterile saline solution (NaCl 0.9%) for Colony-Forming Unit (CFU) determination on TSA medium supplemented by rifampicin (0.1 mg/ml) and kanamycin (0.05 mg/ml). The red fluorescence of randomly chosen colonies was verified by cell observation under epifluorescence microscopy.

2.7. *In vivo* screening PGP activity

The effects of five bacterial isolates, indicated in Table 3, on tomato (*Lycopersicon esculentum*) and pepper (*Capsicum annuum*) plants cultivated under greenhouse conditions inducing drought stress were investigated *in vivo*. Plant seeds were surface sterilized in 70% ethanol for 2 min, followed by 10 min in 1% sodium hypochlorite and, then, rinsed with sterile distilled water. To check the performance of seeds sterilization, 1 ml of the water used for last wash was plated on TSA medium and incubated for 3 days at 30°C, checking the absence of bacterial colonies. Seeds of tomato were germinated on water agar (agar 1.5% in sterile distilled water) in a growth chamber under controlled conditions (temperature 25°C, 55% humidity). Seeds of pepper were incubated on sterile perlite in the same conditions. After germination, plantlets were carefully removed from the germination substrate. Planted pots were maintained in a greenhouse with day/night temperature of

20/25°C with 100 mmol photons m–2 s–1 of light for 12 h. After 10 days of standard irrigation with tap water, plants were bacterized. Bacterial cells at the end of the exponential growth phase were harvested by centrifugation (4000 rpm, 10 min), washed two times with sterile saline solution and counted by phase contrast microscopy using a Thoma chamber. Five ml of cell suspension, containing $10^8$ cells/g of soil, were added to the soil surface of each pot. Each bacterial strain was added to a separate potted plant in a total of three replicated plants. Three control plants were treated in identical conditions except receiving bacterial inoculation. Ten days after bacterization, the plants were exposed to 14 days of drought stress, except the positive controls which were normally irrigated throughout the experiment. Drought conditions corresponded to the water withholds. Positive control plants were not bacterized and fully irrigated (not stressed and not bacterized control). After two weeks all the plants were harvested and the measurement of different growth parameters (shoots and roots lengths, shoot and root fresh weights and shoot and root dry weights) was performed (Marasco et al., 2012).

### 2.8. Statistical analysis

Principal Coordinate Analysis (PCoA) was applied to assess the differences in soil physico-chemical factors between sites. The band patterns of the DGGE gel were analyzed using Image J software and Microsoft Excel XLSTAT software (Addinsoft Inc., NewYork, NY, USA) to perform a statistical analysis of the fingerprints using Primer 6 and PERMANOVA+ for PRIMER routines as previously described (Ferjani et al., 2015). Distance-based multivariate analysis for a linear model (DistLM) was applied to identify which physicochemical factors significantly influenced bacterial assemblages in the rhizosphere. The distance-based redundancy analysis (dbRDA) was used for graphical visualization of the DistLM results. The marginal test was employed to assess the percentage contribution of each variable and their statistical significance and the sequential test was used to explain the biotic similarity taking into consideration all the variable contributions. The diversity indices were calculated in the PAST program version 2.17 (Hammer et al., 2001). Two-way ANOVA test was used to determine the difference between collection sites. The analysis of plant parameters measured in the *in vivo* PGP screening were carried out using the post-hoc tests ($P<0.05$) comparing bacterized plants and control plants.

## 3. Results

### 3.1. Soil physico-chemical factors

Sampling was carried out from six sites located in various parts of Tunisia, along a latitude transect ranging from 35° to 37° N and longitude from 8° to 10° E (Fig. 1A). The sampling sites MB, BK, EK, BZ and BJ were characterized by sub-humid bioclimatic zones while SB was located in an arid zone (You et al., 2016). Bulk (B) soil samples from investigated sites were physico-chemically characterized by analyzing electrical conductivity (EC), proxy of soil salinity, pH and the soil content inorganic matter (OM), total nitrogen (N), organic (C) and Calcium carbonate ($CaCO_3$), total (K) and exchangeable ($K_2O$) potassium, total (P) and assimilable ($P_2O_5$) phosphorus. Granulometric analysis of the soil particles showed that all the sites were classified as sandy soils except for the EK site which was sandy-clay (supplementary Table 1). The recorded EC varied between 0.68 and 2.73mmhos/cm showing that all the analyzed soils were non-saline (Supplementary Table 2). The soil pH was not highly variable between the different sites, ranging between 7.48 and 8.48, indicating a weak or moderately alkaline soil in all the investigated regions. Organic carbon varied between 0.09% and 2.32% and organic matter content showed values comprised between 0.16 % and 3.99%. Soils samples from SB and BJ sampling stations were very poor (0.32% and 0.16% respectively), those collected from BK and MB were moderately poor (0.83% and 1.43% respectively) while soil of sites EK and BZ could be defined as rich (2.1%) and very rich (3.99%) (Supplementary Table 2) (El oumlouki et al., 2014). Phosphorus was highly variable between the stations, ranging between 9.16 ppm in BK and 29.2 ppm in MB. In particular, assimilable phosphorus varied between 21 and 267 ppm (Supplementary Table 2). Both total and exchangeable potassium was in a very low amount in SB (14.57 ppm of K and 33.4 ppm of $K_2O$). On the contrary, the soil of site EK was very rich in this element (507 ppm of K and 600 ppm of $K_2O$) (Supplementary Table 2).

A principal component analysis (PCA) was performed on the physicochemical parameters of the bulk soil collected in the six sites (Supplementary Fig. 1). The PCA showed that soil in the six geographical locations constitutes different environments, nevertheless sites BJ, SB and BK were grouped, indicating more similar physicochemical parameters.

### 3.2. Diversity of bacterial communities associated to *Citrus sinensis*

The phylogenetic diversity and the structure of the bacterial communities in bulk soil and in R and S soil fractions associated to *C. sinensis* in the six different stations was described by DGGE fingerprinting applied to the 16S rRNA gene. The cluster analysis applied to the obtained fingerprinting (Fig 1C) showed that, except for samples of site BZ, the soil bacterial communities are clustered according to the site of collection and not

300 according to the level of association with the plant (B, S, R). Principal coordinate analysis (PCoA) indicates that
301 the soil dwelling bacterial communities changed significantly between the different sampling stations
302 (PERMANOVA, *df* = 5; *F* = 2.41; *P* = 0.022; Supplementary Table 3). To compare the community structure of
303 the six sites, diversity indices were calculated. The diversity indices showed that the abundance of species
304 obtained from EK site was higher than the diversity obtained from the other site as shown by Dominance and
305 Berger-Parker indices. On the contrary, the diversity of the bacterial community detected from the SB site was
306 the lowest (Supplementary Table 4).
307 Soil physicochemical parameters were used in a DistLM multivariate analysis (Fig. 1B) to unravel their possible
308 role as driver of the bacterial community structure associated with Tunisian *Citrus sinensis* rhizosphere. The
309 results of the DistLM analysis showed that soil physico-chemical factors significantly influenced the diversity of
310 the bacterial communities. In fact, marginal tests showed that exchangeable potassium ($K_2O$) and potassium (K)
311 significantly influenced ($P< 0.05$) the selection of bacterial populations (Table 1A). This finding was confirmed
312 by the sequential test (Table 1B), which also revealed that organic matter and carbon content were statistically
313 significant in determining the bacterial soil community structure.
314
315 3.3. Diversity of isolated bacterial communities
316
317 Three-hundred seventy-four bacterial strains have been obtained in pure culture from B, R, S soil fractions
318 collected in the six sampling stations. Phylogenetic redundancy of the collection was reduced by applying ITS-
319 PCR fingerprinting, obtaining 55 polymorphic haplotypes, which correspond to different species/subspecies
320 (Daffonchio et al., 1998). The strains exhibiting unique haplotype and two strains from the groups of two or
321 more strains showing identical ITS fingerprinting have been selected for phylogenetic identification. The
322 identification of the 94 selected strains detected 16 genera, *Pseudomonas, Bacillus, Staphylococus,*
323 *Streptomyces, Flavobacterium, Rhizobacterium, Arthrobacter, Agromyces, Erwinia, Paenibacillus*, *Salmonella,*
324 *Sporosarcina, Raoultella*, *Exiguobacterium, Ensifer* and *Gemmobacter*, affiliated to four phyla. None of the
325 isolates belonged to new taxa, since they showed a percentage of identity varying between 97 and 100% with the
326 closest described strain in NCBI database. The most frequently occurring phyla were *Proteobacteria* (40% of the
327 overall collection) and *Firmicute*s (37%), respectively dominated by the genera *Pseudomonas* (72% of
328 *Proteobacteria*) and *Bacillus* (64% of *Firmicutes*). Other abundant, even if less predominant taxa were
329 *Actinobacteria* (19% of the isolates) and *Bacteriodetes* (4%), mainly represented by the genera *Arthrobacter* and

330 *Flavobacterium,* respectively. An important variability was detected on the distribution of taxa between the six
331 sites. Two-way ANOVA test revealed that each isolate differs significantly between collection sites (*P<0.001*)
332 (Table 2). Strains belonging to the four above mentioned phyla were isolated from all the stations, but with
333 differences in the relative abundance. More than 50% of the genera were detected on the BK and EK sites, while,
334 in SB sites only 31% of genera were present, in agreement with the diversity index (Supplementary Table 5).
335 Sidi Bouzid (SB), the southernmost station, was the most different with the absence of *Actinobacteria* and the
336 higher predominance of *Proteobacteria*. The genera *Pseudomonas* and *Bacillus* were widely distributed in the
337 isolate collection and in most of the samples have been isolated with high frequency (Fig. 2 A).
338 Comparing the different fractions, the higher microbial diversity isolated from R and S soil could be explained
339 by the nutrient availability exerted by the root exudates (Fig. 2 B and Fig. 2 C). Between the sites, SB showed
340 the lower species abundance (Fig. 2D). None of the identified species was exclusively isolated from *C. sinensis*
341 rhizosphere, even if *Rhizobium, Salmonella and Exiguobacterium,* were isolated only in plant related fractions
342 (R, S) and not in B soil. *Erwinia, Sporosarcina and Gemmobacter* were isolated only from S (Supplementary
343 Fig. 2).
344
345 3.4. *In vitro* screening of the isolates: tolerance toward environmental stresses and PGP-related traits
346
347 The 94 identified isolates were screened for the tolerance to abiotic stresses. Fifty-four isolates were able to
348 tolerate pH extremes being able to grow at pH 12 (58% of the collection) and at pH 2 (55% of the collection).
349 None of the strains could be defined as true acidophiles or alkalophiles, as they were originally isolated from soil
350 of pH7, but they showed high tolerance to pH extremes. A significant fraction of the isolates was able to tolerate
351 thermal stress, with 41% of the strains capable of growth at 40°C and 3% of the strains at 50°C, temperatures,
352 >10 degrees above their isolation temperature. Aiming to select bacteria able to grow under drought stress, the
353 collection was tested in cultivation media with reduced water availability by the addition of 30% of PEG and
354 55% of the strains were capable to grow under osmotic stress. Likewise, since salinity often accompanies water
355 shortage in soils, these isolates were tested for salt tolerance. Only 15% of the collection demonstrated to be able
356 to grow in media supplemented with 12% NaCl, and 28% of strains were able to grow with 8% NaCl.
357 Interestingly, 7% isolates showed the ability to tolerate both high salt (10% Nacl) and osmotic stress (30% PEG).
358 The 94 identified bacterial isolates were further evaluated *in vitro* for PGP-related traits (Fig. 3). Most of the
359 isolates (77%) showed more than two PGP-related traits; these isolates were mainly belonging to the *Gamma-*

*Proteobacteria* (28%) and *Firmicutes* (24%) phyla. Bacteria isolated from *C. sinensis* rhizosphere and surrounding soil exhibited higher PGP potential *in vitro*, than those isolated from bulk soil.

Specifically, 49% of the tested isolates were able to solubilize inorganic phosphate and 80% exhibited the potential to fix nitrogen. Eighty-eight percent of the strains were able to produce ammonia. Four isolates isolated from rhizosphere showed the ability to produce siderophores, potentially improving iron nutrition in plants. Sixty-four percent of the collection was able to produce IAA and 49% produced it in high amount, more than 10 µg /ml and up to 70 µg/ml. Eighty-five percent of the strains showed ACC- deaminase activity, thus are potentially able to decrease ethylene level in plant. Production of siderophores, proteases and cellulases was detected on 4%, 16% and 15% of isolates, respectively, thus showing a potential important role in the protection of plants against phytopathogens. The statistical analysis supported a strong correlation between PGP traits and the phylogenetic identity of the strains since a significant difference was detected by comparing the PGP activities of *Firmicutes* and *Proteobacteria* vs *Actinobacteria* and *Bacteriodetes* ($P < 0.05$).

### 3.5. *In vivo* test of PGP activities

Basing on the previous *in vitro* screening, five of the best performing strains, i.e. those exhibiting tolerance toward environmental stresses and multiple potential PGP traits (Table 3), were selected for *in vivo* tests of their beneficial effect on the model plant species tomato and pepper under simulated drought. These strains were all isolated from *C. sinensis* rhizosphere and belonged to different taxa: *Pseudomonas japonica* S3R2.1, *Pseudomonas resinovorans* S4R2.6, *Pseudomonas jessenii* S4R2.1, *Ensifer adhaerens* S1B1.5 and *Ensifer adhaerens S1S2.5*. Bacterial cells were administered with irrigation water to the top soil of potted plants and at the end of the experiments plants were harvested and compared with control plants (non-inoculated) that were i) cultivated in the same condition of drought stress (CN), ii) cultivated at normal irrigation regime (CP).

Compared to CN plants, one or more parameters were significantly improved in all bacterized plants in both the plant species tested (Fig. 4, Supplementary Fig.3 and 4). Bacterial inoculation induced an increase in plant biomass (root, shoot, root dry and shoot dry weight), and number of leaves per plant. *P. resinovorans* S4R2.6 and *E. adhaerens* S1B1.5 showed the highest promotion levels in both tomato and pepper plants, better than in the CP control plants (Supplementary Table 7 and 8). In contrast, *P. japonica* S3R2.1 and *P. jessenii* S4R2.1 showed a selective beneficial effect on tomato and pepper, respectively.

