Amelioration of drought effects in wheat and cucumber by the combined application of super absorbent polymer and potential biofertilizer

Yongbin Li [1], **Haowen Shi** [1], **Haowei Zhang** [1], **Sanfeng Chen** Corresp. [1]

[1] State Key Laboratory of Agrobiotechnology and College of Biological Sciences, China Agricultural University, Beijing, China

Corresponding Author: Sanfeng Chen
Email address: chensf@cau.edu.cn

Biofertilizer is a good substitute for chemical fertilizer in sustainable agriculture, but its effects are often hindered by drought stress. Super absorbent polymer (SAP), showing good capacity of water absorption and retention, can increase soil moisture. However, limited information is available about the efficiency of biofertilizer amended with SAP. This study was conducted to investigate the effects of synergistic application of SAP and biofertilizers (*Paenibacillus beijingensis* BJ-18 and *Bacillus* sp. L-56) on plant growth, including wheat and cucumber. Potted soil was treated with different fertilizer combinations (SAP, BJ-18 biofertilizer, L-56 biofertilizer, BJ-18 + SAP, L-56 + SAP), and pot experiment was carried out to explore its effects on viability of inoculants, seed germination rate, plant physiological and biochemical parameters, and expression pattern of stress-related genes under drought condition. At day 29 after sowing, the highest viability of strain *P. beijingensis* BJ-18 (264 copies ng^{-1} gDNA) was observed in BJ-18 + SAP treatment group of wheat rhizosphere soil, while that of strain *Bacillus* sp. L-56 (331 copies ng^{-1} gDNA) was observed in the L-56 + SAP treatment group of cucumber rhizosphere soil. In addition, both biofertilizers amended with SAP could promote germination rate of seeds (wheat and cucumber), plant growth, soil fertility (urease, sucrose, and dehydrogenase activities). Quantitative real-time PCR analysis showed that biofertilizer + SAP significantly down-regulated the expression levels of genes involved in ROS scavenging (*TaCAT*, *CsCAT*, *TaAPX,* and *CsAPX2*), ethylene biosynthesis (*TaACO2*, *CsACO1,* and *CsACS1*), stress response (*TaDHN3*, *TaLEA,* and *CsLEA11*), salicylic acid (*TaPR1-1a* and *CsPR1-1a*), and transcription activation (*TaNAC2D* and *CsNAC35*) in plants under drought stress. These results suggest that SAP addition in biofertilizer is a good tactic for enhancing the efficiency of biofertilizer, which is beneficial for plants in response to drought stress. To the best of our knowledge, this is the first report about the effect of synergistic use of biofertilizer and SAP on plant growth under drought stress.

Amelioration of drought effects in wheat and cucumber by the combined application of super absorbent polymer and potential biofertilizer

1 **Yongbin Li†, Haowen Shi†, Haowei Zhang, Sanfeng Chen***

2 State Key Laboratory of Agrobiotechnology and College of Biological Sciences, China
3 Agricultural University, Beijing, P. R. China

4 † These authors have contributed equally to this work

5 *** Correspondence:** Sanfeng Chen
6 E-mail: chensf@cau.edu.cn
7 Tel.: +86-10-62731551

ABSTRACT

Biofertilizer is a good substitute for chemical fertilizer in sustainable agriculture, but its effects are often hindered by drought stress. Super absorbent polymer (SAP), showing good capacity of water absorption and retention, can increase soil moisture. However, limited information is available about the efficiency of biofertilizer amended with SAP. This study was conducted to investigate the effects of synergistic application of SAP and biofertilizers (*Paenibacillus beijingensis* BJ-18 and *Bacillus* sp. L-56) on plant growth, including wheat and cucumber. Potted soil was treated with different fertilizer combinations (SAP, BJ-18 biofertilizer, L-56 biofertilizer, BJ-18 + SAP, L-56 + SAP), and pot experiment was carried out to explore its effects on viability of inoculants, seed germination rate, plant physiological and biochemical parameters, and expression pattern of stress-related genes under drought condition. At day 29 after sowing, the highest viability of strain *P. beijingensis* BJ-18 (264 copies ng^{-1} gDNA) was observed in BJ-18 + SAP treatment group of wheat rhizosphere soil, while that of strain *Bacillus* sp. L-56 (331 copies ng^{-1} gDNA) was observed in the L-56 + SAP treatment group of cucumber rhizosphere soil. In addition, both biofertilizers amended with SAP could promote germination rate of seeds (wheat and cucumber), plant growth, soil fertility (urease, sucrose, and dehydrogenase activities). Quantitative real-time PCR analysis showed that biofertilizer + SAP significantly down-regulated the expression levels of genes involved in ROS scavenging (*TaCAT*, *CsCAT*, *TaAPX,* and *CsAPX2*), ethylene biosynthesis (*TaACO2*, *CsACO1,* and *CsACS1*), stress response (*TaDHN3*, *TaLEA,* and *CsLEA11*), salicylic acid (*TaPR1-1a* and *CsPR1-1a*), and transcription activation

