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DROUGHT STRESS SHAPES THE ROOT-ASSOCIATED BACTERIAL AND FUNGAL COMMUNITY STRUCTURE IN SOYBEAN GENOTYPES

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Abstract

Drought stress is one of the key abiotic stresses restraining the crop growth and production worldwide. Drought stress can also influence the structure and function of rhizosphere microbiome. The main objective of current investigation was to explore the effects of drought stress on shaping bacterial and fungal community structure in the wild and cultivated-type soybean genotypes. The results revealed that under drought, higher accumulation of osmolytes (sugar and proline) contents and *NCED1* transcript were found in wild soybean (*Glycine soja*) as compared to the cultivated soybean (*Glycine max*), which elucidate that wild soybean genotype was more drought tolerant. Moreover, dehydration stress significantly suppressed the fungal diversity of the two host plants,though the diversity of the bacterial community in *G. soja* was significantly increased.*Sulfitobacter* sp. was only found in wild soybean. There was an increase in the proportion of *Bradyrhizobium* sp. under drought in two soybean genotypes whereas *Sphingomonas* sp. significantly enhanced in wild genotype. Our results indicated that *G. soja* a wild soybean genotype was highly drought tolerant than *G. max*, and established more microbial association by increasing the number of bacterial community and diversity than *G. max.* Therefore, this study provides a new evidence for improving soybean drought tolerant genotypes by studying the mechanism of plant-microbe interaction.

Key words: Drought stress, Osmolytes, Root-associated bacteria and fungi, Wild soybean.

Introduction

Various biotic and abiotic stresses such as drought, salinity and temperature negatively affect the growth of plant, survival and development and the root-associated micro-flora. Presently, almost 43% of arable land in the world is drought-affected (Zhang *et al*., 2015). Water stress is accounted as one of the major abiotic stresses (Ashraf, 2010), which limits the plant growth and yield world wide (Kramer & Boyer, 1995; Denby & Gehring, 2005; Shukla *et al*., 2012). Consequently, improvement of drought tolerance crops is an imperative problem to be solved to alleviate today's agriculture in terms of yield formation and crop productivity (Shereen *et al*., 2017). Soybean *(Glycine max*) as the main source of proteins and lipids, and has been playing a key role in China's agricultural production and food security (Hussain *et al*., 2017). Water stress has become one of the main factors threatening soybean yield. Drought stress stunts the soybean plant's growth, decreases the number of flowers and pods leading to decline in yield production. Therefore, it is important to enhance drought resistance in soybean to achieve high yield (Busse & Ellis, 1985; Shao *et al*., 2016). *Glycine soja* (wild soybean), the ancestors of *Glycine max* (cultivated soybean), has strong resistance and tolerance to adverse environmental factors and is the main source of vital germplasms for drought resistant breeding practices in China (Chen *et al.*, 2006). Wild and cultivated soybean has been grown in different habitats for a long time and evolved different adaptive mechanism to cope with various abiotic stresses. Epigenetic and genetic factors plays the main role for regulating this process but the role of rhizosphere microorganisms cannot be ignored.

Most of the land plants grow in intimate association with complex microbiota. Rhizosphere microorganisms are pivotal to support the plant growth and development under various abiotic factors (Haldar & Sengupta, 2015; Bahadur *et al*., 2017). Soluble root exudates provide abundant carbon source to rhizosphere microorganisms that enhance the plant growth by several bio-chemical mechanisms. Conversely, the amount and species of rhizosphere microorganism have direct impact on soil physiochemical activity, facilitation in nutrient acquisition and improving the plant tolerance to abiotic stresses (Schippers *et al*., 1990; Berendsen *et al*., 2012; Mendes *et al*., 2013). Berg & Smalla, (2009) demonstrated that soil type and plant species cooperatively shape the function and structure of rhizosphere microbial communities. Alterations in the structure and function of rhizosphere microorganisms are driven by plant species especially under the condition of special habitat (Yergeau *et al*., 2007). Plants species can regulate their rhizosphere microorganisms with the host-dependent manner. Each plant species promotes a particular set of rhizosphere microbial community (Turner *et al*.,

2013), through their mucilage, exudates and root architecture (Badri & Vivanco, 2009; Wu *et al.,* 2014). In previous studies, it is detected that different plant species as well as the diverse genotypes of each species might vary in the microorganism composition of their rhizosphere (İnceoğlu *et al.,* 2011; Weinert *et al.,* 2011; Bulgarelli *et al.,* 2015). Plant roots activities and associated microbial communities are pivotal in altering the soil properties, and to compose a suitable environment where plants can grow well and able to resist diverse environmental challenges (Eisenhauer *et al*., 2012). Remarkable difference between genera of root-associated bacteria and fungi in the roots of wild and cultivated species of plants is reported (Hung *et al*., 2007; Deng *et al*., 2012).

Under unsuitable environment conditions, plants establish certain relationship with rhizosphere microbiome that help plants to absorb essential nutrients and induce specific stress related phytohormones and osmolytes to cope with the abiotic stress factors.According to our hypotheses, *G. soja* (a wild soybean) is more drought tolerant and establish more bacterial and fungal communities as compared to *G. max (*a cultivated soybean). This could be due to the loss of many desirable traits in *G. max* during the process of domestication. The aspect of anti-adversity and difference between rhizosphere microorganisms such as bacteria and fungi and their role in two soybean genotypes is still needed to be explored. The aim of present research was to highlight the rhizosphere bacterial and fungal community structure and diversity in wild and cultivated soybean and its relationship with their native microbiota under drought stress. Insights into the native microbial population will provide new directions for further improvement of profitable soybean drought tolerance genotypes and applicability of beneficial microorganisms in sustainable ecosystem and food production.

Materials and Methods

Plant material and growth conditions: Twosoybean genotypes *G. soja* and *G. max* were selected as a test material in the current study. Healthy seeds were surface sterilized by soaking in 0.9% sodium hypochlorite and 75% alcohol for 3 min and 30 min respectively. Before surface sterilizationplant seeds were treated with 98% concentrated sulfuric acid for 3 min to improve germination rate, then washed several times with sterile water to remove any traces of chemical, and transferred to glass culture and kept in dark at 25±2.0 °C for 2-3 days to germinate.