389 *Pseudomonas resinovorans* S4R2.6 and *Ensifer adhaerens* S1B1.5 differentially altered growth parameters in
390 tomato plants ($P$<0.05) and, in particularly, shoot biomass was almost doubled in bacterized plants compared
391 with CN ones. The same strains induced a significant increase in root biomass on pepper plants (Fig.4), thereby
392 demonstrating an effective *in vivo* PGP effect. Compared with the negative controls, the plants inoculated with
393 the bacterial isolates showed a significantly higher growth in comparison to the non-stressed positive control
394 plantlets (Fig.4, Table 4). *Pseudomonas jessenii* S4R2.1 produced a significant increase in dry weight of pepper
395 roots (0.77 mg ± 0.03 mg) and shoots (0.24 mg ± 0.007 mg) and *Pseudomonas japonica* S3R2.1 showed an
396 increase on dry weights of tomato roots (1.22 mg ± 0.38 mg) and shoots (0.87 mg ± 0.01 mg). *Pseudomonas*
397 *resinovorans* S4R2.6 and *Ensifer adhaerens* S1B1.5 increased the growth of both plant species (Table 4).
398
399 3.6. Plant colonization by *Pseudomonas japonica* S3R2.1
400
401 To confirm the capability of bacteria to colonize plant-roots, one of the best performing strains, *P. japonica*
402 S3R2.1, was tested for its ability to overcome the root barrier and colonize the plant as a root endophyte. This
403 strain was chromosomally tagged with genes conferring fluorescence and antibiotic resistance, in order to be
404 able to unequivocally detect it in the plant. Roots of the model plant *A. thaliana* were dipped for a short time (1-4
405 hours) in a suspension of cells, and then cultivated in sterile hydroponic solution. After 16 days from bacterial
406 exposure, *P. japonica* S3R2.1_DR was detected in plant leaves at a density of $5*10^4$ CFU per gram of tissue,
407 thereby confirming the ability of the labelled strain to establish in *A. thaliana* plant as endophyte (Supplementary
408 Fig 5).
409
410 **4. Discussion**
411
412 In this study, we investigated the community structure and PGP potential of the microbiota associated to *Citrus*
413 *sinensis* plants located in different geographical regions of Tunisia. The bacteria community structure was
414 correlated to soil physiochemical parameters and we identified potassium, carbon and organic matter content as
415 drivers of the *C. sinensis* microbiota composition.
416 All six sites hosted unique bacterial communities. The soil of the EK site, differentiating from the other for the
417 texture, showed a higher abundance of species, than the other sites, supporting the hypothesis of a positive
418 correlation between clay soil content and microbiota diversity (Wakelin et al., 2008). K in the soil, both total and

419 the exchangeable fraction, was a driver of bacterial community assemblages ($P$<0.05). Although previous works
420 showed the important role of N and P in assembling the bacterial community in the soil (Cao et al., 2010; Friedel
421 and Scheller, 2002; Wakelin et al., 2008), these parameters were not identified as significant in the present work.
422 Instead, we found that K, which is one of the three macronutrients needed for plant growth and development (He
423 et al., 2016), played a crucial role in differentiating the structure of *C. sinensis* associated bacterial communities.
424 The role of potassium in affecting the overall soil community structure is still poorly described but its significant
425 correlation to rhizosphere bacterial diversity has been recently reported in cold deserts (Mapelli et al., 2018).
426 Moreover, it is well known that soil microorganisms can promote soil K fertility by dissolving K bioweathering
427 processes (Mapelli et al., 2012). For example, the weathering capacity was previously demonstrated for bacteria
428 isolated from the rhizoplane of cacti plants growing in a desertic area and was linked to their role in plant growth
429 promotion (Puente et al., 2004). Similarly, the inoculation of different plant species with rhizobacteria isolated
430 from Mediterranean shrubs growing in a semiarid ecosystem resulted in higher levels of plant tissue K (Armada
431 et al., 2015). The result is in agreement with a previous study that indicated such parameters among the
432 significant physicochemical factors influencing the structure of the soil dwelling microbiota independently from
433 the land-use type (Kuramae et al., 2012). Although previous studies reported pH as the best predictor of soil
434 bacterial diversity (Fierer and Jackson 2006) and low pH was indicated as a major constraint on the soil
435 microorganism's diversity (Frostegard et al., 1993; Lauber et al., 2009; Xu et al., 2006), soil pH was not a
436 significant factor influencing the bacterial community structure in our study.
437
438 Among the 374 bacterial strains isolated from the *C. sinensis* associated environment, only 24% belong to
439 species previously isolated from soil (Wang et al., 2010). The majority of the strains affiliate with taxa typically
440 isolated from plant (Reva et al., 2002), animal (Takahashi et al., 1997), atmosphere (Shivaji et al., 2009) or other
441 origin (Vandamme et al., 2013; Die et al., 2012). The strains belonged to only six ubiquitous phyla, retrieved in
442 all the analyzed samples, showing nevertheless a different distribution among the sub-collection obtained from
443 different sites and niches. The phylogenetic diversity was higher in the root surrounding soil (fraction S) and in
444 the rhizosphere soil (fraction R), than in the bulk soil (fraction B). This could be explained by a stimulation of
445 rhizosphere activity by the root exudates, which increased the abundance and/or cultivability of a higher number
446 of taxa. A significant difference was also detected in sub-collections obtained from the six sites. This result,
447 based on the cultivable fraction of soil microbiota, is in agreement with previous results obtained by a cultivation
448 independent PCR-fingerprinting approach. Taken together, these results confirm the importance of

449 environmental factors and soil texture on bacterial shaping, as has been noted in other studies (Hackl et al., 2005;
450 Schlecht-pietsch et al., 1994; Wakelin et al., 2008). The genera *Pseudomonas* and *Bacillus* were ubiquitously
451 abundant in all the various fractions retrieved from all the studied sites. Previous studies targeting the cultivable
452 bacterial community also reported the presence of these genera in association with the rhizosphere of several
453 plants (Marasco et al., 2012; Mapelli et al., 2013). As a result, we can suggest that these taxa are not specifically
454 selected by *C. sinensis* species but rather are typical soil and rhizosphere inhabitant. Roots exudates are also
455 known to enhance bacterial density and change the metabolic fingerprint of the soil bacterial communities
456 (Baudoin et al., 2003), leading to the so-called "rhizosphere effect" (Smalla et al., 2001). The rhizobacterial
457 community of *C. sinensis* Florida described by Trivedi et al. (2012) and mandarin orange in India analyzed by
458 Thokchom et al. (2014) were partially in agreement with our findings regarding the dominance of
459 *Proteobacteria*, especially of the *Pseudomonas* genus. Likewise, *Bacillus, Staphylococus, Streptomyces,*
460 *Arthrobacter, Agromyces* gernus were also detected among Florida citrus rhizobacteria (Trivedi et al., 2012).
461 However, it is worthy to note that the diversity of culturable soil microbes is significantly lower than the
462 diversity of total microbes present in the soil. As a result, our study only tested a very small fraction of the
463 potential PGPR.
464
465 PGP microorganisms have been shown to play an essential role in improving the plant growth especially under
466 stressful conditions (Zelicourt et al., 2013). In some cases, the PGP effect was not inherent trait of the tested
467 bacterial strains, but rather an effect that was expressed under environmental stress (Rolli et al., 2015). Our
468 hypothesis was thus that the microbiome associated to *C. sinensis* adapted to semi-arid conditions could exhibit
469 the potential to improve the growth of plant subjected to drought stress. The screening of 94 identified isolates *in*
470 *vitro* for PGP-related traits showed that 98% percent of the tested isolates demonstrated at least one *in vitro* trait
471 related to PGP activity, and that the soil environment hosts a rich, cultivable and potentially beneficial
472 microbiome. By comparing the PGP potential of strains isolated from different soil niches, our results
473 demonstrated that bacterial strains from the rhizosphere plane active plant-bacteria interaction are occurring
474 displayed a higher number of PGP –related traits than those isolated from bulk soil. This result indicates that the
475 plant enriched its proximity to develop a beneficial microbiome with the potential to improve its growth and
476 survival under adverse conditions (Marasco et al., 2012; Naveed et al., 2014; Sarwar and Kremer, 1995). In fact,
477 88% of the isolates were able to produce ammonia, a finding in agreement with many studies reporting ammonia
478 production as a common trait in rhizosphere bacteria (Ahmad et al., 2008; Saleem et al., 2007). Moreover, more

than 40% of the strains were able to solubilize phosphorus and potentially fix nitrogen, major compounds in plant nutrition (Schachtman et al., 1998; Wani et al., 2007). Siderophore producing strains have been isolated from *C. sinensis* in different geographical areas, belonging especially to the *Pseudomonas* and *Bacillus* genera, in accordance with our results (Araújo et al., 2002; Trivedi et al., 2011). Most of the tested strains demonstrated potential biostimulation activity by their ability to produce indol acetic acid (IAA), a phytohormone which stimulates shoot and root elongation (Srinivasan et al., 1996) and ACC-deaminase activity, a compound which is involved in the decrease of ethylene level in plant. Ethylene is a stress- and senescence-related hormone in plants (Saleem et al., 2007; Zahir et al., 2008) and bacteria able to hydrolyze its precursor ACC could have a significant potential role in decreasing plant response to stress perception. Production of proteases and cellulases detected on some isolates of our collection indicates also a potential biocontrol activity for the protection of plants against phytopathogens (Kloepper et al., 1980). Overall, our results confirmed that many bacterial genera isolated from *C. sinensis* root environment demonstrated PGP potential (Trivedi et al., 2011).

Comparing the tolerance to environmental stresses of the strains according to the soil niche, we found that the strains isolated from the rhizosphere fraction (78%) were, on the average, more tolerant than those isolated from the bulk soil (55%). Although further studies are needed to explain this observation, we can suggest that the rhizosphere environment constitutes a more dynamic environment than the free bulk soil, hence inferring a lower selection pressure toward the enrichment of stress tolerant organisms. In contrast, there was no significant difference in the environmental stress tolerance of strains isolated from the different sites. This result further emphasizes the importance of the rhizosphere in the selection of PGP.

Five *in vitro* best performer strains were selected from the bacterial collection based on multiple PGP related traits and higher tolerance toward temperature, pH, osmotic and saline stress. The strains, belonging to the class of *Alpha-proteobacteria* and *Gamma-proteobacteria,* were evaluated *in vivo* for plant growth promotion on model tomato and pepper plants cultivated in potted soil under drought stress. Comparing the fully irrigated and the water stressed not bacterized controls, we observed that deficit irrigation reduced root proliferation, stem extension and leaf number and size, and yellowing of tissues in the shoots and leaves, in agreement with literature data (Farooq et al., 2009). *Pseudomonas japonica* S3R2.1 and *Ensifer adhaerens* S1B1.5 significantly increased in *vivo* physiological parameters of tomato and pepper plantlets. Both the strains produced IAA, thus we hypothesize that this activity could be implicated in the PGP effect. *Pseudomonas resinovorans* S4R2.6, in particular, produced high quantity of this phytohormone (62.5µg/ml) which could explain the changes in root length observed in both plant species inoculated by this strain. Several previous reports have described the

beneficial traits of isolates belonging to the *Pseudomonas* genus (Nowak 1997; Siddikee et al., 2011), that promote plant growth under drought stress conditions (Sandhya et al., 2010; Mayaka et al., 2005). Likewise, higher PGP activity under salt stress conditions was reported also for the *Ensifer* genus (Djedidi et al., 2011; Payakapong et al., 2006). The capacity to produce exopolysaccharides and create a hydrophilic biofilm around the roots, improve soil water retention in dry soils (Rossi et al. 2012), as has been reported for the species *E. adhaerens*.

To efficiently support water stress, a close association should be realized between plant and bacteria (Marasco et al., 2012; Rodrigues and Hemert, 2001). The most intimate association is realized between plants and endophytic bacteria, potentially conferring a strong advantage for PGP bacteria (Podile and Kishore, 2006). Previous works showed that different rhizospheric bacterial strains belonging to *Pseudomonas* genus showed the ability to colonize roots (Maurhofer et al., 1993; Wang et al., 2000; Marascoet al., 2016). Using a tagged strain, *P. japonica* S3R2.1 had shown an endophytic lifestyle. After soil inoculation, the PGP strain overcame the root barrier and colonized the plant leaf tissues.

## 5. Conclusions

The results of the *in vitro* characterization of a large collection of bacterial isolates associated to *C. sinensis* from different geographical areas in Tunisia allowed the identification of strains with a promising potential to promote plant growth in dry environments, which now a day's represent an emerging problem also at higher latitudes. Two strains showed the ability to mitigate the effects of drought in different crop species, tomato and pepper, significantly improving plant growth at the same levels than fully irrigated plants. Moreover, one of the strains was able to overcome root barrier and establish as endophyte an intimate relationship with the plant. We propose the strains *Ensifer adhaerens* S1B1.5 and *Pseudomonas resinovorans* S4R2.6 as biofertilizer candidates, particularly suited for desert farming practices.

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**Figures captions**

**Fig. 1.** Sampling locations and the diversity of the bacterial community associated with *Citrus sinensis* rhizosphere.

A. Map of Tunisia showing the location of sampling sites.

B. DistLM analysis to assess the physicochemical factors significantly influencing the structure of the bacterial communities in the analyzed soil fractions. E.C: electric conductivity, SP: saturated Paste, pH, $CaCo_3$: inorganic carbon, C: carbon, M.O: organic matter, $P_2O_5$: phosphorus assimilable. P: phosphorus. K: potassium. $K_2O$: exchangeable potassium, N: nitrogen.