(*TaNAC2D* and *CsNAC35*) in plants under drought stress. These results suggest that SAP addition in biofertilizer is a good tactic for enhancing the efficiency of biofertilizer, which is beneficial for plants in response to drought stress. To the best of our knowledge, this is the first report about the effect of synergistic use of biofertilizer and SAP on plant growth under drought stress.

INTRODUCTION

Traditional agricultural practices have negative influences on continental ecosystem due to huge inputs of chemical fertilizer (Simonsen et al., 2015), which has given rise to serious problems such as soil degradation, genetic diversity loss, nitrous oxide emission, and nitrate leaching (Webb, Harrison & Ellis 2000; Kaur, Brar & Dhillon 2007). Biofertilizer containing plant growth-promoting rhizobacteria (PGPR) is an ideal candidate for reducing application of chemical fertilizer in sustainable agriculture (Rajkumar et al. 2010; Choudhury, Kecskes & Kennedy 2014). Numerous studies have been reported with respect to growth promotion and biocontrol (Vrieze et al., 2015) and colonization of PGPR in different plants. Some PGPR could promote plant growth by enhancing phosphorus (P) solubilization, nitrogen (N) fixation, zinc solubilization, potassium solubilization, and indole-3-acetic acid (IAA) production (Calvo et al., 2010; Khan et al., 2010; Islam et al., 2013; Gontia-Mishra et al., 2016). Previous studies have confirmed the roles of *Pseudomonas*, *Azospirillum*, *Bacillus*, *Burkholderia,* and *Paenibacillus* on the growth of pea, strawberry, rice, rape, and maize, respectively (Chen et al., 2013b; Guerrero-Molina et al., 2015; Oteino et al., 2015; Shakeel et al., 2015; Li et al., 2017). PGPR, such as *Lysinibacillus sphaericus* ZA9, showed great potential in plant growth promotion and biocontrol (Naureen et al., 2017). The inoculation with endophytic *P. aeruginosa* PW09 ameliorated both biotic (*Sclerotium rolfsii*) and abiotic (NaCl stress) stresses in cucumber seedlings (Pandey et al., 2012). Similarly, the tolerance of chickpea to biotic (*Sclerotinia sclerotiorum*) and abiotic (NaCl stress) stresses was also significantly enhanced after inoculation with *P. putida* S1 or *P. aeruginosa* Cgr (Ankita et al.,

2014). The application of endophytic *Burkholderia phytofirmans* PsJN mitigated drought stress, thereby improving the growth and development of wheat (Naveed et al., 2013). PGPR could also successfully colonize the tissues of plants (Yang et al. 2013; Gontia-Mishra et al. 2016; Hao & Chen 2017; Wang et al. 2017). However, the application of some biofertilizers still remains limitation (Zhang et al. 2012; Grady et al. 2016). The reason might be that the reproduction of PGPR was affected by adverse climatic conditions (Zhang et al., 2012;Bashan et al., 2013) such as limited precipitation. Therefore, in arid and semi-arid regions of northern China (Islam et al., 2010), drought is a crucial limiting factor, and water shortage hindered the application of biofertilizer in practices.

Drought stress is one of the most destructive environmental factors affecting agricultural production worldwide (Gontia-Mishra et al., 2016). Wheat often suffered from periodic drought stress in the growth cycle (Naveed et al., 2013; Naresh et al., 2014). Super absorbent polymer (SAP), a macromolecular cross-linked and environmentally-friendly polymer, had high capacity of water adsorption and retention (Johnson & Veltkamp 1985), which is conductive to improving seed germination and seedling survival. SAP could be degraded through physical or chemical processes (Mikkelsen, 1994). Hence, increasing number of SAP commercial products have been developed to raise water use efficiency, alleviate drought stress, improve soil physical properties and increase crop yield (Terry et al., 1986; Mikkelsen, 1994; Gray, 2011). Hydrogel supply enhanced soil moisture and seed germination rate (Rehman, Ahmad & Safdar 2011). The application of a hydrophilic polymer (Superab A200) increased dry matter accumulation and water

74 use efficiency of maize (Dorraji, Golchin & Ahmadi 2010). Most studies only focused on the
75 application effect of SAP alone or mixed with a chemical fertilizer, however, to the authors'
76 knowledge, studies on synergistic use of SAP with biofertilizer are rarely reported, particularly
77 under water deficit conditions.