Six uniform two-days-old seedlings of each genotype were individually transplanted into pots. Each pot (18cm long × 12cm wide × 16cm high) filled with 2 kg of growth substrate that consist of a mixture of sand and native soil (1:2 v/v) and was randomly arranged in the greenhouse conditions. Field soil characteristics were as follows: pH 6.6, 16.2 g/kg organic matter, 109.2 mg/kg available nitrogen, 7.48 mg/kg available phosphorus, and 88.66 mg/kg available potassium. After seven days of emergence, the seedlings were thinned to four in each pot. Plants were grown in the greenhouse under a temperature ranging from 15±2°C to 25±2°C and the photoperiod was maintained about 16/8 h (day/night). According to their nutritional requirements, they were irrigated every third day with full strength Hoagland nutrient solution (Steinkellner *et al*., 2005).

Experimental design: A factorial experiment was used to study the plant responses with two factors including crop variety (*G. soja and G. max*) and water treatments (drought-stress and well-watered). Before starting water treatments all plant individuals were watered daily and kept well at 75% field capacity (FC). Afterwards, at the flowering stage (six weeks after planting), the pots were maintained at two water regimes: Drought-stress (55% FC) and well-watered (75% FC). Five replicates for each treatment combination were used in total 20 pots. Drought condition was imposed by withholding water in pots until the soil water content reached to 55% of field capacity. After that, drought stress was maintained at this level by irrigating with appropriate amounts of water for 10 days before harvesting. Theta Probe TZS-IW soil moisture sensor was used for measuring water contents in all pots.

Determination of free proline and soluble sugar: The free proline from the leaf tissues was extracted and quantified by colorimetric method following the Bates *et al.* (1973). Soluble sugar was extracted as elucidated by Curran *et al.* (2001) with some amendments.

Determination of gene expression using quantitative real-time PCR (qRT-PCR): Total RNA was extracted by using Trizol® Plus RNA Purification Kit (Invitrogen, Carlsbad, USA) according to Liu *et al.* (2014). Approximately 1 µg of RNA was transcribed into cDNA using Superscript III Reverse Transcriptase (Invitrogen, Karlsruhe, Germany). The quality of the cDNA was assessed through qRT-PCR using primers for the 18S rRNA genes and used as a reference gene.

Gene expression analysis was performed by qRT-PCR in an Agilent Mx3000P RT-qPCR system (Agilent Technologies Ltd., Santa Clara, CA, USA). The reaction medium contained 1 µL cDNA, 2 µl primer mix, 7.5 µl SYBR® Green qRT-PCR Master Mix from Agilent (Santa Clara, CA, USA) and 4.5 µl ultra-pure sterile water. The primer sets *NCED*1F (5'-TTCTTCCAAATGGTGTCGACG-3'), *NCED1*R (5'-GGCATTGACAATCTGCAGCTC-3') and *18SrRNA*F (5'-5'-TCACGACTACTGCTGAACGG-3'), *18SrRNA*R (5'-GGAGCCTCCAATCCAAACAC-3') were used for qRT-PCR. The PCR program was as follows:an initial cycle at 95°C for 30 s,followed by40 cycles in 5 s at 95°C, 30 s at 55°C and 1 min at 72°C, respectively. The melting curve was produced according to the following program: 30 s at 95°C, 30 s at 61°C, and heating to 95°C at a rate of 0.2°C s^{-1} and data were collected persistently. The data was analyzed by the ΔΔCT method (Winer *et al.,* 1999).

Total DNA extraction, PCR and DGGE analysis: Total DNA was extracted from the root samples (SN, SD, WN, WD) according to Porebski *et al.* (1997) method. Bacterial DNA was tested by two rounds of PCR. The extracted DNA was used to first round PCR amplification of bacteria. The amplification reaction of the bacterial variable region fragments for 16S rRNA V3 was conducted in a final volume of 20 µL containing 10 µL 2 × Premix Taq Version 2.0 (TAKARA), 2 µL DNA (approximately 50 ng), 1 µL 27F primer (10 pmol), 1 µL 1492R primer (10 pmol), and 6 µL ultra-pure sterile

water. The amplification reaction was performed in a thermocycler (PCR TC-96, Bio-Rad, America) programmed for an initial cycle of 94°C for 5 min, followed by 32 cycles of 30 s at 94°C, 50 s at 54°C , 90 s at 72°C and 10 min at 72°C. Afterwards, first round PCR amplification of 16S rRNA gene fragments were used to perform second round PCR amplification with 10-fold dilution by using the GC-clamped primer GC-338F (5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3') and primer 518R (5'-ATTACCGCGGCTGCTGG-3'). The second round PCR reaction mediums were the similar to the first round. The PCR program was as follows: an initial cycle of 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 52°C for 50 s, 72°C for 40 s, followed by a final extension at 72°C for 10 min. Fungal DNA tested by single-round PCR. The PCR amplification reaction of fungi used the extracted DNA with GC-clamped primer GC-Fung (5'- CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCCATTCCCCGTTACCCGTTG -3') and NS1 (5'-GTAGTCATATGCTTGTCTC-3'). The PCR program was as the first round PCR amplification of bacteria. All PCR products were confirmed by electrophoresis on a 1.4% (w/v) agarose gel.

Denaturing gradient gel electrophoresis (DGGE) analysis of the bacterial PCR products was performed in an apparatus (JY-CZ-B) as described by Muyzer *et al.* (1993) with some modifications. Linear denaturing gradient of urea and formamide was ranged from 40% to 60% and 20% to 40% for bacteria and fungi, respectively. All gels were run for 12 h at 80V in 1×TAE buffer at a constant temperature of 60°C and were stained with 5 mmol/l ethidium bromide (Dingguo, Beijing, China) for 30 min and then visualized by ultraviolet imager (GenoSens, Shanghai, China). Prominent bands in the DGGE gels were excised and re-amplified for subsequent sequence analysis. Closest known relative species of the sequence data were determined by BLAST searches of both the NCBI Genbank database (http://www.ncbi.nlm.nih.gov/). The sequences obtained in this study were deposited in GenBank under accession number KX896952-KX896982.