C. sample clustering patterns in relation to sites and soil.

**Fig. 2.** Cultivable bacterial community structure associated to *Citrus sinensis* in the different site of sampling.

A. Distribution of different bacterial phyla based on sampling sites

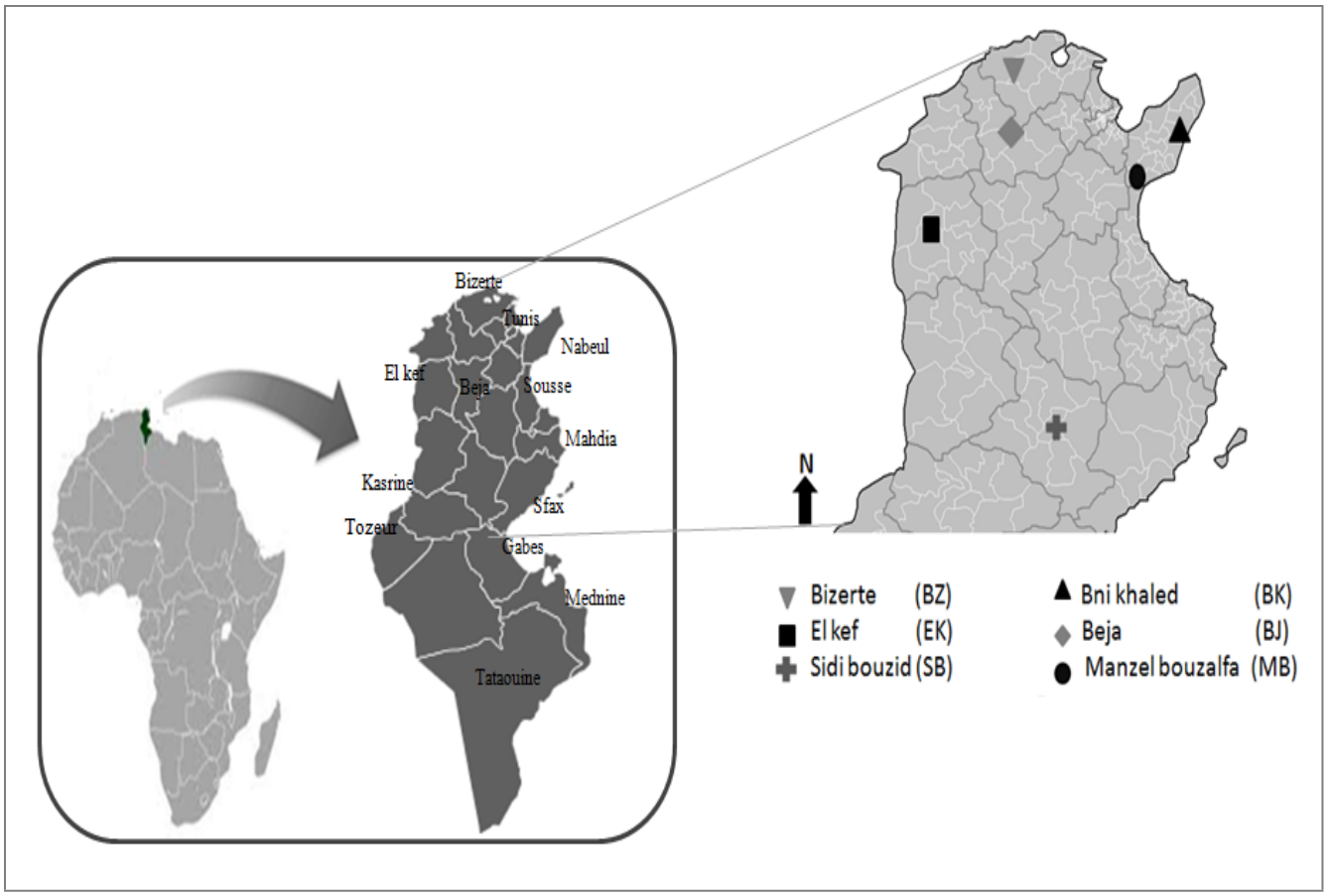
B. Percentages of bacterial genera in the rhizosphere soil factions. R.

C. Percentages of bacterial genera in the root surrounding soil fractions S.

D. Percentages of bacterial genera in the bulk soil factions B.

**Fig. 3.** Percentage of strains showing PGP activity and tolerance to abiotic stress. Strains are shown in different colors according to their phylogenetic classification.

**Fig. 4.** Evaluation of PGP selected bacteria on pepper and tomato growth promotion under drought stress. The graphs show the increase in root fresh biomass and root dry biomass of pepper (A) and tomato (B) shoot fresh biomass and shoot dry biomass of pepper (C) and tomato (D) of pepper plants treated compared with the untreated plants. Statistical analysis using Student's *t*-test showing significant difference reported as $*P \leq 0.05$.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Gabes
Mednine
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N
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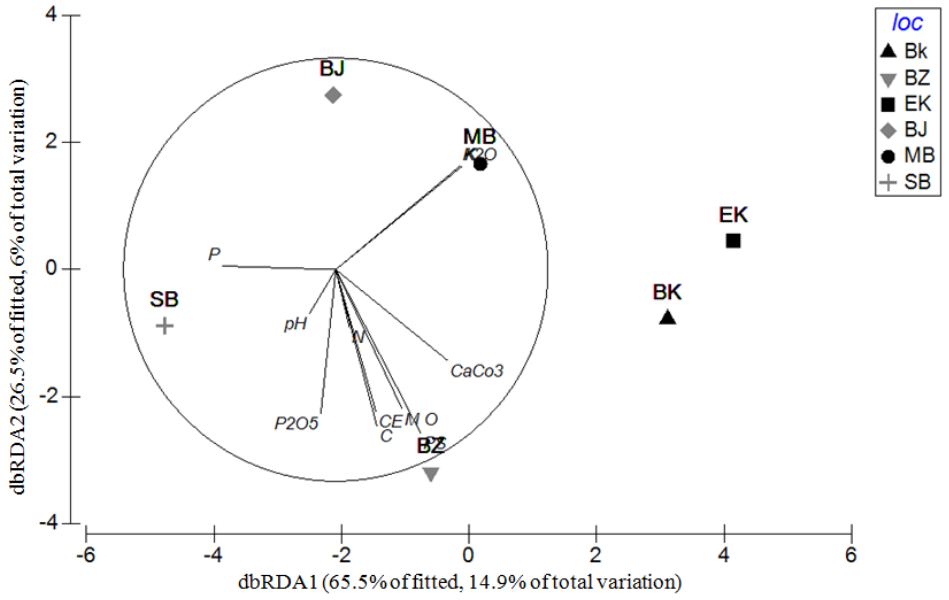
El kef (EK)
Sidi bouzid (SB)
Bni khaled (BK)
Beja (BJ)
Manzel bouzalfa (MB)

A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dbRDA2 (26.5% of fitted, 6% of total variation)
dbRDA1 (65.5% of fitted, 14.9% of total variation)
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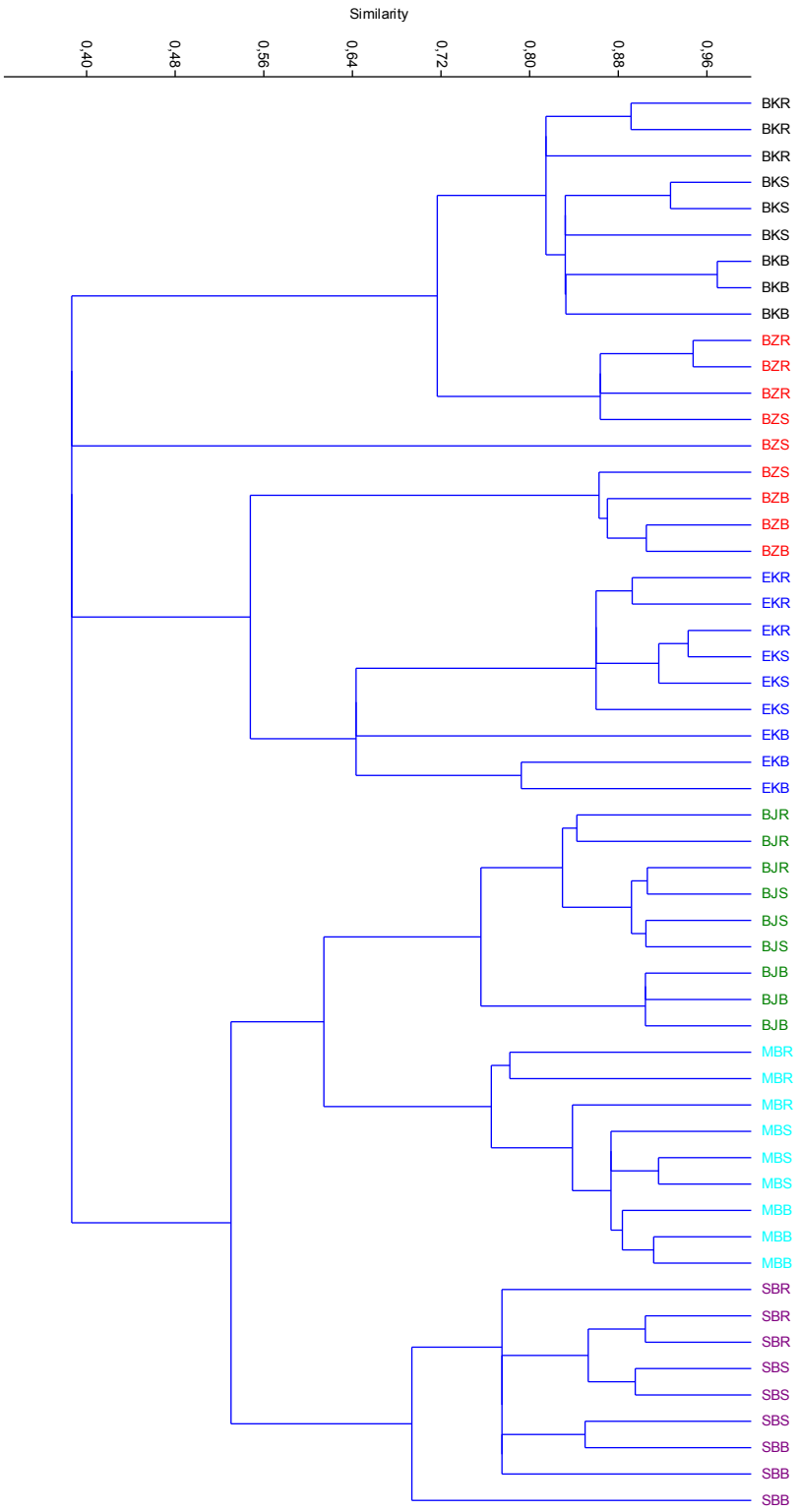
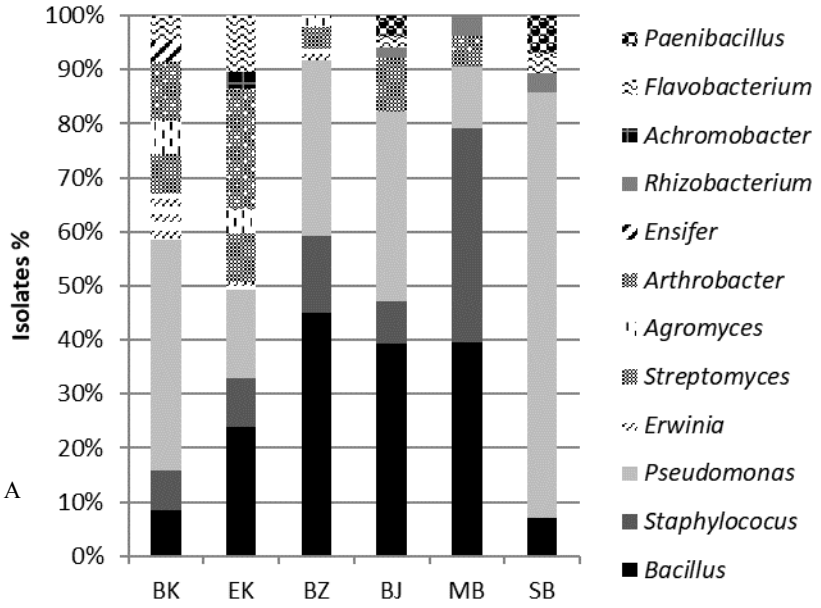
B




833

834 C

835 **Fig.1**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Achromobacter
Rhizobacterium
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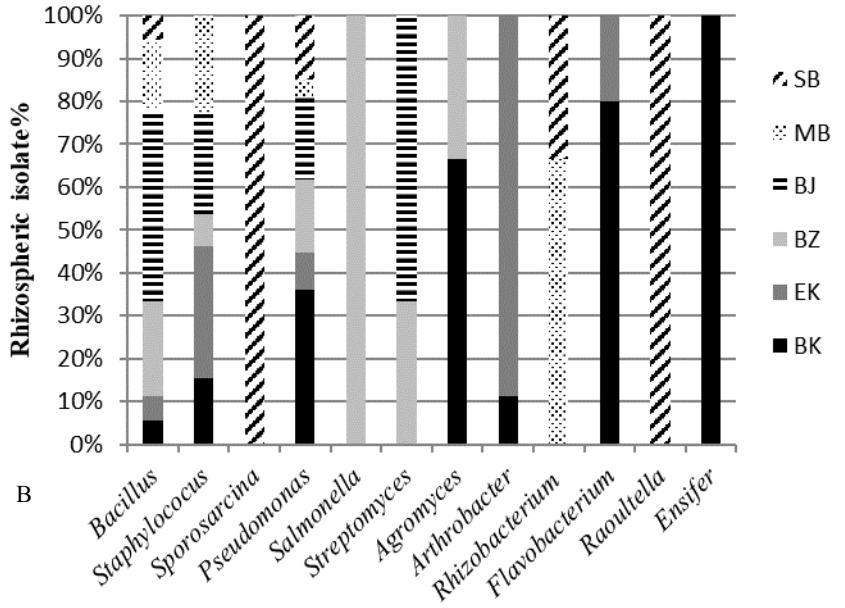
Arthrobacter
Agromyces
Streptomyces
Erwinia
Pseudomonas
Staphylococcus
Bacillus

A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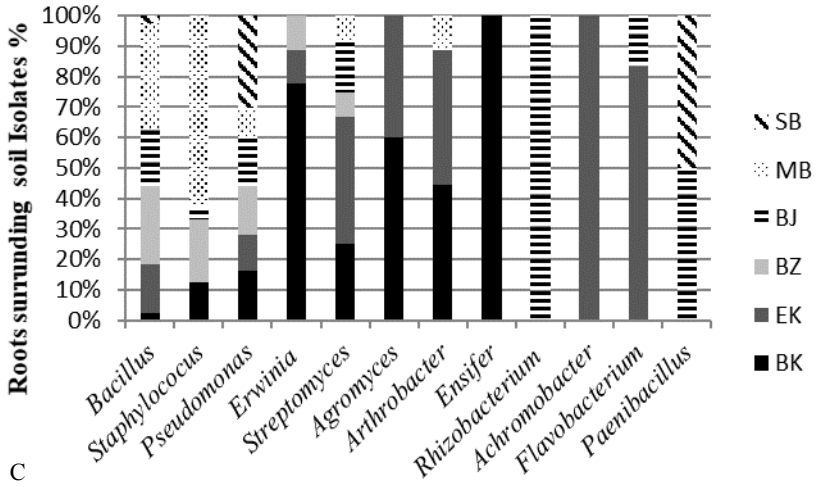
Staphylococus
Sporosarcina
Pseudomonas
Salmonella
Streptomyces
Agromyces
Arthrobacter
Rhizobacterium
Flavobacterium
Raoultella
Ensifer