78 It is of great significance to investigate whether the application of SAP can retain more water
79 and create a suitable environment for reproduction of inoculants.

80 Soil enzyme activity, as a susceptive indicator of soil health, has potential of comprehensively
81 evaluating soil microbial functional diversity and its functional changes (Hestrin & Goldblum
82 1953; Lebrun et al. 2012). Some soil enzymes paticipate in N, P, C cycling (Hestrin & Goldblum
83 1953), and microbial activity. N cycling is mainly governed by urease, protease, and asparaginase;
84 P cycling by alkaline and acidity phosphatase; C cycling by cellulase, sucrase, and β-glucosidase;
85 and microbial activity is regulated by catalase (Kandeler, Kampichler & Horak 1996). *Fusarium*
86 could produce and secrete extracellular enzymes such as phosphatase (Meyer, Garber & Shaeffer
87 1964). SAP application had little effect on soil microbial metabolism (Sojka, Entry & Fuhrmann
88 2006), but it still needs more understanding regarding the effect of synergistic use of biofertilizer
89 and SAP on soil enzymes involved in C, N, P cycling, and microbial activity.

90 In this paper, two important but different crops (wheat: monocotyledon; cucumber: dicotyledon)
91 were selected to comparatively investigate the effects of synergistic use of biofertilizer and SAP
92 on the following aspects: (i) viability of inoculants and soil enzyme activity; (ii) seed germination
93 and plant growth promotion; (iii) plant biochemical indexes and expression of drought stress-

related genes under severe drought condition (40% relative soil moisture). Our results are expected to popularize the synergistic use of biofertilizer and SAP, especially in the areas suffered from long-term drought stress.

MATERIALS & METHODS

Bacterial strains and potted plant soil

Table 1 details the bacteria used in this study.

The topsoil (0-20 cm depth) was collected from Shangzhuang Experimental Station of China Agricultural University, Beijing, China (40°08′12.15″ N, 116°10′44.83″ E, 50.21 m above sea level). After air-dried at room temperature, the soil was screened by a 10-mesh sieve to remove plant residues and reduce soil heterogeneity. Then the soil was sterilized at 121°Cfor 30 min. The experimental soil were low N-content sandy loam (pH: 7.7, Olsen-P: 7.3 mg kg^{-1}, N_{min}: 7.8 mg kg^{-1}, NH_4OAc-K: 115.8 mg kg^{-1}, organic matter, 7.2 g kg^{-1}).

Preparation of biofertilizer and super absorbent polymer (SAP)

Two PGPR, *P.beijingensis* BJ-18 and *Bacillus* sp. L-56, were inoculated in Erlenmeyer flasks (250 mL) containing 100 mL of Luria Bertani (LB) broth and cultured at 30°Cfor 36 h at 180 rpm, respectively. The culture was centrifuged at 6000 rpm for 5 min and adjusted to 5×10^8 cells mL^{-1} with sterile normal saline (0.89% w/v NaCl in water). Rice hull was used as carrier, which was ground and screened by a 200-mesh sieve. Then the powder was sterilized at 121°C for 20 min.

112 The above bacterial suspension was mixed aseptically with sterile rice hull powder (1:1, v/w), and
113 then the mixture was air dried separately in shade as biofertilizer.
114 The SAP used in this study is polyacrylamide (Dongying Huaye New Material Co., Ltd), which
115 is developed for agriculture and forestry use only. It was sterilized by ultraviolet (UV) light for 1 h
116 in super clean bench (SW-CJ-1F(D)/2F(D), AIRTECH, China), and then mixed with biofertilizer
117 (1:100, w/w) according to the instructions of SAP.