Multivariate analysis and statistical analysis: Three methods of multivariate analysis were applied to perform data analysis: (1) Principal component analysis (PCA); (2) Venn diagram;and (3) Phylogenetic tree. Image quantifications were performed using Quantity One 4.2.3 software (Bio-Rad). PCA and Venn diagrams were based on the intensity (optical density) of each band generated using the Vegan package in the R program. The phylogenetic tree constructed by the sequences of the excised DGGE bands from bacteria and fungi. The multiple alignment and phylogenetic tree were constructed by the neighbor-joining method using Kimura 2-parameter distance, as implemented in MEGA version 6.0 (Tamura *et al.*, 2013). Bootstrap support (>50%) from 1000 replications is shown at the nodes of the trees.

The expression of *NCED1* gene, content of free proline (Pro) and soluble sugar, and Shannon-Wiener Index of bacteria and fungi were analyzed in triplicate. SPSS 15.0 was used for the statistical analysis for the mean and standard deviation. ANOVA and Duncan's test were used for the comparison of the treatments based on three biological replicates of each treatment. The means between treatments and significant values are presented at $p \leq 0.05$.

Results

Comparison of dehydration-induced *NECD1* transcript level, proline and sugar contents in the *Glycine soja* and *Glycine max*: ABA (abscisic acid) known as the major drought-induced hormone and its signaling play key regulatory role in plant adaptation to adverse environmental conditions. Genetic and biochemical findings have shown that 9-*cis*-epoxycarotenoid dioxygenase (*NCED*) is the key enzyme in the ABA biosynthetic pathway in plant and across various *NCED* genes.*NCED1* is believed to be the stress-marker gene induced during stress. In this experiment, we studied the expression pattern of *NCED1* marker gene by qRT-PCR in *G. soja* and *G. max* under drought stress condition. *G. soja* (wild soybean) showed higher *NCED1* gene expression level than *G. max* (cultivated soybean) under water stress conditions. The data indicated that *G. soja* was more resistant to drought stress as compared to *G. max* (Fig. 1a).

The adaptive mechanism of plants to drought stress also includes the accumulation of different organic solutes like proline and sugar. Under well-watered condition, the proline contents in both wild and cultivated soybean leaves were similar, while in drought stress it was increased significantly in wild soybean leaves however in cultivated ones no significant increase was found (Fig. 1b). Moreover, thesoluble sugarcontentswere also higher in wild soybeanleavesthan thecultivated leaves (Fig. 1b) indicate that drought condition had significant effect on the amount of osmoregulatory substances which increased the plant tolerance especially in wild soybean genotype. Comparative analysis of *NCED1* gene expression pattern and incline in osmolytes (proline and sugar) suggested that wild soybean genotype plants were more tolerant to drought than cultivated soybean genotype plants.

Diversity analysis of the root-associated bacteria and fungi under dehydration stress: PCA analysis showed high similarity among the replicates of each treatment (Fig. 2) and high similarity for bacteria between SD and WN (Fig. 2a). While the fungi samples of SD, SN, WD and WN were quite separate and showed significant differences among the four groups (Fig. 2b).

The diversity of bacterial communities was compared and the significance level tests were made based on the Shannon-wiener index for bacterial community. The results showed that the diversity of bacterial community in SD was higher than WN. Moreover, the diversity of bacterial community in SN and WD was significantly higher than SD and WN. While under drought stress condition the diversity of fungal community in both wild and cultivated soybean roots were significantly decreased (Fig. 3). These data suggest that under drought the diversity of bacterial community in wild soybean was significantly increased, while in cultivated soybean it was decreased. Drought stress reduced fungal diversity in both cultivated and wild soybean. In addition, wild soybean showed more significant loss in the fungal community than cultivated soybean.

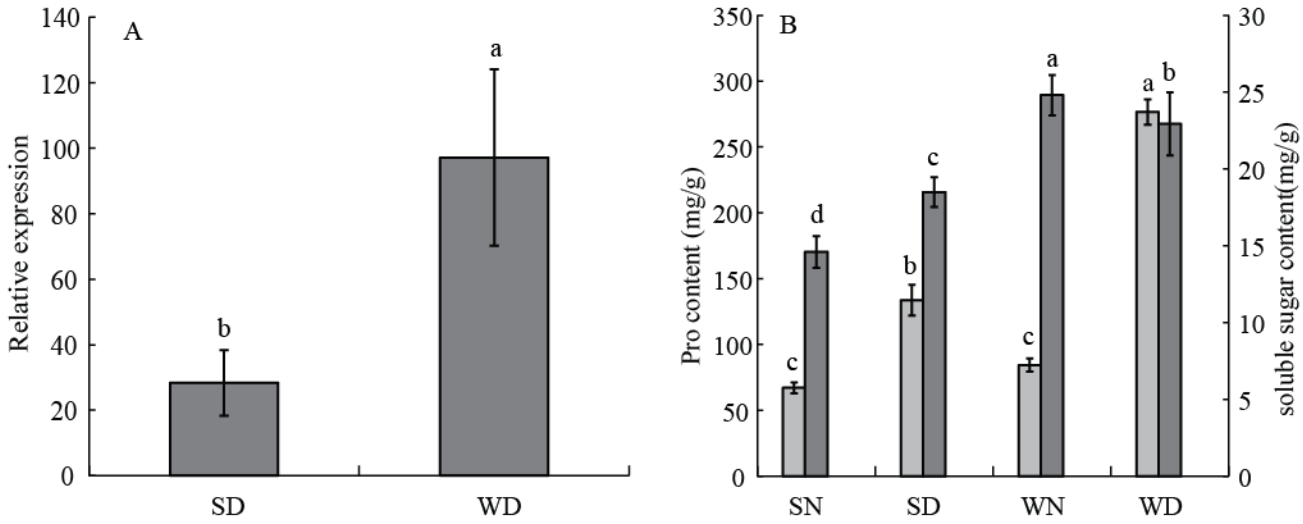

Fig. 1. a: Relative expression levels of *NCED1* gene in soybean leaves (*G. soja* and *G. max*) under drought stress; b: content of free proline and soluble sugar in *G. soja* and *G. max*. SD: cultivated soybean under drought stress; SN: cultivated soybean under well-watered; WD: wild soybean under drought stress; WN: wild soybean under well-watered. Values are means (n = 3) ± standard errors. Means were compared with Duncan's multiple-range test at the $p \leq 0.05$ level.