B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Roots surrunding soil Isolates %
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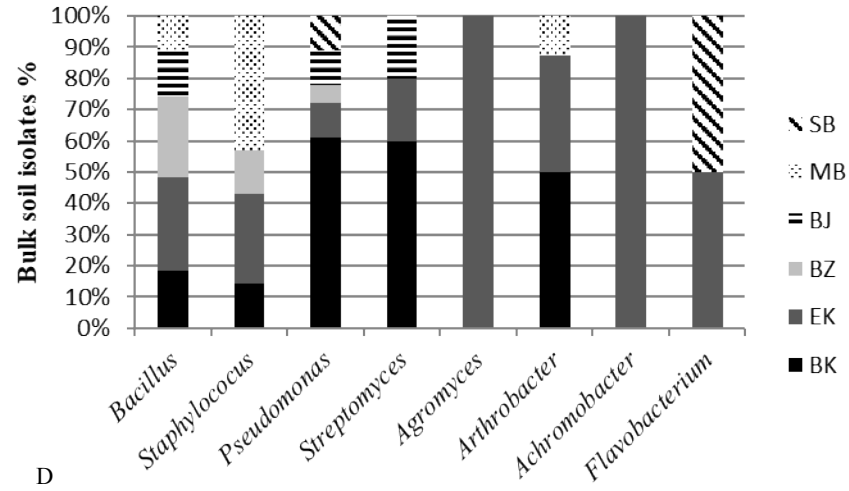
EK
BK
Bacillus
Staphylococus
Pseudomonas
Erwinia
Streptomyces
Agromyces
Arthrobacter
Ensifer
Rhizobacterium
Achromobacter
Flavobacterium
Paenibacillus

C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Pseudomonas
Streptomyces
Agromyces
Arthrobacter
Achromobacter
Flavobacterium

D

**Fig. 2**

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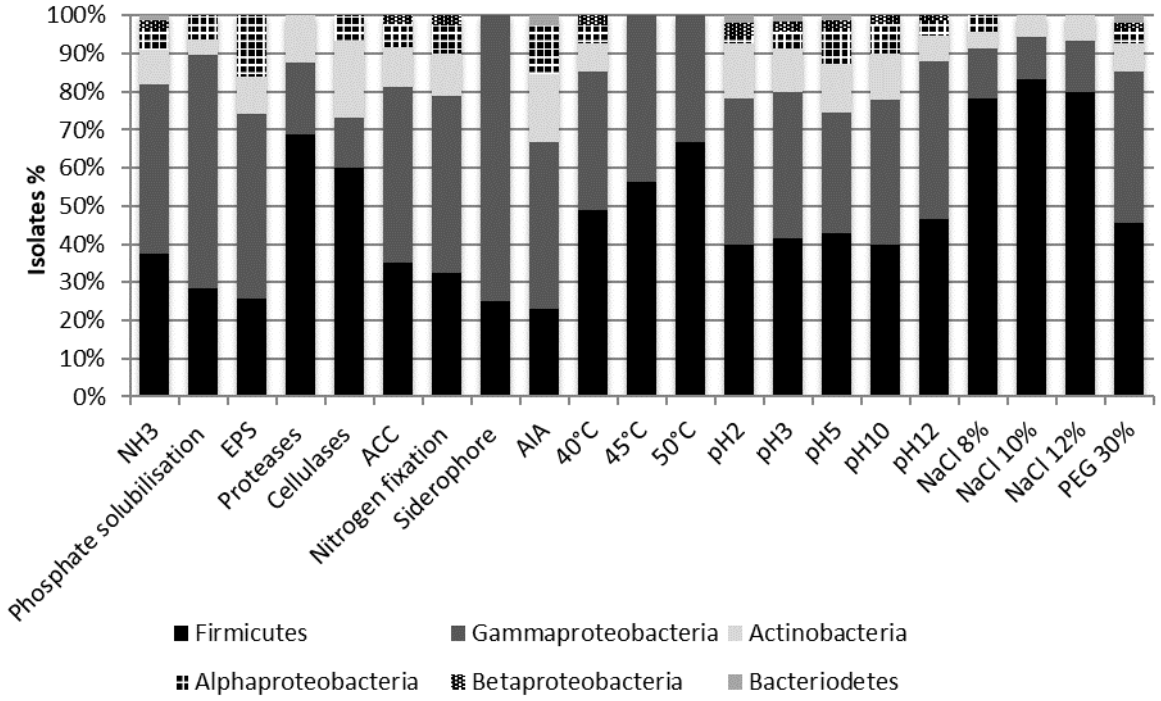


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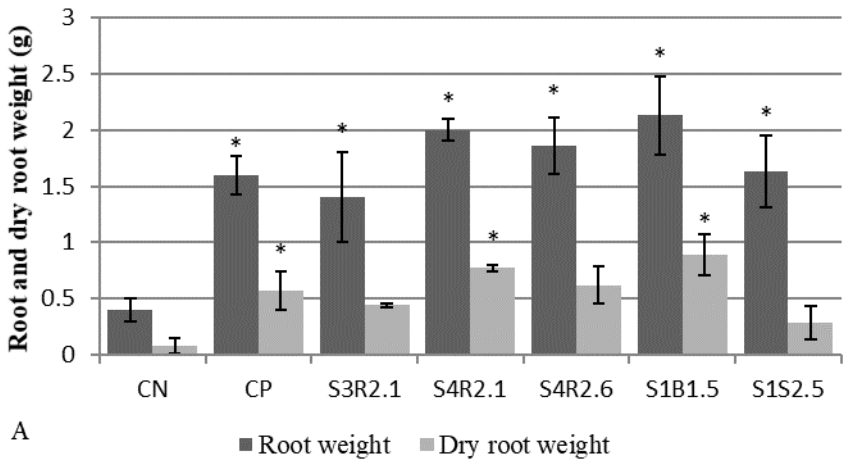
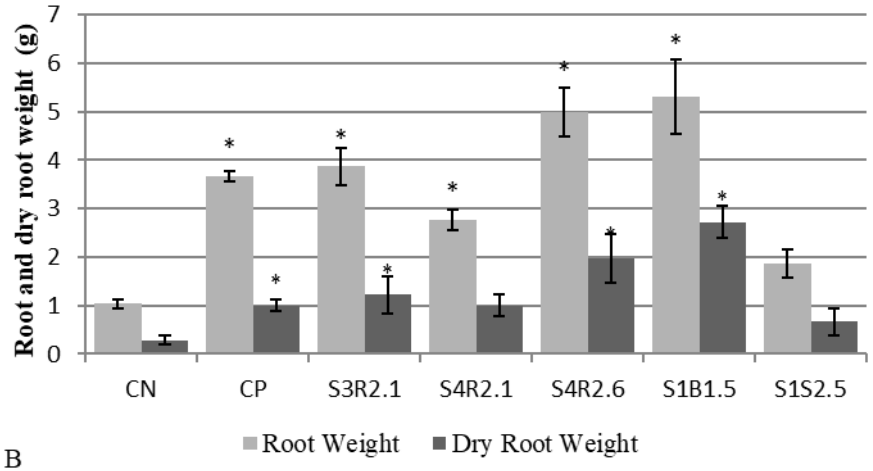
841 **Fig. 3**




A




B

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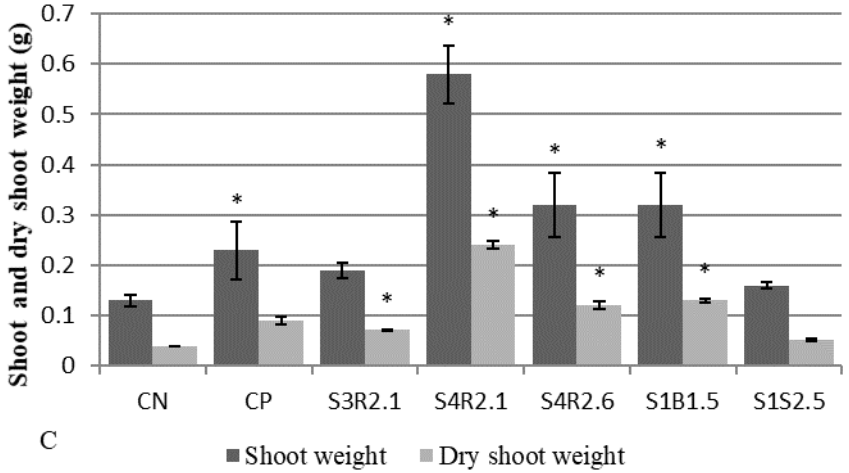
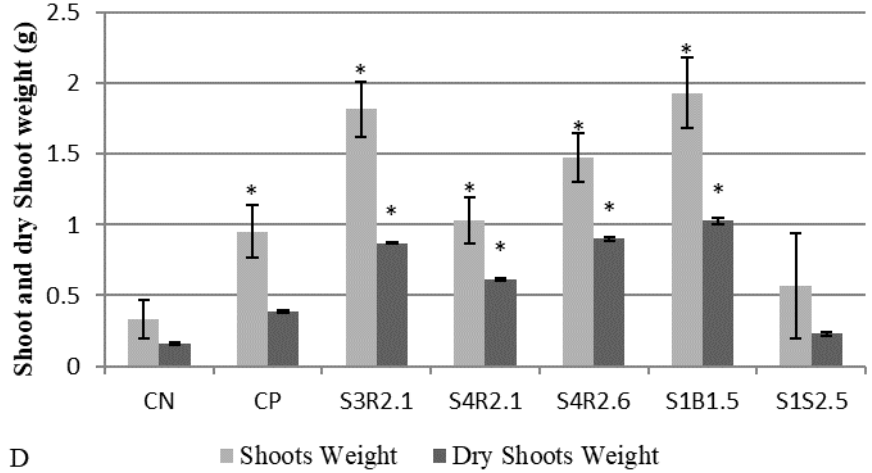



C




D

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**Fig.4**

846 **Table1.** Correlation between physico-chemical factors and the structure of *Citrus sinensis* soil bacterial community according to the Distance-based Linear Model (DISTLM).
847 (a) Marginal test. (b) Sequential test. Organic matter (OM), pH, Carbon (C), Calcium carbonate ($CaCO_3$), Potassium (K), Nitrogen (N), Phosphorus (P), Assimilable sodium
848 phosphorus ($P_2O_5$) and exchangeable potassium ($K_2O$) . Salinity was analyzed by measuring electrical conductivity (EC) and saturated Paste (SP).*F*: statistic *F*; *P*: probability
849 (Variables statistically significant in bold; *P*<0.05); Prop: proportion of total variation explained; Cumul: cumulative variation clarified by the variables listed; Res *df*: residual
850 degrees of freedom.

851 (A) Marginal test

| | SS (trace) | *F* | *P* | *Prop* |
|---|---|---|---|---|
| SP | 201.24 | 3.3876 | 0.061 | 0.061162 |
| EC | 114.96 | 1.882 | 0.158 | 0.03494 |
| pH | 29.342 | 0.4679 | 0 .0584 | 0.0089178 |
| $CaCO_3$ | 186.67 | 3.1276 | 0.065 | 0.056734 |
| C | 132.66 | 2.1847 | 0.117 | 0.040319 |
| OM | 141.44 | 2.3357 | 0.114 | 0.042986 |
| $P_2O_5$ | 104.93 | 1.713 | 0.158 | 0.031892 |
| P | 159.72 | 2.6531 | 0.083 | 0.048544 |
| **K** | **227.58** | **3.864** | **0.04** | **0.069168** |
| **$K_2O$** | **223.5** | **3.7896** | **0.037** | **0.067927** |
| N | 33.63 | 0.53698 | 0.57 | 0.010221 |

852

853 (B) Sequential test

| | SS | F | P | Prop | Cumul | Res.df |
|---|---|---|---|---|---|---|
| SP | 201.24 | 3.3876 | 0.057 | 0.061162 | 0.16118 | 52 |
| EC | 31.948 | 0.53298 | 0.512 | 0.0097099 | 0.070872 | 51 |
| pH | 166.65 | 2.8829 | 0.079 | 0.050651 | 0.12152 | 50 |
| $CaCO_3$ | 57.912 | 1.0018 | 0.366 | 0.017601 | 0.13912 | 49 |
| **C** | **292.7** | **5.5317** | **0.008** | **0.088959** | **0.22808** | **48** |
| **OM** | **219.95** | **3.694** | **0.043** | **0.066849** | **0.07707** | **47** |
| $P_2O_5$ | 104.93 | 1.713 | 0.186 | 0.031892 | 0.031892 | 46 |
| P | 159.48 | 2.688 | 0.066 | 0.04847 | 0.080362 | 45 |
| **K** | **276.42** | **5.0073** | **0.015** | **0.084013** | **0.16108** | **44** |
| **$K_2O$** | **187.07** | **3.5623** | **0.036** | **0.056856** | **0.21794** | **43** |
| N | 33.63 | 0.53698 | 0.531 | 0.010221 | 0.010221 | 43 |

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856

**Table 2.** Anova two way to determine the frequency of each isolate between collection sites.

ANOVA

| *Source of variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F-crit* |
|---|---|---|---|---|---|---|
| Samples | 27.2156863 | 5 | 5.44313725 | 1.83032967 | 0.10835983 | 2.25834248 |
| Species | 885.986928 | 16 | 55.374183 | 18.6203297 | **1.0619E-31** | 1.69329572 |
| Interaction | 463.895425 | 80 | 5.79869281 | 1.94989011 | **0.00009** | 1.3449069 |
| Within | 606.666667 | 204 | 2.97385621 | | | |
| | | | | | | |
| Total | 1983.76471 | 305 | | | | |

**Table 3.** Plant Growth Promoting Activities of rhizobacteria.$NH_3$= ammonia production; EPS = Exopolysaccharide production; P Sol. = inorganic phosphate solubilization; Prot. = protease activity; Cellu = cellulase activity; IAA = auxin production; N fix. = nitrogen fixation; Sid. = siderophore production; ACCd= ACC deaminase activity; NaCl = sodium chloride and PEG = polyethylene glycol.

| | | Potential of Plant Growth Promoting Traits | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sites** | **Strains** | **$NH_3$** | **EPS** | **P. sol** | **Prot** | **Cellu** | **IAA** | **N.fix** | **Sid** | **ACCd** | **40°C** | **45°C** | **50°C** | **pH2** | **pH3** | **pH5** | **pH10** | **pH12** | **NaCl 8%** | **NaCl 10%** | **NaCl 12%** | **PEG 30%** | **PGP score** | **Species Affiliation** | **Accession nomber** |
| BZ | S3R2.1 | ■ | | ■ | | | ■ | ■ | | ■ | | | | ■ | ■ | ■ | ■ | ■ | | | | ■ | 11 | *Pseudomonas japonica* | MG569879 |
| BJ | S4R2.1 | ■ | | ■ | ■ | | ■ | ■ | | ■ | | | | | | | ■ | ■ | | | | ■ | 9 | *Pseudomonas jessenii* | MG569826 |
| BJ | S4R2.6 | ■ | | ■ | | | ■ | ■ | | ■ | | | | | | | ■ | ■ | | | | ■ | 8 | *Pseudomonas resinovorans* | MG569797 |
| EK | S1B1.5 | ■ | ■ | ■ | | | ■ | ■ | | ■ | | | | | | ■ | ■ | | | | | ■ | 9 | *Ensifer adhaerens* | MG569841 |
| EK | S1S2.5 | ■ | ■ | ■ | | | ■ | ■ | | ■ | ■ | | | | | ■ | ■ | | ■ | | | ■ | 11 | *Ensifer adhaerens* | MG569853 |