118 **Plant culture and collection**

119 The experiment was performed to evaluate the role of SAP + biofertilizer under drought
120 condition. Plastic pots (35 cm in diameter; 25 cm in height), which were sterilized by soaking in
121 0.5 N nitric acid for 24 h, were filled with the above sterilized soil (5 kg). The treatments were
122 listed in Table 2. The mixture (rice hull powder, biofertilizer, and SAP) of each treatment was
123 applied as base manure (9 g/per pot). Before sowing, the pots were watered to 40% relative soil
124 moisture by weighing method. Then, the plants were watered (200 mL each pot) every 5 days till
125 harvest.

126 **Seed germination assay**

127 For assessment of seed germination, plump seeds of wheat and cucumber were surface sterilized
128 with sodium hypochlorite (10% v/v) for 10 min, followed by rinsing with sterile deionized water.
129 One hundred seeds of each plant were sown in each plastic pot. The experiment design was
130 completely randomized with three replicates for each treatment. The pots were arranged to the

131 greenhouse under optimum condition (15 h day/25°C-30°C/day temperature and 9 h night/15°C-
132 20°C/night temperature).

133 The numbers of germinated seeds were recorded daily from 6 days to 14 days after sowing, and
134 the germination rate was calculated according to the reported method (Sudisha et al., 2010).

135 **Viability assessment of inoculants in rhizosphere soil**

136 Two weeks after sowing, the seedlings (wheat and cucumber) were thinned to about 10 cm apart,
137 with only nine uniform and healthy seedlings were left in each pot. At the same time, the mixture
138 (rice hull powder, biofertilizer, and SAP) was applied again according to the description of Table 2
139 (9 g / per pot, ignoring the weight of SAP). To analyze the viability of inoculants in the rhizosphere
140 soil, the population density was measured by quantitative PCR (qPCR) (Savazzini et al., 2008) at
141 day 14, 19, 24, 29, 34, 39, and 44 after sowing. In order to collect the rhizosphere soil, the seedling
142 was uprooted and shaken gently to remove the loosely adhering soil, and the tightly adhering soil
143 regarded as rhizosphere soil. For the qPCR counting, DNA was extracted form rhizosphere soil
144 using an TIANamp Soil DNA Kit (Tiangen Biotech CO., LTD., Beijing, China) according to the
145 manufacturer's protocol. Suitable primers for qPCR were designed based on *nifB* of *P.*
146 *beijingensis* BJ-18 and *amye* of *Bacillus* sp. L-56 with the AlleleID 6.01 (PREMIER Biosoft
147 International, Palo Alto CA, USA; Table 3). The 107 bp (*nifB*) and 138 bp (*amye*) fragments were
148 amplified by conventional PCR and then gel-purified. The PCR products were ligated to PMD 19-
149 T Vector (Takara, Otsu, Japan) and transformed into *Escherichia coli* JM109 competent cells by

150 electrotransformation. The successfully transformed *E. coli* JM109 was cultured in LB liquid
151 medium, afterwards, the plasmids were extracted and purified using a TIANprep Mini Plasmid Kit
152 (TIANGEN BIOTECH(BEIJING) CO., LTD). A standard curve was generated for each run with a
153 dilution range of the recombinant plasmids from 2×10^1 to 2×10^7 copies. The above gDNA
154 isolated from different treatments was mixed with the SYBR® Premix Ex Taq™ (Takara, Kyoto,
155 Japan), primer pairs and ddH_2O to a total volume of 20 uL for qPCR. Target DNA was quantified
156 based on the above standard curve.

157 **Sample collection and preparation**

158 The plant and rhizosphere soil samples of wheat and cucumber were harvested at 44 days after
159 sowing. The growth parameters, i.e., root length, shoot length and fresh weight (FW) of each plant,
160 were immediately determined. Four plants per pot were oven dried at 105°C for 30 min, followed
161 by 65°C for 72 h. The dry weight (DW) was recorded. Chlorophyll content of seedlings was
162 determined at 8:30-9:30 am on the harvest day using the SPAD-502 chlorophyll meter (Minolta
163 Camera Co. Ltd. Japan). The remaining plant samples were rapidly frozen in liquid N and then
164 stored at -80°C for further use. Rhizosphere soil samples were screened by a 10-mesh sieve, and
165 then stored in plastic bags for soil enzyme activity determination.

166 **Determination of free proline content and total soluble sugars (TSS) in plant leaves**

167 Under drought stress, accumulation of osmolytes (free proline content and total soluble sugars)
168 can promote cell growth under adverse osmotic conditions under adverse osmotic conditions.