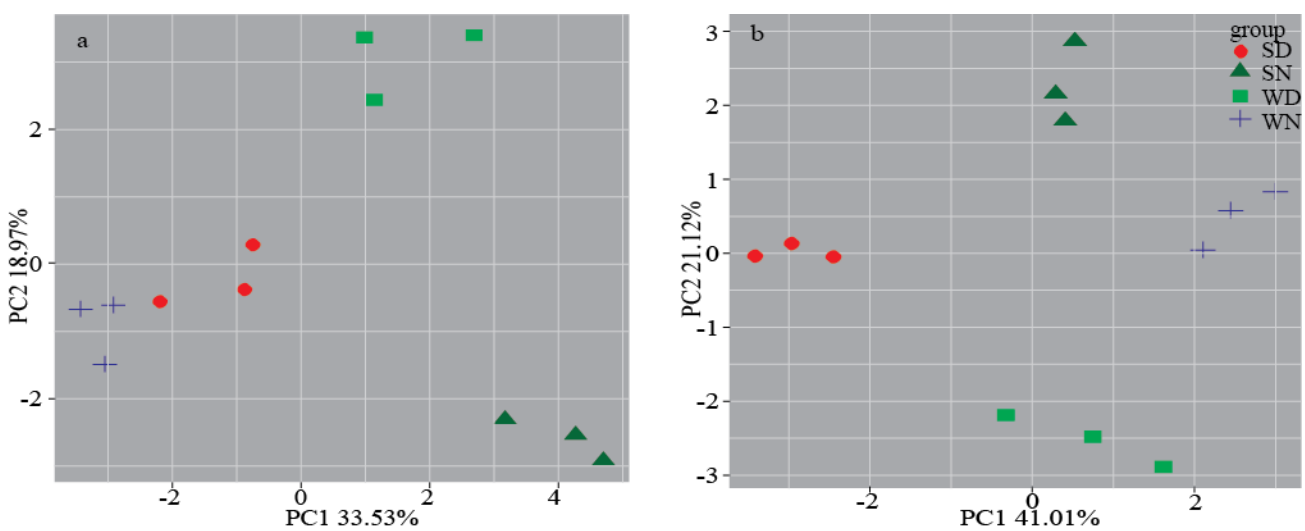

Fig. 2. Principal component analysis (PCA) for bacteria (a) and fungi (b) based on the intensity of DGGE bands. Symbols in the diagrams indicate the different treatments.

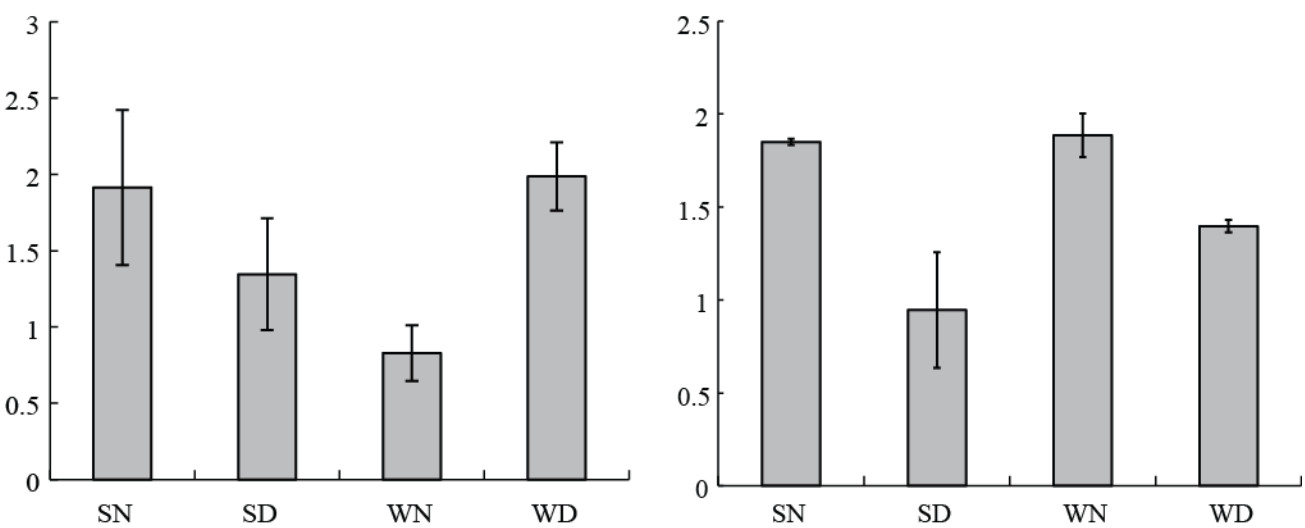

Fig. 3. Shannon-Wiener Index of bacteria (a) and fungi (b) under different drought treatments.