YES ■ NO □

872 **Table 4.** Dry weight of shoots and roots of tomato and pepper seedlings.

| | CN | CP | S3R2.1 | S4R2.1 | S4R2.6 | S1B1.5 | S1S2.5 |
|---|---|---|---|---|---|---|---|
| Tomato Dry Shoots Weight (mg) | 0.16 ± 0.01 | 0.39 ± 0.01 | 0.87 ± 0.01 | 0.62 ± 0.01 | 0.9 ± 0.02 | 1.03 ± 0.02 | 0.23 ± 0.01 |
| Tomato Dry Root Weight (mg) | 0.29 ± 0.1 | 1 ± 0.12 | 1.22 ± 0.38 | 0.99 ± 0.22 | 1.98 ± 0.5 | 2.72 ± 0.33 | 0.67 ± 0.28 |
| Pepper Dry Shoots Weight (mg) | 0.04 ± 0.001 | 0.09 ± 0.006 | 0.07 ± 0.001 | 0.24 ± 0.007 | 0.12 ± 0.007 | 0.13 ± 0.003 | 0.05 ± 0.002 |
| Pepper Dry Root Weight (mg) | 0.08 ± 0.07 | 0.57 ± 0.17 | 0.44 ± 0.02 | 0.77 ± 0.03 | 0.62 ± 0.17 | 0.89 ± 0.18 | 0.28 ± 0.15 |

874

**SUPPLEMENTARY FIGURES**

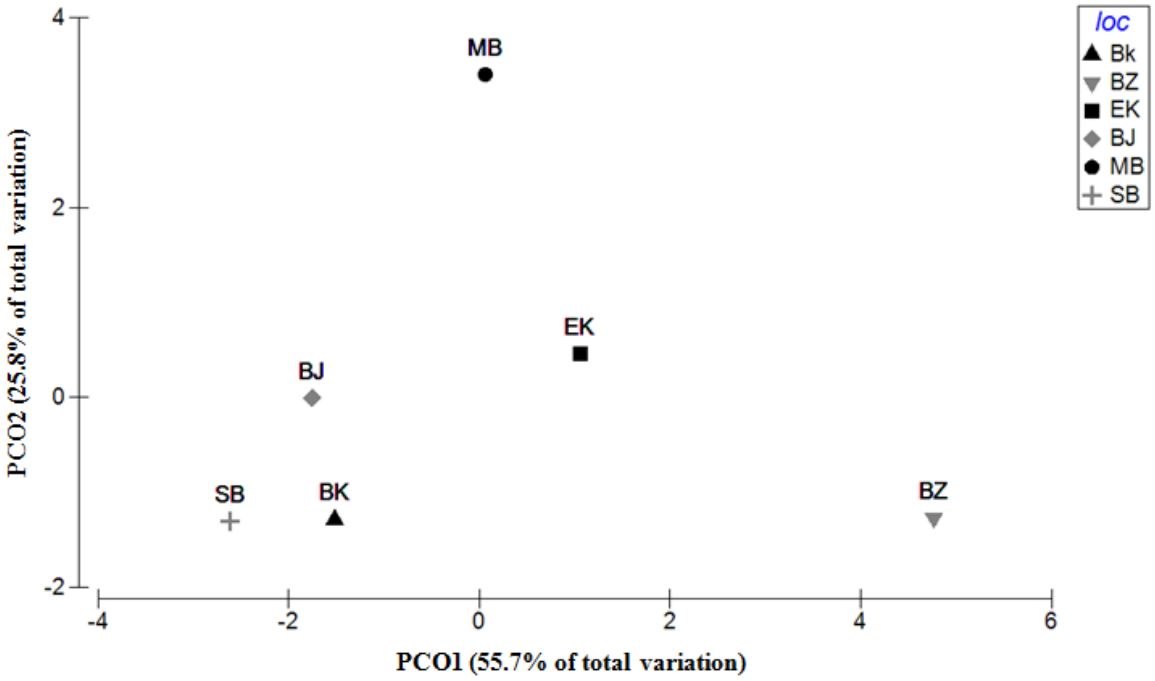
**Supplementary Fig.1.** Principal coordinate analysis (PCoA) of soil samples according to their physicochemical data and bacterial communities.

**Supplementary Fig.2.** Distribution of bacterial genera in the different soil factions associated to *Citrus sinensis*: rhizosphere, root surrounding soil and bulk soil.

**Supplementary Fig.3.** Evaluation of PGP selected bacteria on pepper growth promotion under drought stress. The graphs show the increase in root and shoot length (A) number of leaves (B) of pepper plants treated compared with the untreated plants. Statistical analysis using Student's *t*-test showing significant difference reported as $*P \leq 0.05$.

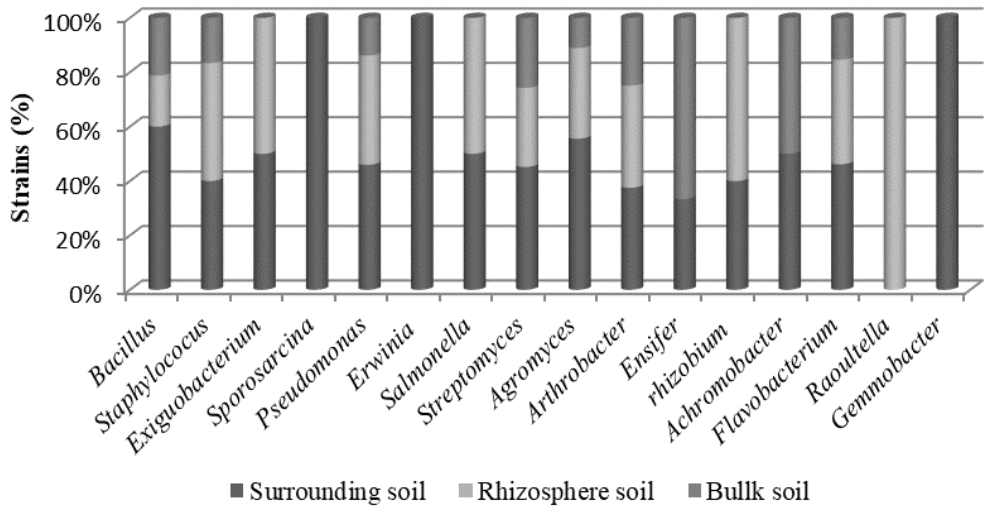
**Supplementary Fig.4.** Evaluation *in vivo* of selected bacteria on tomato growth promotion under drought stress. Root and shoot length (A) number of leaves (B). (CP) untreated plants not subjected to drought stress. (CN) untreated plants subjected to drought stress. Statistical analysis using Student's *t*-test showing significant difference are reported as $*P \leq 0.05$.

**Supplementary Fig.5.** Images by epifluorescence microscope showing colonization of *Arabidopsis thaliana* plants by *Pseudomonas japponica* (dsRed).

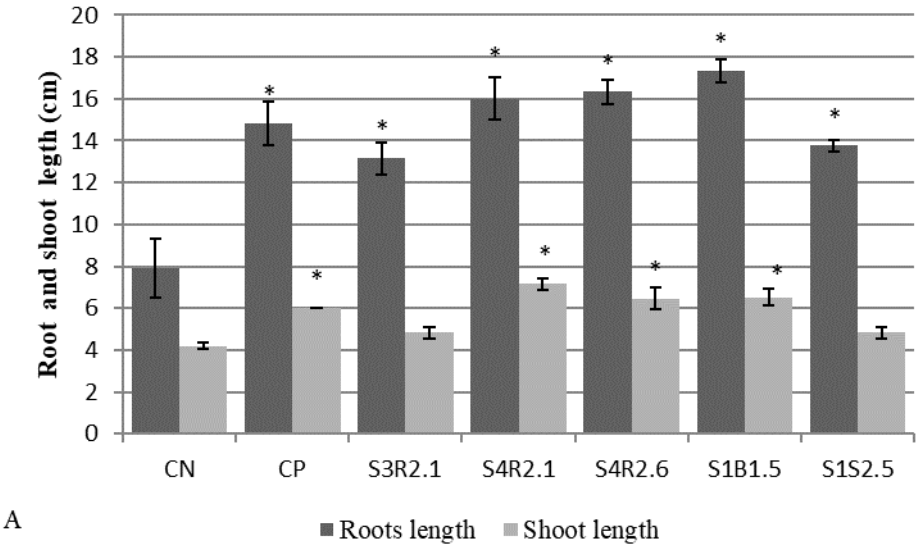



(A)

**Supplementary Fig.**




**Supplementary Fig.2**




A

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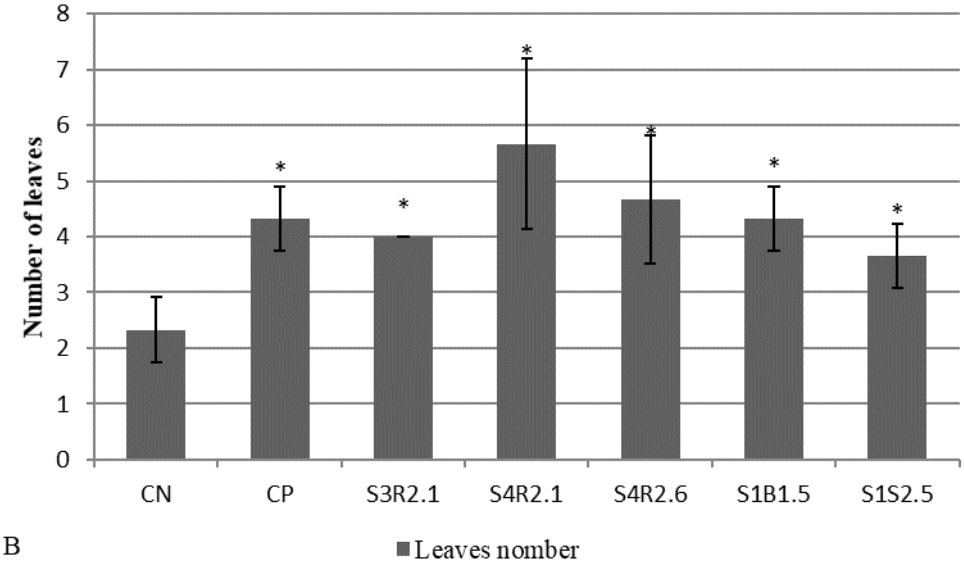


B

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43 **Supplementary Fig.3**

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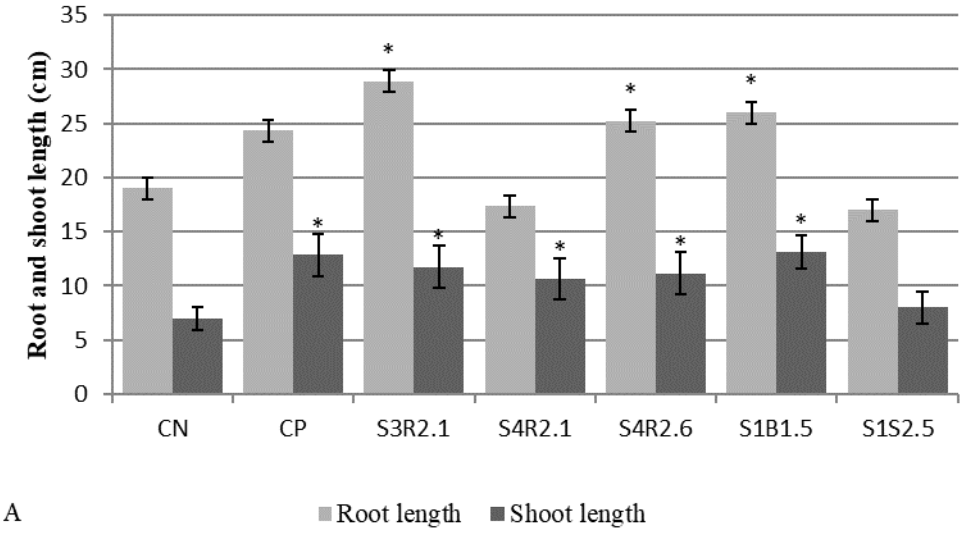
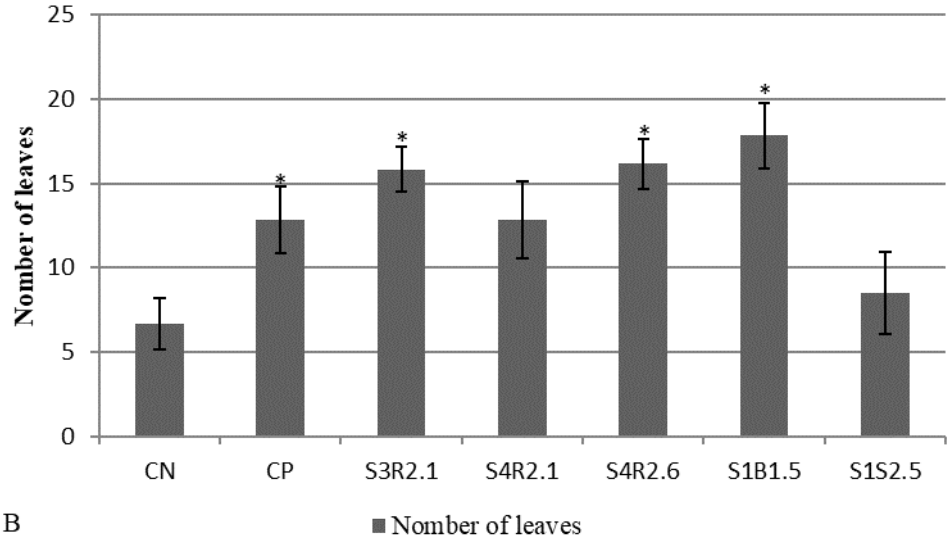
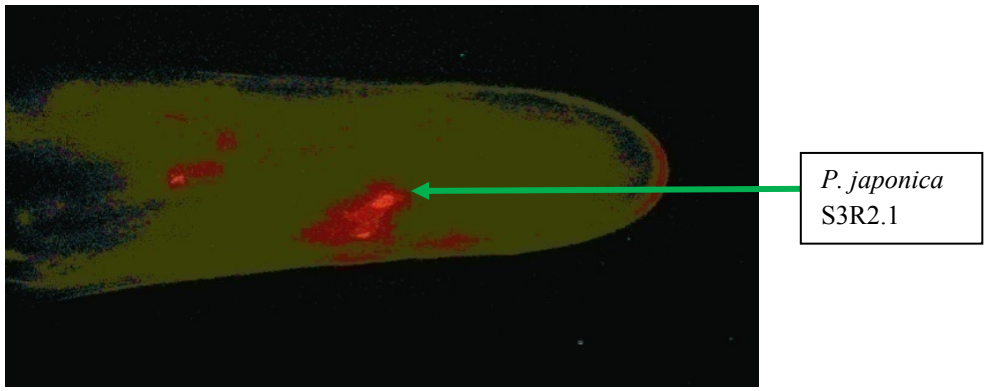