(Sandhya et al. 2010). Free proline content in leaves was measured using acid ninhydrin (Sharma et al., 2010), and total soluble sugars (TSS) was determined using anthrone reagent (Shukla, Agarwal & Jha 2012).

Soil enzyme activity determination

A total of 5 g soil was taken to measure activities of enzymes, which was related to N cycling: urease (EC 3.5.1.5); P cycling: alkaline phosphatase (EC 3.1.3.1) and acid phosphatase (EC 3.1.3.2); C cycling: invertase (EC 3.2.1.26) as well as microbial activity: dehydrogenase (EC 1.1.1). Urease activity was determined with urea as a substrate (Kaur, Brar & Dhillon 2007) and the released $N\text{-}NH_4^+$ amount (mg $N\text{-}NH_4^+$ g $soil^{-1}$ h^{-1}) was determined spectrophotometrically at OD_{578}. Alkaline phosphatase and acid phosphatase were assessed with *p*-nitrophenyl phosphate as a substrate (Tabatabai & Bremner 1969) and the released *p*-nitrophenol (pNP) amount (mg pNP g $soil^{-1}$ h^{-1}) was measured spectrophotometrically at OD_{660}. Sucrase activity was measured with sucrose as a substrate (Chen et al., 2013a) and the released glucose amount (mg glucose g $soil^{-1}$ h^{-1}) was determined spectrophotometrically at OD_{508}. Dehydrogenase activity was determined with 2,3,5-triphenyltetrazolium chloride (TTC) as a substrate (Schinner et al., 1996) and the reduced triphenylformazan (TPF) amount (mg TPF g $soil^{-1}$ h^{-1}) was determined spectrophotometrically at OD_{485}. All assays were carried out in triplicate.

Quantitative real-time (qRT) PCR analysis of drought stress-related genes

Total RNA was extracted from leaf samples of wheat and cucumber using Trizol reagent according to the manufacturer's protocol. RNA concentration was determined at 260 nm using a spectrophotometer (Nanodrop 1000, Thermo Scientific, USA). Strand cDNA was synthesized using PrimeScript™ RT reagent kit (Takara, Japan) following the instructions of the manufacturer. Then, cDNA was used as template to determine gene expression levels. qRT-PCR was performed by using the SYBR® Premix Ex Taq™ (TaKaRa, Kyoto, Japan). The specific primers of *ACTIN* were used as reference. The specific primers used for qRT-PCR were designed by using the AlleleID 6.01 (PREMIER Biosoft International, Palo Alto CA, USA) or from previous literature (Table 3). The relative expression levels were calculated according to the standard comparative $C(t)$ method (Livak & Schmittgen 2001). Each treatment had three biological replicates, with three technical replicates for each biological replicate.

Statistical analysis

All data was statistically analyzed using SPSS software version 20 (SPSS Inc., Chicago, IL, USA). The significant differences among treatments were analyzed using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) at 0.05 level of probability. Graphs were prepared with SigmaPlot software version 12.5 (Systat Software, Inc, California, USA).

RESULTS

205 **Effects of biofertilizer and SAP on seed germination**

206 The number of germination seeds was dynamically observed from day 5 to day 14 after sowing
207 (Table 4). The synergistic use of biofertilizer and SAP had significant effects on germination rate
208 and germination time of wheat and cucumber seeds. The germination time of wheat seeds treated
209 with biofertilizer + SAP shortened by 4 days compared with that of the control, while 2 days
210 compared with that of SAP treatment group. The germination rate of wheat seeds was 22.70% in
211 the control; while 70.0% in BJ-18 + SAP treatment group and 62.0% in L-56 + SAP treatment
212 group. The results indicated that adding SAP to the biofertilizer of *P. beijingensis* BJ-18 and
213 *Bacillus* sp. L-56 increased the seed germination rate by 47.3% and 39.3%, respectively, compared
214 with the control.

215 Similarly, biofertilizer + SAP treatment group shorten the germination time of cucumber seed
216 by 2-4 days compared with other treatment groups. Among all the treatments of cucumber, L-56 +
217 SAP treatment group had the highest germination rate, with a 35.7% increase compared to the L-
218 56 treatment group.