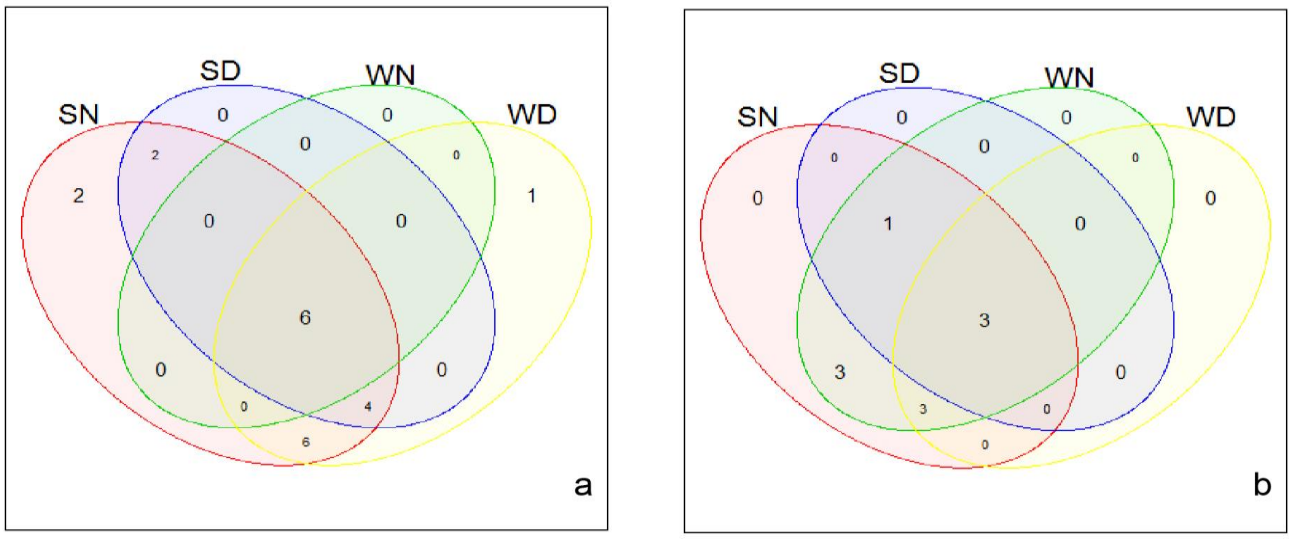

Fig. 4. Venn diagram showing specific and common OTUs (a. bacteria; b. fungi) in SN, SD, WN and WD. Numbers in the circles indicate numbers of method-specific and shared OTUs.

To evaluate the distribution of OTUs among the different treatments, Venn diagrams were constructed that indicate the numbers of shared and specific OTUs for the four samples. Venn diagrams showed the presence of bacteria at 21 OTUs and fungi at 10 OTUs, respectively. The number of bacterial OTUs shared by the four treatments was 6, while 2 were only recovered from SN and 1 was unique to WD. 3 OTUs of fungus were shared by the four samples (Fig. 4). The number of bacterial OTUs of SN and WD was 20 and 17, while SD and WN were 8 and 6 (Fig. 4a). Under drought stress condition the number of fungal OTUs of wild soybean and cultivated soybean was decreased and WD (6 OTUs) was more than SD (4 OTUs) (Fig. 4b). These data advocate that under drought conditions the OTUs of bacteria in wild soybean was increased, while it was decreased in cultivated soybean. Drought stress decreased the number of fungal OTUs, and the OTUs of fungi in wild soybean outnumbered that cultivated soybean. Overall our results indicate that drought stress badly affect the bacterial and fungal community structure of both genotypes but comparatively, the loss in the community structure was more profound in cultivated soybean than wild soybean plants indicating that cultivated soybean lost their desirable traits during process of domestication and remained hypersensitive to abiotic stresses.

Phylogenetic analysis of the root-associated bacteria and fungi: For phylogenetic analysis our obtained sequences were blasted against NCBI (http://www.ncbi.nlm.nih.gov/), and sequences were selected based on highest homology and phylogenetic tree was constructed according to that. The phylogenetic analysis, shown in Figs. 5 and 6 revealed that OTU-11, OTU-14, OTU-15, OTU-17, OTU-20 and OTU-21 separately belonged to *Sphingobacterium* sp., *Bradyrhizobium* sp., *Mesorhizobium* sp., *Caldimonas* sp., and *Rhizobium* sp. *Nitratireductor* sp., were shared by the four treatments irrelevant to the two host plants and water conditions. Some bacterial strains found in a certain treatments such as *Sulfitobacter* sp., was unique to wild soybean under drought stress condition. OTU-1, OTU-2, OTU-4 and OTU-6 were found in cultivated soybean only, and their closest strains were *Acidobacteria bacterium*, *Sphingomonas sp.*, *Bacteroidia bacterium*, and *Terrimonas* sp. The proportion of OTU-20 (*Rhizobium* sp.) under drought stress condition was decreased compared to control condition in both soybean genotypes, while OTU-14 (*Bradyrhizobium* sp.) was increased. But, OTU-16 (*sphingomonas* sp.) was increased only in wild soybean genotype under drought stress (Fig. 5, Fig. 7a). As shown in Fig. 6 and Fig. 7b OTU-5 *(Rhizophlyctis rosea*), OTU-8 (*Fusarium graminearum*) and OTU-9 (*Halosarpheia fibrosa*) were shared by the four treatments while OTU-1 (*Fusarium* sp.), OTU-2 (*Fusarium* sp.) and OTU-4 (*Fusarium graminearum*) were only found under well-watered conditions. These results revealed that drought stress is responsible for changing in the structure and function of bacteria and fungi communities in the rhizosphere of both soybean genotypes. Furthermore, it was inferred that wild soybean genotype cope with the drought stress by selectively binding specific bacteria and fungi of rhizosphere.