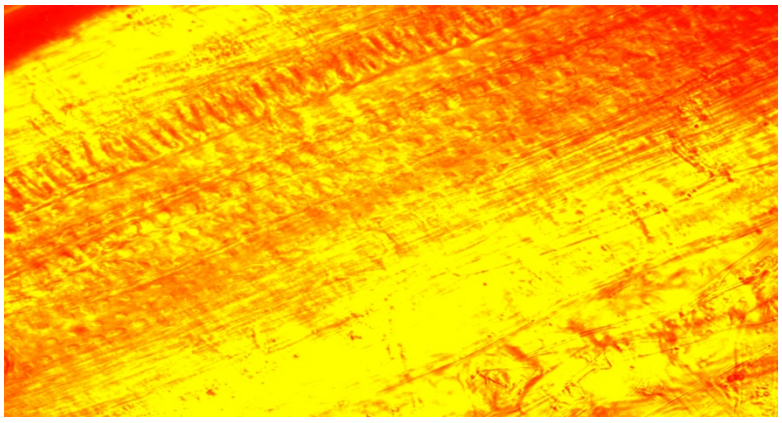

A

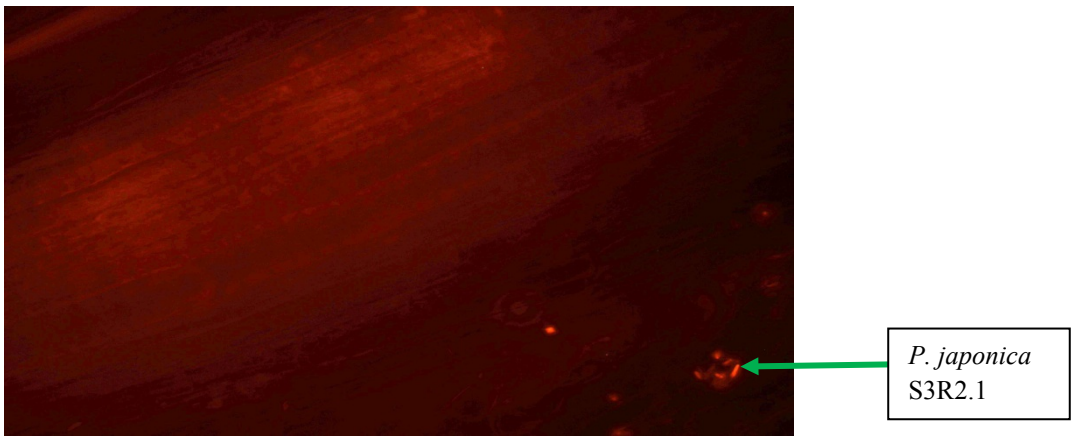



B

**Supplementary Fig.4**






**Supplementary Fig.5**

## SUPPLEMENTARY TABLES

**Supplementary Table 1.** Station, Sample code, type of sample collected, coordinates and Physico-chemical analysis of samples soil. Organic matter (OM), pH, carbon (C), inorganic carbon ($CaCo_3$), potassium (K), nitrogen (N), phosphorus (P), assimilable sodium phosphorus (P2O5), exchangeable potassium (K2O), electrical conductivity (EC) and saturated paste (S.P.).

| Region | Coordinates | Altitude | Sample code | Type of sample | S.P | C.E.25°C mmhos /cm | pH½.5( H2O) | $CaCo_3$ % | C % | M .O % | $P_2O_5$ PPm | P PPm | K PPm | $K_2O$ PPm | N PPm | Type of soil |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Beni-khalled** | 36° 39′ North. 10° 36′ Est | $14^2$ m | S1R1.2.3 | Rhizosphere | 45.0 | 2.73 | 8.48 | 6.00 | 0.48 | 0.83 | 21.0 | 9.16 | 218.40 | 263.17 | 108 | sandy |
| | | | S1S1.2.3 | Surrounding soil | | | | | | | | | | | | |
| | | | S1B1 | Bulk soil | | | | | | | | | | | | |
| **El kef** | 36° 11′ 10″ N orth. 8° 42′ 00 ″ Est | $582^3$ m | S2R1.2.3 | Rhizosphere | 54.0 | 1.32 | 7.98 | 13.00 | 1.22 | 2.10 | 32.0 | 13.95 | 507.00 | 610.93 | 185 | sandy-clay |
| | | | S2S1.2.3 | Surrounding soil | | | | | | | | | | | | |
| | | | S2B2 | Bulk soil | | | | | | | | | | | | |
| **Bizerte** | 37° 16′ North. 9° 52′ Est | $33^3$ m | S3R1.2.3 | Rhizosphere | 60 | 0.94 | 7.80 | 14.00 | 2.32 | 3.99 | 26.7 | 16.41 | 195.00 | 234.96 | 420 | sandy |
| | | | S3S1.2.3 | Surrounding soil | | | | | | | | | | | | |
| | | | S3B3 | Bulk soil | | | | | | | | | | | | |
| **Beja** | 36° 43′ 30″ N orth. 9° 10′ 55 ″ Est | $222^3$ m | S4R1.2.3 | Rhizosphere | 35.0 | 0.68 | 8.06 | 6.00 | 0.09 | 0.16 | 26 | 11.34 | 249.60 | 300.77 | 210 | sandy |
| | | | S4S1.2.3 | Surrounding soil | | | | | | | | | | | | |
| | | | S4B4 | Bulk soil | | | | | | | | | | | | |
| **Manzel bouzalfa** | 36° 41′ North. 10° 35′ Est | $14^2$ m | S5R1.2.3 | Rhizosphere | 32.5 | 1.32 | 7.48 | 2.00 | 0.83 | 1.43 | 67 | 29.20 | 624.00 | 751.92 | 280 | sandy |
| | | | S5S1.2.3 | Surrounding soil | | | | | | | | | | | | |
| | | | S5B5 | Bulk soil | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sidi bouzid** | 35° 02′ North. 9° 30′ Est | $327^2$ m | S6R1.2.3 | Rhizosphere | 40 | 0 .68 | 8.44 | 1 | 0.56 | 0.32 | 23.4 | 28.2 | 14.57 | 33.4 | 70 | sandy |
| | | | S6S1.2.3 | Surrounding soil | | | | | | | | | | | | |
| | | | S6B6 | Bulk soil | | | | | | | | | | | | |

**Supplementary Table 2.** Parameters determinating the nature of soil (El oumlouki et al. 2014).

| **pH** | **CE%** | **MO%** | **P / $P_2O_5$ ppm** | **K / $K_2O$ ppm** |
|---|---|---|---|---|
| Acid pH <6 | Non-saline < 4 | Very poor <0.7 | Very weak <15 | Very weak <60 |
| Low acid pH 6-6.5 | Little Salt 4-8 | Poor 0.7–1.5 | weak 15-30 | weak 60-100 |
| Neutral pH 6.5-7.3 | Saline 8- 16 | Moderately poor1.5-3 | Fully Furnished 30-45 | Fully Furnished 100-180 |
| Low basic pH 7.3-7.8 | Highly saline 16-32 | Rich 3-6 | High 45- 100 | High 180- 300 |
| Moderately basic pH 7.8- 8.5 | Very strongly saline >32 | Very rich >6 | Very High >100 | Very High >300 |
| Alkaline tendency 8.5-9 | | | | |
| High alkalinity >9 | | | | |

**Supplementary Table 3.** PERMANOVA analysis of bacterial assemblage of soils according to the different sampling site. df= degrees of freedom; F= statistic F; p= probability (in bold the variables statistically significant; $p<0.05$).

| Factors | df | MS | F | *P* |
|---|---|---|---|---|
| **sampling site** | **5** | **131.99** | **2.408** | **0.022** |
| Res | 48 | 54.798 | | |
| Total | 53 | | | |

**Supplementary Table 4.** Index of molecular diversity for the samples in the different sites.

| | BK | BZ | EK | BJ | MB | SB |
|---|---|---|---|---|---|---|
| **Taxa_S** | 318 | 323 | 323 | 320 | 318 | 316 |
| **Individuals** | 63604.5 | 70019.5 | 79549 | 65197 | 73058.5 | 59731.5 |
| **Dominance_D** | 0.003289 | 0.003335 | 0.003139 | 0.003236 | 0.003316 | 0.003444 |
| **Simpson_1-D** | 0.9967 | 0.9967 | 0.9969 | 0.9968 | 0.9967 | 0.9966 |
| **Shannon_H** | 5.738 | 5.735 | 5.771 | 5.749 | 5.732 | 5.709 |
| **Evenness_e^H/S** | 0.9759 | 0.9587 | 0.9929 | 0.9811 | 0.9699 | 0.9544 |
| **Brillouin** | 5.72 | 5.719 | 5.756 | 5.732 | 5.716 | 5.691 |
| **Menhinick** | 1.261 | 1.221 | 1.145 | 1.253 | 1.176 | 1.293 |
| **Margalef** | 28.66 | 28.86 | 28.54 | 28.78 | 28.31 | 28.64 |
| **Equitability_J** | 0.9958 | 0.9927 | 0.9988 | 0.9967 | 0.9947 | 0.9919 |
| **Fisher_alpha** | 43.65 | 43.78 | 42.92 | 43.8 | 42.71 | 43.77 |
| **Berger-Parker** | 0.004237 | 0.004335 | 0.003752 | 0.004042 | 0.004414 | 0.004897 |
| **Chao-1** | 318 | 323 | 323 | 320 | 318 | 316 |

**Supplementary Table 5.** Index of diversity for the cultivable bacteria in the different sites.

| | BK site | | | |
|---|---|---|---|---|
| | Number | pi | lnpi | pi* Lnpi |
| ***Bacillus*** | 7 | 0.08536585 | -2.4608091 | -0.21006907 |
| ***Staphylococus*** | 6 | 0.07317073 | -2.61495978 | -0.19133852 |
| ***Pseudomonas*** | 35 | 0.42682927 | -0.85137119 | -0.36339014 |
| ***Erwinia*** | 7 | 0.08536585 | -2.4608091 | -0.21006907 |
| ***Streptomyces*** | 6 | 0.07317073 | -2.61495978 | -0.19133852 |
| ***Agromyces*** | 5 | 0.06097561 | -2.79728133 | -0.17056594 |
| ***Arthrobacter*** | 9 | 0.1097561 | -2.20949467 | -0.24250551 |
| ***Ensifer*** | 3 | 0.03658537 | -3.30810696 | -0.1210283 |
| ***Rhizobacterium*** | 0 | 0 | 0 | 0 |
| ***Achromobacter*** | 0 | 0 | 0 | 0 |
| ***Flavobacterium*** | 4 | 0.04878049 | -3.02042489 | -0.1473378 |
| ***Paenibacillus*** | 0 | 0 | 0 | 0 |
| | **Diversity index** | | **1.84764287** | |

| | BZ site | | | |
|---|---|---|---|---|
| | Number | pi | lnpi | pi* Lnpi |
| ***Bacillus*** | 22 | 0.44897959 | -0.80077784 | -0.35953291 |
| ***Staphylococus*** | 7 | 0.14285714 | -1.94591015 | -0.27798716 |
| ***Pseudomonas*** | 16 | 0.32653061 | -1.11923158 | -0.36546337 |
| ***Erwinia*** | 1 | 0.02040816 | -3.8918203 | -0.0794249 |
| ***Streptomyces*** | 2 | 0.04081633 | -3.19867312 | -0.13055809 |
| ***Agromyces*** | 1 | 0.02040816 | -3.8918203 | -0.0794249 |
| ***Arthrobacter*** | 0 | 0 | 0 | 0 |
| ***Ensifer*** | 0 | 0 | 0 | 0 |
| ***Rhizobacterium*** | 0 | 0 | 0 | 0 |
| ***Achromobacter*** | 0 | 0 | 0 | 0 |
| ***Flavobacterium*** | 0 | 0 | 0 | 0 |
| ***Paenibacillus*** | 0 | 0 | 0 | 0 |
| | **Diversity index** | | **1.29239134** | |

| | MB site | | | |
|---|---|---|---|---|
| | Number | pi | lnpi | pi* Lnpi |
| ***Bacillus*** | 21 | 0.39622642 | -0.92576948 | -0.36681432 |
| ***Staphylococus*** | 21 | 0.39622642 | -0.92576948 | -0.36681432 |
| ***Pseudomonas*** | 6 | 0.11320755 | -2.17853244 | -0.24662a631 |
| ***Erwinia*** | 0 | 0 | 0 | 0 |
| ***Streptomyces*** | 1 | 0.01886792 | -3.97029191 | -0.07491117 |
| ***Agromyces*** | 0 | 0 | 0 | 0 |
| ***Arthrobacter*** | 2 | 0.03773585 | -3.27714473 | -0.12366584 |
| ***Ensifer*** | 0 | 0 | 0 | 0 |
| ***Rhizobacterium*** | 2 | 0.03773585 | -3.27714473 | -0.12366584 |
| ***Achromobacter*** | 0 | 0 | 0 | 0 |
| ***Flavobacterium*** | 0 | 0 | 0 | 0 |
| ***Paenibacillus*** | 0 | 0 | 0 | 0 |
| | **Diversity index** | | **1.3024978** | |