219 **Effect of biofertilizer and SAP on viability of inoculant**

220 The qPCR method was used to determine the inoculant population density in the rhizosphere
221 soil, and the viability of inoculants was significantly enhanced when biofertilizer was applied with
222 SAP as compared to other treatments (Table 5). The inoculant populations increased at first and
223 then declined. In wheat rhizosphere, the strain of BJ-18 + SAP treatment group showed the highest

bacterial population (264 copies ng^{-1} gDNA) at day 29 after sowing. After reaching the highest population, both strains of *P. beijingensis* BJ-18 and *Bacillus* sp. L-56 showed a continuous declining trend. In cucumber rhizosphere, the L-56 + SAP treatment group showed the highest bacterial population (331.7 copies ng^{-1} gDNA) at day 29; and retained a higher level (75.0 copies ng^{-1} gDNA) till day 44 than others. No such inoculants (*P. beijingensis* BJ-18 and *Bacillus* sp. L-56) populations were detected in the rhizosphere soil of un-inoculated seedlings (wheat and cucumber).

Effect of biofertilizer and SAP on plant growth parameters

The treatments of adding SAP to biofertilizer significantly increased the growth parameters of both wheat and cucumber seedlings, including plant length, FW and DW (Fig. 1). The BJ-18 + SAP treatment group showed maximum increase over control in shoot length (28.2%), root length (42.3%), shoot FW (86.9%), root FW (83.4%), shoot DW (104.9%) and root DW (79.7%) of wheat seedlings. However, no significant effect was found in BJ-18 treatment group and L-56 treatment group in comparison to control.

In case of cucumber plants, the treatment group of L-56 + SAP showed maximum increase over control in shoot length (47.8%), root length (50.8%), shoot FW (71.9%), root FW (175.7%), shoot DW (68.9%) and root DW (76.9%). All the biometric growth parameters showed an order of L-56 + SAP > BJ-18 + SAP > L-56 > BJ-18 > Control. Similarly, cucumber treated with biofertilizer alone had no significant difference in all the biometric growth parameters as compared to control.

Effect of biofertilizer and SAP on biochemical parameters of seedlings

For both wheat and cucumber, the biochemical parameters (proline content, TSS content, and chlorophyll content) of leaves were quantified (Fig. 2). The results indicated that L-56 + SAP treatment group showed the lowest proline content, followed by BJ-18 + SAP group. The seedlings also showed a significant decline in total soluble sugar content in contrast to corresponding control. The chlorophyll content was expressed as SPAD value. A significant increase in chlorophyll content of both wheat and cucumber seedlings was observed in the BJ-18 + SAP treatment group compared to corresponding control (SPAD value of 59.0 and 46.3, respectively).

Effect of biofertilizer and SAP on soil extracellular enzyme activities

The activities of extracellular enzymes (urease, sucrose, acid phosphate, alkaline phosphate, and catalase) in rhizosphere soil were determined (Fig. 3). In the wheat and cucumber rhizosphere soil, urease activity in the BJ-18 + SAP group was greater than others (Fig. 3A). The BJ-18 + SAP treatment had significant positive effects on sucrose activity of the wheat rhizosphere soil as compared to control (Fig. 3B). However, the L-56 + SAP group showed the lowest sucrose activity in the cucumber rhizosphere soil (Fig. 3B). It was observed that the activity of alkaline phosphatase in different treatments was higher than that of acid phosphatase, and all treatment groups showed no significant difference in both acid and alkaline phosphatase activities in comparison to corresponding control (Fig. 3C and 3D). In the Figure 3E, the dehydrogenase

activity was the lowest in BJ-18 + SAP group of wheat rhizosphere soil, while it was the lowest in the L-56 + SAP group of cucumber rhizosphere soil.

Effect of PGPR and SAP on expression of stress-responsive genes

The qRT-PCR results showed the drought stress-related genes had different expression patterns under drought stress in biofertilizer + SAP treatment group and SAP treatment group (Fig. 4). The transcript levels of *TaCAT*, *TaAPX, TaACO*, *TaDHN*, *TaLEA*, *TaPR1,* and *TaNAC* were down-regulated in wheat treated with BJ-18+SAP and L-56 + SAP as compared to the control.