Discussion

Plants have evolved different molecular, biochemical and physiological mechanisms to defend dehydration stress. For instance the accumulation of ABA, different organic solutes such as proline and sugar are among these adaptive mechanisms. ABA is defined as a stress hormone because of its rapid accumulation in response to abiotic stresses. Under stress conditions, ABA reduces water loss through lowering transpiration rate by inducing stomatal closure (Kriedemann *et al*., 1972; Zhang & Davies, 1989; Assmann, 2010). Meanwhile, ABA increases the activities and contents of the antioxidant enzymes in plants (Zhou *et al*., 2005), and induce expression of stress-related genes (Ingram & Bartels, 1996; Bray, 1997). Previous reports showed that ABA biosynthetic gene *NCED1* induced during dehydration stress and overexpression of *AhNCED1* transgenic lines in *Arabidopsis* can improve drought-stress tolerance. Wan & Li, (2006) demonstrated the significant upregulation of *AhNCED1* transcriptand ABA accumulation in the *Arabidopsis* under dehydration. Here, we studied the *NCED1* marker gene expression in *G. soja* and *G. max* under water deficit and found significantly greater induced expression of *NCED1* in *G. soja* than *G. max* (Fig. 1a) which demonstrated that *G. soja* genotype is more drought tolerant than that of *G. max*.

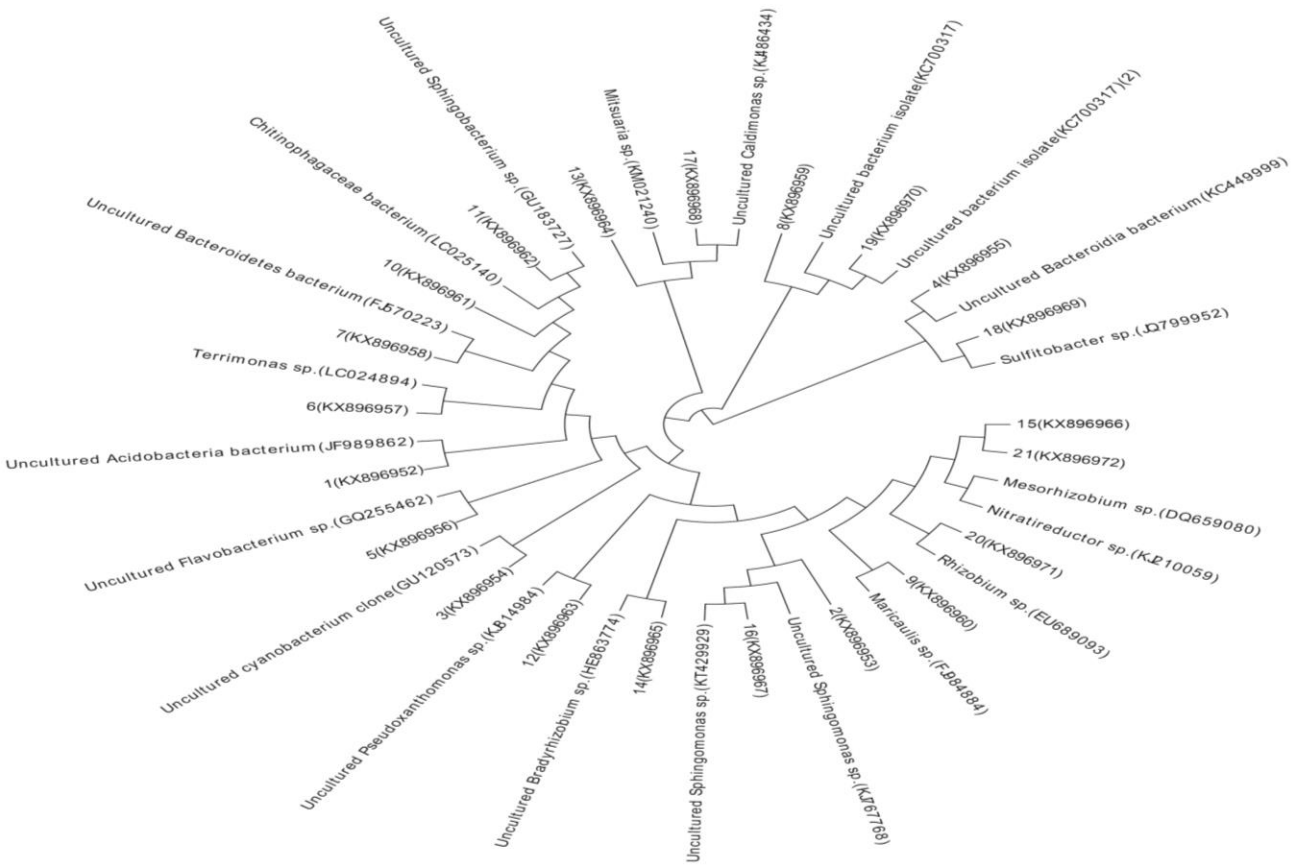

Fig. 5. Phylogenetic tree constructed by the sequences of the excised DGGE bands of bacteria. Nodal support in neighbor joining was evaluated by 1,000 bootstrap replications. The accession numbers of the closest BLAST-N matches are listed.