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| | EK site | | | |
|---|---|---|---|---|
| | Number | pi | lnpi | pi* Lnpi |
| ***Bacillus*** | 16 | 0.23880597 | -1.4321039 | -0.34199496 |
| ***Staphylococus*** | 6 | 0.08955224 | -2.41293315 | -0.21608357 |
| ***Pseudomonas*** | 11 | 0.1641791 | -1.80679735 | -0.29663837 |
| ***Erwinia*** | 1 | 0.01492537 | -4.20469262 | -0.06275661 |
| ***Streptomyces*** | 6 | 0.08955224 | -2.41293315 | -0.21608357 |
| ***Agromyces*** | 3 | 0.04477612 | -3.10608033 | -0.13907822 |
| ***Arthrobacter*** | 15 | 0.2238806 | -1.49664242 | -0.3350692 |
| ***Ensifer*** | 0 | 0 | 0 | 0 |
| ***Rhizobacterium*** | 0 | 0 | 0 | 0 |
| ***Achromobacter*** | 2 | 0.02985075 | -3.51154544 | -0.10482225 |
| ***Flavobacterium*** | 7 | 0.10447761 | -2.25878247 | -0.2359922 |
| ***Paenibacillus*** | 0 | 0 | 0 | 0 |
| | **Diversity index** | | **1.94851894** | |

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| | BJ site | | | |
|---|---|---|---|---|
| | Number | pi | lnpi | pi* Lnpi |
| ***Bacillus*** | 20 | 0.39215686 | -0.93609336 | -0.36709543 |
| ***Staphylococus*** | 4 | 0.07843137 | -2.54553127 | -0.19964951 |
| ***Pseudomonas*** | 18 | 0.35294118 | -1.04145387 | -0.36757196 |
| ***Erwinia*** | 0 | 0 | 0 | 0 |
| ***Streptomyces*** | 5 | 0.09803922 | -2.32238772 | -0.22768507 |
| ***Agromyces*** | 0 | 0 | 0 | 0 |
| ***Arthrobacter*** | 0 | 0 | 0 | 0 |
| ***Ensifer*** | 0 | 0 | 0 | 0 |
| ***Rhizobacterium*** | 1 | 0.01960784 | -3.93182563 | -0.07709462 |
| ***Achromobacter*** | 0 | 0 | 0 | 0 |
| ***Flavobacterium*** | 1 | 0.01960784 | -3.93182563 | -0.07709462 |
| ***Paenibacillus*** | 2 | 0.03921569 | -3.23867845 | -0.127007 |
| | **Diversity index** | | **1.44319821** | |

| | SB site | | | |
|---|---|---|---|---|
| | Number | pi | lnpi | pi* Lnpi |
| ***Bacillus*** | 2 | 0.07142857 | -2.63905733 | -0.18850409 |
| ***Staphylococus*** | 0 | 0 | 0 | 0 |
| ***Pseudomonas*** | 22 | 0.78571429 | -0.24116206 | -0.18948447 |
| ***Erwinia*** | 0 | 0 | 0 | 0 |
| ***Streptomyces*** | 0 | 0 | 0 | 0 |
| ***Agromyces*** | 0 | 0 | 0 | 0 |
| ***Arthrobacter*** | 0 | 0 | 0 | 0 |
| ***Ensifer*** | 0 | 0 | 0 | 0 |
| ***Rhizobacterium*** | 1 | 0.03571429 | -3.33220451 | -0.1190073 |
| ***Achromobacter*** | 0 | 0 | 0 | 0 |
| ***Flavobacterium*** | 1 | 0.03571429 | -3.33220451 | -0.1190073 |
| ***Paenibacillus*** | 2 | 0.07142857 | -2.63905733 | -0.18850409 |
| | **Diversity index** | | **0.80450727** | |

**Supplementary Table 6.** Statistical analysis of the PGP traits detected in different bacteria groups. Statistical analysis using Post-hoc tests showing significant difference reported as $P \leq 0.05$.

| | *Firmicutes* | *Proteobacteria* | *Firmicutes* | *Actinobacteria* | *Firmicutes* | *Bacteriodetes* |
|---|---|---|---|---|---|---|
| **Mean** | 18.71428571 | 21.0952381 | 4.523809524 | 21.0952381 | 4.523809524 | 0.285714286 |
| **Variance** | 118.8142857 | 274.0904762 | 14.26190476 | 274.0904762 | 14.26190476 | 0.214285714 |
| **Observations** | 21 | 21 | 21 | 21 | 21 | 21 |
| **Hypothesized Mean** | 0 | | 0 | | 0 | |
| **df** | 35 | | 22 | | 21 | |
| **t Stat** | -0.550448523 | | -4.472062101 | | 5.104500079 | |
| **P(T<=t) one-tail** | 0.292754068 | | 9.52159E-05 | | 2.3425E-05 | |
| **t Critical one-tail** | 1.68957244 | | 1.717144335 | | 1.720742871 | |
| **P(T<=t) two-tail** | 0.585508136 | | 0.000190432 | | 4.68499E-05 | |
| **t Critical two-tail** | 2.030107915 | | 2.073873058 | | 2.079613837 | |

Suite

| | *Proteobacteria* | *Actinobacteria* | *Proteobacteria* | *Bacteriodetes* | *Actinobacteria* | *Bacteriodetes* |
|---|---|---|---|---|---|---|
| **Mean** | 21.0952381 | 4.523809524 | 21.0952381 | 0.285714286 | 4.523809524 | 0.285714286 |
| **Variance** | 274.0904762 | 14.26190476 | 274.0904762 | 0.214285714 | 14.26190476 | 0.214285714 |
| **Observations** | 21 | 21 | 21 | 21 | 21 | 21 |
| **Hypothesized Mean** | 0 | | 0 | | 0 | |
| **df** | 22 | | 20 | | 21 | |
| **t Stat** | 4.472062101 | | 5.757780653 | | 5.104500079 | |
| **P(T<=t) one-tail** | 9.52159E-05 | | 6.18483E-06 | | 2.3425E-05 | |
| **t Critical one-tail** | 1.717144335 | | 1.724718218 | | 1.720742871 | |
| **P(T<=t) two-tail** | 0.000190432 | | 1.23697E-05 | | 4.68499E-05 | |
| **t Critical two-tail** | 2.073873058 | | 2.085963441 | | 2.079613837 | |

**Supplementary Table 7.** Statistical analysis of the effect of PGP selected bacteria on pepper and tomato growth promotion under drought stress. Root length (A) root fresh biomass (B) root dry biomass (C) shoot length (D) shoot fresh biomass (E) shoot dry biomass (F) number of leaves (G).Statistical analysis using Post-hoc tests showing significant difference reported as $P \leq 0.05$.

**t-Test: Two-Sample Assuming Equal Variances**

**A**

| | CN | *CP* | *CN* | **S3R2.1** | *CN* | **S4R2.1** | *CN* | *S4R2.6* | *CN* | *S1B1.5* | *CN* | *S1S2.5* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mean** | 7.9 | 14.83333333 | 7.9 | 13.16666667 | 7.9 | 16 | 7.9 | 16.33333333 | 7.9 | 17.33333333 | 7.9 | 13.76666667 |
| **Variance** | 1.93 | 1.083333333 | 1.93 | 0.583333333 | 1.93 | 1 | 1.93 | 0.333333333 | 1.93 | 0.333333333 | 1.93 | 0.063333333 |
| **Observations** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **Pooled Variance** | 1.506666667 | | 1.256666667 | | 1.465 | | 1.131666667 | | 1.131666667 | | 0.996666667 | |
| **Hypothesized Mean** | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| **df** | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | |
| **t Stat** | -6.917977094 | | -5.754020622 | | -8.196186577 | | -9.709248889 | | -10.86054322 | | -7.197175217 | |
| **P(T<=t) one-tail** | 0.001145528 | | 0.002261948 | | 0.000603613 | | 0.000314973 | | 0.000203966 | | 0.000987524 | |
| **t Critical one-tail** | 2.131846782 | | 2.131846782 | | 2.131846782 | | 2.131846782 | | 2.131846782 | | 2.131846782 | |
| **P(T<=t) two-tail** | **0.002291056** | | **0.004523896** | | **0.001207226** | | **0.000629946** | | **0.000407933** | | **0.001975049** | |
| **t Critical two-tail** | 2.776445105 | | 2.776445105 | | 2.776445105 | | 2.776445105 | | 2.776445105 | | 2.776445105 | |

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**B**

| | *CN* | *CP* | *CN* | **S3R2.1** | *CN* | **S4R2.1** | *CN* | ***S4R2.6*** | *CN* | ***S1B1.5*** | *CN* | ***S1S2.5*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mean** | 0.4 | 1.6 | 0.4 | 1.4 | 0.4 | 2.2 | 0.4 | 1.86666667 | 0.4 | 2.43333333 | 0.4 | 1.63333333 |
| **Variance** | 0.01 | 0.03 | 0.01 | 0.16 | 0.01 | 0.01 | 0.01 | 0.06333333 | 0.01 | 0.12333333 | 0.01 | 0.10333333 |
| **Observations** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **Pooled Variance** | 0.02 | | 0.085 | | 0.01 | | 0.03666667 | | 0.06666667 | | 0.05666667 | |
| **Hypothesized Mean** | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| **df** | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | |
| **t Stat** | -10.3923048 | | -4.20084025 | | -22.0454077 | | -9.38083152 | | -9.64494686 | | -6.34544765 | |
| **P(T<=t) one-tail** | 0.00024206 | | 0.00684328 | | 1.2529E-05 | | 0.00035971 | | 0.00032316 | | 0.00157972 | |
| **t Critical one-tail** | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | |
| **P(T<=t) two-tail** | **0.00048413** | | **0.01368656** | | **2.5058E-05** | | **0.00071942** | | **0.00064633** | | **0.00315945** | |
| **t Critical two-tail** | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | |

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**C**

| | *CN* | *CP* | *CN* | **S3R2.1** | *CN* | **S4R2.1** | *CN* | ***S4R2.6*** | *CN* | ***S1B1.5*** | *CN* | ***S1S2.5*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mean** | 0.08133333 | 0.57 | 0.08133333 | 7.50333333 | 0.08133333 | 0.77533333 | 0.08133333 | 0.478 | 0.08133333 | 0.89766667 | 0.08133333 | 0.28866667 |
| **Variance** | 0.00512433 | 0.029169 | 0.00512433 | 157.615033 | 0.00512433 | 0.00108133 | 0.00512433 | 0.078579 | 0.00512433 | 0.16234133 | 0.00512433 | 0.02162633 |
| **Observations** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **Pooled Variance** | 0.01714667 | | 78.8100788 | | 0.00310283 | | 0.04185167 | | 0.08373283 | | 0.01337533 | |
| **Hypothesized Mean** | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| **df** | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | |
| **t Stat** | -4.5705503 | | -1.0239428 | | -15.2589931 | | -2.37473393 | | -3.45513695 | | -2.19564667 | |
| **P(T<=t) one-tail** | 0.00512826 | | 0.18187219 | | 5.3788E-05 | | 0.03821298 | | 0.01296628 | | 0.04655178 | |
| **t Critical one-tail** | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | |
| **P(T<=t) two-tail** | **0.01025652** | | 0.36374437 | | **0.00010758** | | 0.07642595 | | 0.02593255 | | 0.09310357 | |
| **t Critical two-tail** | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | |

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**D**

| | *CN* | *CP* | *CN* | **S3R2.1** | *CN* | **S4R2.1** | *CN* | ***S4R2.6*** | *CN* | ***S1B1.5*** | *CN* | ***S1S2.5*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mean** | 4.2 | 6 | 4.2 | 4.83333333 | 4.2 | 7.16666667 | 4.2 | 6.46666667 | 4.2 | 6.53333333 | 4.2 | 4.83333333 |
| **Variance** | 0.03 | 0 | 0.03 | 0.08333333 | 0.03 | 0.08333333 | 0.03 | 0.25333333 | 0.03 | 0.17333333 | 0.03 | 0.08333333 |
| **Observations** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **Pooled Variance** | 0.015 | | 0.05666667 | | 0.05666667 | | 0.14166667 | | 0.10166667 | | 0.05666667 | |
| **Hypothesized Mean** | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| **df** | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | |
| **t Stat** | -18 | | -3.25847312 | | -15.2633741 | | -7.37563557 | | -8.9625816 | | -3.25847312 | |
| **P(T<=t) one-tail** | 2.7999E-05 | | 0.01556282 | | 5.3727E-05 | | 0.00090051 | | 0.00042872 | | 0.01556282 | |
| **t Critical one-tail** | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | |
| **P(T<=t) two-tail** | **5.5999E-05** | | 0.03112563 | | **0.00010745** | | **0.00180103** | | **0.00085745** | | 0.03112563 | |
| **t Critical two-tail** | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | |

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**E**

| | *CN* | *CP* | *CN* | **S3R2.1** | *CN* | **S4R2.1** | *CN* | ***S4R2.6*** | *CN* | ***S1B1.5*** | *CN* | ***S1S2.5*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mean** | 0.13333333 | 0.23333333 | 0.13333333 | 0.19666667 | 0.13333333 | 0.58333333 | 0.13333333 | 0.32666667 | 0.13333333 | 0.32666667 | 0.13333333 | 0.16666667 |
| **Variance** | 0.00013333 | 0.00333333 | 0.00013333 | 0.00023333 | 0.00013333 | 0.00333333 | 0.00013333 | 0.00413333 | 0.00013333 | 0.00413333 | 0.00013333 | 3.3333E-05 |
| **Observations** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **Pooled Variance** | 0.00173333 | | 0.00018333 | | 0.00173333 | | 0.00213333 | | 0.00213333 | | 8.3333E-05 | |
| **Hypothesized Mean** | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| **df** | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | |
| **t Stat** | -2.94174203 | | -5.72871555 | | -13.2378391 | | -5.12652416 | | -5.12652416 | | -4.47213595 | |

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| | | | | | | |
|---|---|---|---|---|---|---|
| **P(T<=t) one-tail** | 0.02115783 | 0.0022985 | 9.4083E-05 | 0.00342751 | 0.00342751 | 0.00552825 |
| **t Critical one-tail** | 2.13184678 | 2.13184678 | 2.13184678 | 2.13184678 | 2.13184678 | 2.13184678 |
| **P(T<=t) two-tail** | 0.04231566 | **0.00459701** | **0.00018817** | **0.00685502** | **0.00685502** | **0.01105649** |
| **t Critical two-tail** | 2.77644511 | 2.77644511 | 2.77644511 | 2.77644511 | 2.77644511 | 2.77644511 |