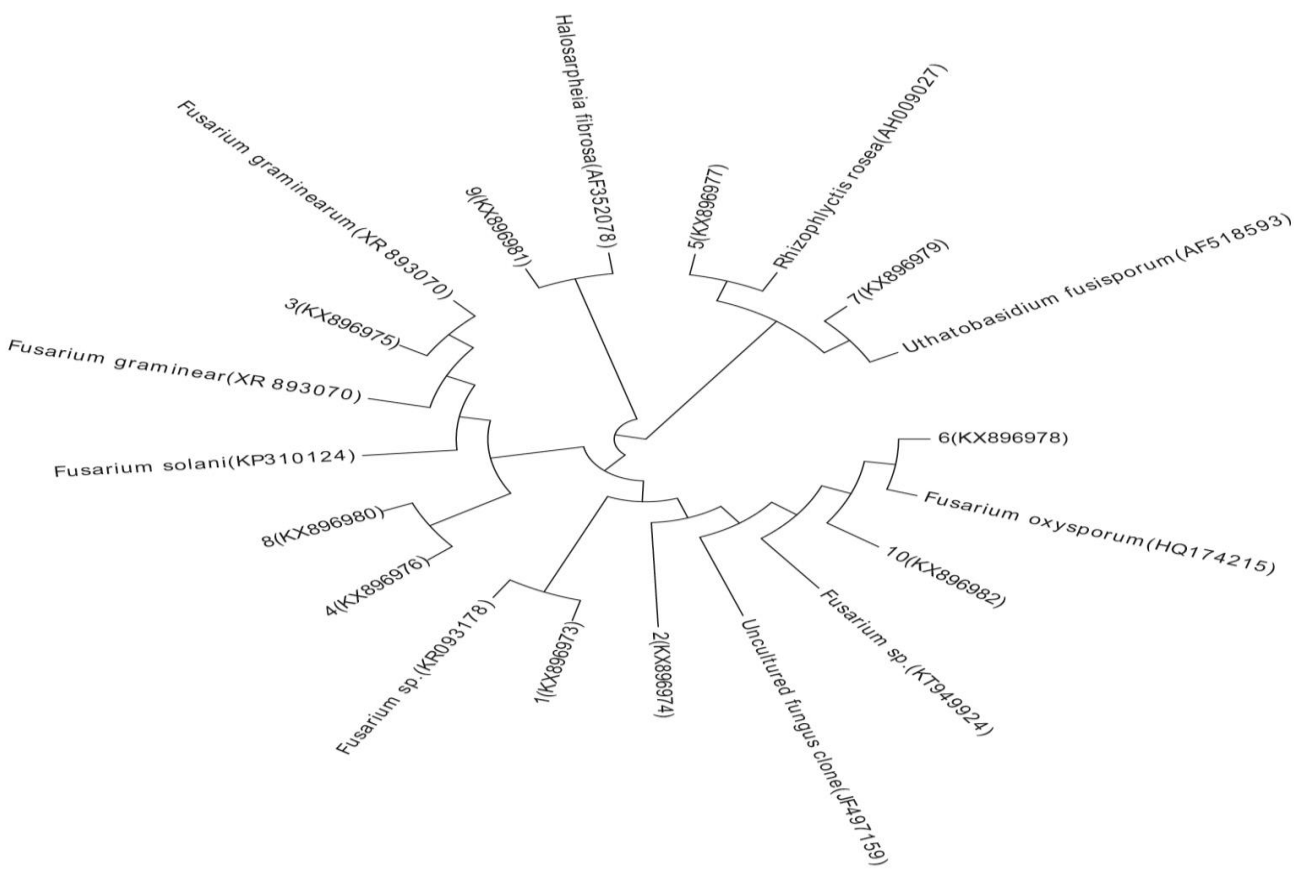

Fig. 6. Phylogenetic tree constructed by the sequences of the excised DGGE bands of fungi. Nodal support in neighbor joining was evaluated by 1,000 bootstrap replications. The accession numbers of the closest BLAST-N matches are listed.

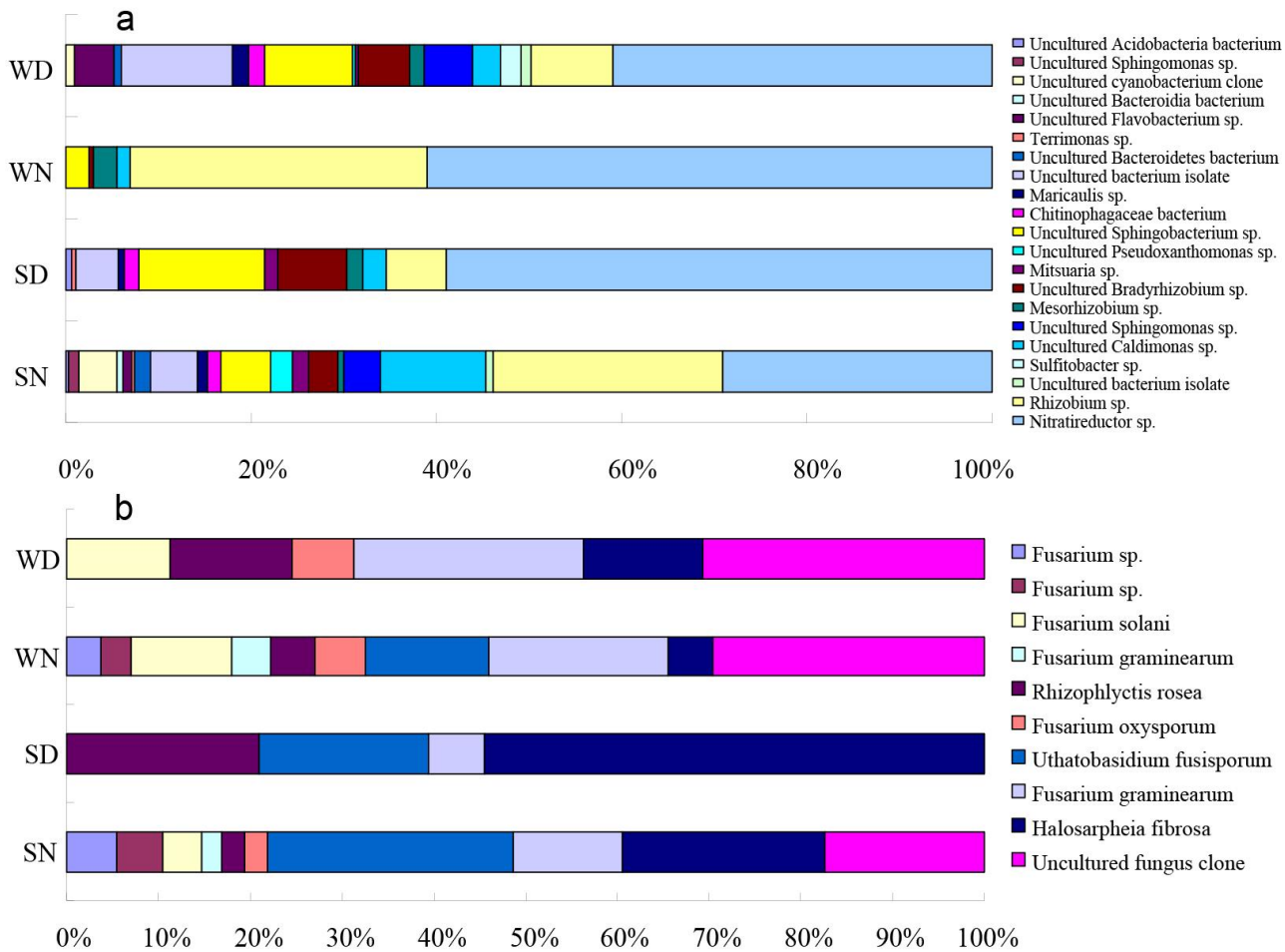

Fig. 7. Percentage distribution of main bacterial (a) and fungal (b) groups in four treatments.