**F**

| | ***CN*** | ***CP*** | ***CN*** | **S3R2.1** | ***CN*** | **S4R2.1** | ***CN*** | ***S4R2.6*** | ***CN*** | ***S1B1.5*** | ***CN*** | ***S1S2.5*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mean** | 0.006 | 0.01866667 | 0.006 | 0.011 | 0.006 | 0.0436 | 0.006 | 0.02033333 | 0.006 | 0.021 | 0.006 | 0.01133333 |
| **Variance** | 1.1285E-36 | 4.2333E-05 | 1.1285E-36 | 0.000003 | 1.1285E-36 | 5.908E-05 | 1.1285E-36 | 5.8333E-05 | 1.1285E-36 | 0.000013 | 1.1285E-36 | 4.3333E-06 |
| **Observations** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **Pooled Variance** | 2.1167E-05 | | 0.0000015 | | 2.954E-05 | | 2.9167E-05 | | 0.0000065 | | 2.1667E-06 | |
| **Hypothesized Mean** | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| **df** | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | |
| **t Stat** | -3.37195474 | | -5 | | -8.47282485 | | -3.25049447 | | -7.20576692 | | -4.43760157 | |
| **P(T<=t) one-tail** | 0.01399651 | | 0.00374522 | | 0.00053176 | | 0.01568054 | | 0.0009831 | | 0.0056775 | |
| **t Critical one-tail** | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | |
| **P(T<=t) two-tail** | 0.02799302 | | **0.00749043** | | **0.00106352** | | 0.03136107 | | **0.00196621** | | **0.011355** | |
| **t Critical two-tail** | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | |

| G | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***CN*** | ***CP*** | ***CN*** | **S3R2.1** | ***CN*** | **S4R2.1** | ***CN*** | ***S4R2.6*** | ***CN*** | ***S1B1.5*** | ***CN*** | ***S1S2.5*** |
| **Mean** | 2.33333333 | 4.33333333 | 2.33333333 | 4 | 2.33333333 | 5.66666667 | 2.33333333 | 4.66666667 | 2.33333333 | 4.33333333 | 2.33333333 | 3.66666667 |
| **Variance** | 0.33333333 | 0.33333333 | 0.33333333 | 0 | 0.33333333 | 2.33333333 | 0.33333333 | 1.33333333 | 0.33333333 | 0.33333333 | 0.33333333 | 0.33333333 |
| **Observations** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **Pooled Variance** | 0.33333333 | | 0.16666667 | | 1.33333333 | | 0.83333333 | | 0.33333333 | | 0.33333333 | |
| **Hypothesized Mean** | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| **df** | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | |
| **t Stat** | -4.24264069 | | -5 | | -3.53553391 | | -3.13049517 | | -4.24264069 | | -2.82842712 | |
| **P(T<=t) one-tail** | 0.0066178 | | 0.00374522 | | 0.01205506 | | 0.01758423 | | 0.0066178 | | 0.02371033 | |
| **t Critical one-tail** | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | |
| **P(T<=t) two-tail** | **0.0132356** | | **0.00749043** | | 0.02411011 | | 0.03516845 | | **0.0132356** | | 0.04742066 | |
| **t Critical two-tail** | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | |

**Supplementary Table 8.** Statistical analysis of the effect of PGP selected bacteria on tomato growth promotion under drought stress. Root length (A) root fresh biomass (B) root dry biomass (C) shoot length (D) shoot fresh biomass (E) shoot dry biomass (F) number of leaves (G).Statistical analysis using Post-hoc tests showing significant difference reported as $P \leq 0.05$.

**t-Test: Two-Sample Assuming Equal Variances**

**A**

| | *CN* | *CP* | *CN* | S3R2.1 | *CN* | S4R2.1 | *CN* | *S4R2.6* | *CN* | *S1B1.5* | *CN* | *S1S2.5* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mean** | 15.9166667 | 19 | 15.9166667 | 24.0833333 | 15.9166667 | 16.2 | 15.9166667 | 23 | 15.9166667 | 25.6666667 | 15.9166667 | 17.5833333 |
| **Variance** | 9.04166667 | 8.8 | 9.04166667 | 29.4416667 | 9.04166667 | 6.88 | 9.04166667 | 4.4 | 9.04166667 | 2.26666667 | 9.04166667 | 6.24166667 |
| **Observations** | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| **Pooled Variance** | 8.92083333 | | 19.2416667 | | 7.96083333 | | 6.72083333 | | 5.65416667 | | 7.64166667 | |
| **Hypothesized Mean** | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| **df** | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| **t Stat** | -1.78804479 | | -3.22466136 | | -0.17393182 | | -4.73245774 | | -7.10200445 | | -1.04427608 | |
| **P(T<=t) one-tail** | 0.05202872 | | 0.00455147 | | 0.43269483 | | 0.0004006 | | 1.6438E-05 | | 0.16047216 | |
| **t Critical one-tail** | 1.8124611 | | 1.8124611 | | 1.8124611 | | 1.8124611 | | 1.8124611 | | 1.8124611 | |
| **P(T<=t) two-tail** | 0.10405744 | | **0.00910294** | | 0.86538966 | | **0.00080119** | | **3.2875E-05** | | 0.32094432 | |
| **t Critical two-tail** | 2.22813884 | | 2.22813884 | | 2.22813884 | | 2.22813884 | | 2.22813884 | | 2.22813884 | |

**B**

| | *CN* | *CP* | *CN* | S3R2.1 | *CN* | S4R2.1 | *CN* | *S4R2.6* | *CN* | *S1B1.5* | *CN* | *S1S2.5* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mean** | 1.03333333 | 3.16666667 | 1.03333333 | 3.7 | 1.03333333 | 2.76666667 | 1.03333333 | 4.98333333 | 1.03333333 | 5.3 | 1.03333333 | 1.86666667 |
| **Variance** | 0.01066667 | 1.40666667 | 0.01066667 | 1.172 | 0.01066667 | 0.81066667 | 0.01066667 | 1.07366667 | 0.01066667 | 0.332 | 0.01066667 | 0.96266667 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Observations** | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| **Pooled Variance** | 0.70866667 | | 0.59133333 | | 0.41066667 | | 0.54216667 | | 0.17133333 | | 0.48666667 | |
| **Hypothesized Mean** | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| **df** | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| **t Stat** | -4.38933112 | | -6.00638518 | | -4.68487481 | | -9.29161736 | | -17.8537073 | | -2.06901472 | |
| **P(T<=t) one-tail** | 0.00067874 | | 6.5495E-05 | | 0.00043056 | | 1.5519E-06 | | 3.2455E-09 | | 0.03269586 | |
| **t Critical one-tail** | 1.8124611 | | 1.8124611 | | 1.8124611 | | 1.8124611 | | 1.8124611 | | 1.8124611 | |
| **P(T<=t) two-tail** | **0.00135748** | | **0.00013099** | | **0.00086111** | | **3.1038E-06** | | **6.4911E-09** | | 0.06539171 | |
| **t Critical two-tail** | 2.22813884 | | 2.22813884 | | 2.22813884 | | 2.22813884 | | 2.22813884 | | 2.22813884 | |

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**C**

| | ***CN*** | ***CP*** | ***CN*** | **S3R2.1** | ***CN*** | **S4R2.1** | ***CN*** | ***S4R2.6*** | ***CN*** | ***S1B1.5*** | ***CN*** | ***S1S2.5*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mean** | 0.17918333 | 0.61066667 | 0.17918333 | 1.2195 | 0.17918333 | 74.1951667 | 0.17918333 | 1.76133333 | 0.17918333 | 1.9895 | 0.17918333 | 0.43216667 |
| **Variance** | 0.03311928 | 0.01481307 | 0.03311928 | 0.1454931 | 0.03311928 | 32291.1559 | 0.03311928 | 0.45562627 | 0.03311928 | 0.6845487 | 0.03311928 | 0.18225897 |
| **Observations** | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| **Pooled Variance** | 0.02396617 | | 0.08930619 | | 16145.5945 | | 0.24437277 | | 0.35883399 | | 0.10768912 | |
| **Hypothesized Mean** | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| **df** | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| **t Stat** | -4.82753351 | | -6.02955694 | | -1.00892555 | | -5.54347219 | | -5.23441786 | | -1.33526239 | |
| **P(T<=t) one-tail** | 0.00034717 | | 6.3505E-05 | | 0.16839963 | | 0.00012317 | | 0.00019093 | | 0.10569424 | |
| **t Critical one-tail** | 1.8124611 | | 1.8124611 | | 1.8124611 | | 1.8124611 | | 1.8124611 | | 1.8124611 | |
| **P(T<=t) two-tail** | **0.00069434** | | **0.00012701** | | 0.33679926 | | **0.00024634** | | **0.00038186** | | 0.21138849 | |
| **t Critical two-tail** | 2.22813884 | | 2.22813884 | | 2.22813884 | | 2.22813884 | | 2.22813884 | | 2.22813884 | |

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**D**

| | *CN* | *CP* | *CN* | S3R2.1 | *CN* | S4R2.1 | *CN* | *S4R2.6* | *CN* | *S1B1.5* | *CN* | *S1S2.5* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mean** | 6.5 | 9.86666667 | 6.5 | 9.51666667 | 6.5 | 9.33333333 | 6.5 | 9.38333333 | 6.5 | 10.0333333 | 6.5 | 7.41666667 |
| **Variance** | 2.376 | 24.8866667 | 2.376 | 19.5416667 | 2.376 | 11.0666667 | 2.376 | 13.8736667 | 2.376 | 21.5386667 | 2.376 | 4.54966667 |
| **Observations** | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| **Pooled Variance** | 13.6313333 | | 10.9588333 | | 6.72133333 | | 8.12483333 | | 11.9573333 | | 3.46283333 | |
| **Hypothesized Mean** | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| **df** | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| **t Stat** | -6.59220319 | | -8.5 | | -8.1408063 | | -4.90189468 | | -6.164414 | | -3.18198052 | |
| **P(T<=t) one-tail** | 0.00137135 | | 0.00052529 | | 0.00061941 | | 0.00401652 | | 0.00175776 | | 0.01673587 | |
| **t Critical one-tail** | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | |
| **P(T<=t) two-tail** | **0.0027427** | | **0.00105058** | | **0.00123882** | | **0.00803305** | | **0.00351552** | | 0.03347174 | |
| **t Critical two-tail** | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | |

**E**

| | *CN* | *CP* | *CN* | S3R2.1 | *CN* | S4R2.1 | *CN* | *S4R2.6* | *CN* | *S1B1.5* | *CN* | *S1S2.5* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mean** | 0.4 | 1.06666667 | 0.4 | 1.83333333 | 0.4 | 1.06666667 | 0.4 | 1.43333333 | 0.4 | 2 | 0.4 | 1.26666667 |
| **Variance** | 0.03 | 0.04333333 | 0.03 | 0.08333333 | 0.03 | 0.05333333 | 0.03 | 0.06333333 | 0.03 | 0.13 | 0.03 | 1.54333333 |
| **Observations** | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| **Pooled Variance** | 0.03666667 | | 0.05666667 | | 0.04166667 | | 0.04666667 | | 0.08 | | 0.78666667 | |
| **Hypothesized Mean** | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| **df** | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| **t Stat** | -4.26401433 | | -7.37443916 | | -4 | | -5.85844933 | | -6.92820323 | | -1.196747 | |
| **P(T<=t) one-tail** | 0.00650597 | | 0.00090107 | | 0.00806504 | | 0.00211842 | | 0.00113921 | | 0.14874226 | |
| **t Critical one-tail** | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | | 2.13184678 | |
| **P(T<=t) two-tail** | **0.01301195** | | **0.00180213** | | **0.01613009** | | **0.00423684** | | **0.00227843** | | 0.29748452 | |
| **t Critical two-tail** | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | | 2.77644511 | |

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**F**

| | *CN* | *CP* | *CN* | **S3R2.1** | *CN* | **S4R2.1** | *CN* | ***S4R2.6*** | *CN* | ***S1B1.5*** | *CN* | ***S1S2.5*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mean** | 0.0155 | 0.01883333 | 0.0155 | 0.05433333 | 0.0155 | 0.05483333 | 0.0155 | 0.05333333 | 0.0155 | 0.064 | 0.0155 | 0.02283333 |
| **Variance** | 7.35E-05 | 5.0167E-05 | 7.35E-05 | 0.00226827 | 7.35E-05 | 0.00014257 | 7.35E-05 | 0.00160587 | 7.35E-05 | 0.0027104 | 7.35E-05 | 0.00015777 |
| **Observations** | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| **Pooled Variance** | 6.1833E-05 | | 0.00117088 | | 0.00010803 | | 0.00083968 | | 0.00139195 | | 0.00011563 | |
| **Hypothesized Mean** | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| **df** | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| **t Stat** | -0.7342231 | | -1.96566039 | | -6.55454413 | | -2.26140042 | | -2.25159641 | | -1.18119209 | |
| **P(T<=t) one-tail** | 0.23983727 | | 0.03885128 | | 3.218E-05 | | 0.02362798 | | 0.02402463 | | 0.13242811 | |
| **t Critical one-tail** | 1.8124611 | | 1.8124611 | | 1.8124611 | | 1.8124611 | | 1.8124611 | | 1.8124611 | |
| **P(T<=t) two-tail** | 0.47967454 | | 0.07770256 | | **6.4359E-05** | | **0.04725596** | | **0.04804926** | | 0.26485623 | |
| **t Critical two-tail** | 2.22813884 | | 2.22813884 | | 2.22813884 | | 2.22813884 | | 2.22813884 | | 2.22813884 | |

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**G**

| | *CN* | *CP* | *CN* | **S3R2.1** | *CN* | **S4R2.1** | *CN* | ***S4R2.6*** | *CN* | ***S1B1.5*** | *CN* | ***S1S2.5*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mean** | 6.66666667 | 10.6666667 | 6.66666667 | 11.6666667 | 6.66666667 | 10 | 6.66666667 | 12 | 6.66666667 | 12.8333333 | 6.66666667 | 8.5 |
| **Variance** | 2.26666667 | 16.6666667 | 2.26666667 | 34.2666667 | 2.26666667 | 11.6 | 2.26666667 | 24.4 | 2.26666667 | 34.9666667 | 2.26666667 | 5.9 |
| **Observations** | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| **Pooled Variance** | 9.46666667 | | 18.2666667 | | 6.93333333 | | 13.3333333 | | 18.6166667 | | 4.08333333 | |
| **Hypothesized Mean** | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| **df** | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| **t Stat** | -19 | | -26 | | -2.19264505 | | -6.93375245 | | -6.54653671 | | -1.94145069 | |
| **P(T<=t) one-tail** | 2.2601E-05 | | 6.5007E-06 | | 0.02654891 | | 0.0011358 | | 0.00140724 | | 0.06208512 | |
| **t Critical one-tail** | 2.13184678 | | 2.13184678 | | 1.8124611 | | 2.13184678 | | 2.13184678 | | 2.13184678 | |
| **P(T<=t) two-tail** | **4.5202E-05** | | **1.3001E-05** | | 0.05309782 | | **0.00227161** | | **0.00281447** | | 0.12417024 | |
| **t Critical two-tail** | 2.77644511 | | 2.77644511 | | 2.22813884 | | 2.77644511 | | 2.77644511 | | 2.77644511 | |

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