Proline and soluble sugar have the ability to maintain osmotic adjustment, scavenge free radicals and stabilize subcellular structures and are considered to store carbon and nitrogen to benefits plants under drought conditions (Hare & Cress, 1997; Redillas *et al*., 2012). Moreover, in plants, proline not only acts as a reactive oxygen species scavenger (Smirnoff & Cumbes, 1989) but also regulates redox reaction, thus influencing energy transfer and storage (Liu *et al*., 2015; Szabados & Savoure, 2010). Therefore proline and soluble sugar are considered important plant osmolytes and the accumulation of these osmolytes can drive a path for buffering the cytosolic pH and regulating the status of cell redox that is considered to play adaptive approach in stress tolerance of plants. In present study we compared the sugar and proline in wild and cultivated soybean during well-watered and drought stress and observed that under drought, higher amount of sugar and proline were accumulated in wild soybean leaves (Fig. 1b), than the cultivated ones, representing that wild soybean plants were more drought tolerant, while cultivated soybean genotype was hypersensitive under dehydration condition.

It is well-known that drought stress not only affects plant physiology and development but also adversely affects the root-associated microbiota of the host plant. Bacteria and fungi are two large groups of rhizosphere microorganisms. Mycorrhiza is comparatively more important to plant growth in dry conditions as compared to sufficient water conditions(Michelsen & Rosendahl, 1990; Wu & Xia, 2006). Arbuscular mycorrhizal fungi affect the water status of the host plant and improve the drought resistance under drying soil (Augé, 2001). Root-associated bacteria not only augment plant immunity and productivity but also elicit abiotic stress tolerance by inducing physical or chemical changes (Yang *et al.*, 2009). In current study, many dominant OTUs were detected in all four treatments (Figs. 6, Fig. 7) and diversities between bacterial and fungal communities were compared (Fig. 4). Under drought stress, the Shannon-wiener index and OTUs of bacteria in wild soybean was increased significantly, while decreased in cultivated soybean. Drought stress reduced fungal diversity in both cultivated and wild soybean, additionally cultivated soybean showed more significant loss in fungal diversity than wild soybean. Work by several group showed that advanced plant nutrition enhanced drought resistance and yield productivity when crops were subjected to different evels of water stress (Begg & Turner, 1976; Wu & Xia, 2006). Phosphorus can alter plant metabolic pathways (Radin, 1984; Ackerson, 1985) by improving water use efficiency (Payne *et al*., 1992), stimulating root growth (Edwards, 1991), and promoting crop absorption of other elements that can enhance plant tolerance to drought stress. Many of bacteria could dissolve insoluble phosphorus that cannot be utilized by plants directly and transform them

into soluble phosphorus. In our experiment, we presumed that under drought stress condition, the structure of root-associated bacteria of wild soybean was modulated to enhance drought resistant capacity by releasing soil phosphorus. Results indicated that drought stress reduced the proportion of *Rhizobium* sp. of rhizosphere microorganism groups in both soybean genotypes. It was due to adverse effects of drought stress on nodule formation and development of legume plants (Gil-Quintana *et al.*, 2013). Through sequences analysis, we found that *Sulfitobacter* sp. was unique to wild soybean under drought stress condition. A previous study has shown that most of *Sulfitobacter* sp. obtained from the East China Sea possesses evident free radical scavenging capacities (Long *et al.*, 2009). So it would be vital to explore that whether *Sulfitobacter* sp. improves the drought resistance of wild soybean by removing free radicals. There was an increase in the proportion of *Bradyrhizobium* sp. under drought stress condition in both soybean genotypes and S*phingomonas* sp. (OUT-16) was much increased in wild genotype. Aliasgharzad *et al.* (2006) revealed that *Bradyrhizobium japonicum* in cooperation with AM fungi could improve plant water uptake and nutritional level by improving the drought avoidance mechanism of plants, thus leading to alleviation of plant microbe association in soybean under water stress. A number of studies have described that *Sphingobacteria* sp. isolated from plant rhizosphere was closely related to plant released carbohydrates into the rhizosphere for promoting the plants nutrients absorption level and resistance to variety of plant pathogens (Berg & Ballin, 1994; Takeuchi *et al.*, 1995). Another possible explanation might be that *Sphingobacterium* sp. as a bio-surfactant producer can produce surfactants to change the surface or interfacial properties of the cell or surroundings to have an effective relationship with the environment (Burgos-Diaz *et al.*, 2011).

Conclusion

Drought stress reduces the growth and production of the plats mostly in arid/semiarid regions of the world. Plant adapts different strategies to cope with the adverse environmental conditions including drought tolerance and it varies with different plant genotypes. Plant-growth-promoting rhizobacteria/PGPR can potentially augment the plant water status under drought conditions. Plant-microbe association can alleviate the drought tolerance mechanism by improving the nutrients and water uptake from soil and increasing the plant productivity. Our study indicated that wild soybean has a stronger drought tolerance profile than cultivated soybean owing to the changes in the structure of root-associated bacterial and fungal communities. These wild soybean genotype-specific bacteria and fungi could be used in future studies andcontribute to the long-term sustainable development of the agricultural and soil ecosystem. Moreover, this study provides a novel perspective to boost the production of soybean drought tolerant genotypes and a potential approach to uplift the agroecosystem sustainability and food security.

Acknowledgement

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