Variability in expression was also observed in the cucumber seedlings. The transcript levels of *CsCAT, CsAPX, CsACO, CsACS, CsLEA, CsPR1, and CsNAC* were down-regulated in cucumber treated with BJ-18+SAP and L-56 + SAP as compared to the control

DISSCUSSION

Water deficiency suppresses PGPR reproduction of biofertilizer in the arid and semiarid areas of North China, and the application of biofertilizer was limited because no positive effect on crop growth and yield was observed. This study was aimed at minimizing the negative effect of water deficiency on biofertilizer by adding SAP to biofertilizer. The results revealed that synergistic use of biofertilizer and SAP decreased adverse effect of water deficiency on PGPR, and improved the efficiency of biofertilizer.

279 Germination is one of the most vital stages in a crop life cycle, which is significantly impacted
280 by drought stress (Sleimi et al., 2013). Both SAP treatment group and PGPR + SAP treatment
281 group significantly enhanced the germination rate of wheat and cucumber seeds. The use of SAP
282 could improve the water-holding capacity of soil (Johnson, 1984), which is beneficial to seed
283 germination. The application of SAP improved *Caragana korshinskii* seed germination rate (Su et
284 al., 2017). Inoculation with PGPR (*P. fluorescens*, *Enterobacter hormaechei*, and *P. migulae*)
285 could promote foxtail millet seed germination and seedling growth under drought condition (Niu
286 et al., 2018). PGPR of *Burkholderia* sp. L2 and *Bacillus* sp. A30 could increase tomato seed
287 germination (Tripti et al., 2017). In addition, the inoculation of *Pseudomonas*, *Azospirillum,* and
288 *Azotobacter* significantly improve maize seed germination (Gholami, Shahsavani & Nezarat
289 2009). In this study, the SAP application created an optimal environment for PGPR reproduction
290 and seed germination by increasing soil moisture. Therefore, both BJ-18 + SAP and L-56 + SAP
291 groups presented the maximum germination rate of wheat and cucumber.
292 The survival and persistence of PGPR introduced into rhizospher soil is a most vital factor for
293 successful application of biofertilizer (Naqqash et al., 2016). Here, the survival and persistence of
294 inoculants (*P. beijingensis* BJ-18 and *Bacillus* sp. L-56) in the rhizosphere soil was dynamically
295 observed during the period of day 14-44 using qPCR. All groups except control exhibited
296 consistent trend in populations of inoculants, which were increased firstly and declined afterwards.
297 Similar trend has been observed in the strain of *Phomopsis liquidambari* (Wang et al., 2014). In
298 general, the combination use of biofertilizer and SAP significantly increased the bacterial

299 populations compared with corresponding biofertilizer use alone, in both wheat and cucumber
300 rhizosphere soil. Even at day 44, the largest bacterial population was observed in BJ-18 + SAP
301 group of wheat rhizosphere (68.5 copies ng^{-1} gDNA), and in L-56 + SAP group of cucumber
302 rhizosphere (75.0 copies ng^{-1} gDNA). Numerous studies have reported the populations of
303 introduced microorganisms were significantly reduced in the natural soil (Pujol et al. 2006; Kumar,
304 Trivedi & Pandey 2007; Longa et al. 2009) due to the hostility of adverse biotic factor
305 (competition with native organisms for finite nurture) and abiotic factors (temperature, humidity,
306 pH values) within the soil (Avrahami & Bohannan 2007). Our results indicated that adding SAP to
307 biofertilizer could minimize the adverse effect of water deficiency, thus improving the inoculant
308 viability and prolonging persistence. It has been reported that the population density of *Pseudome*
309 *fluorescents* in biochar and/or compost treated soil significantly higher than that in un-treated soil,
310 which is achieved by increasing soil moisture (Nadeem et al., 2017).
311 In wheat and cucumber, both BJ-18 group and L-56 group did not significantly improve plant
312 growth parameters (length, FW, DW) as compared to the control, which was not consistent with
313 the results of previous studies, where *Klebsiella* sp. MBE02, *Paenibacillus* sp. NSY50, *Bacillus,*
314 and *Pseudomonas* sp. MS16 enhanced the growth of peanut, cucumber, rice, wheat and
315 respectively (Sharma, Kulkarni & Jha 2016; Du et al. 2016; Feng et al. 2017; Muhammad et al.
316 2018). However, with the combination use of SAP (BJ-18 + SAP group and L-56 + SAP group),
317 the above parameters (length, FW, DW) were significantly elevated in both wheat and cucumber
318 seedlings as compared to the corresponding control. Particularly, BJ-18 + SAP treatment

319 significantly promoted the root length, root FW, shoot DW and root DW compared with the SAP
320 treatment in wheat, and also increased shoot FW, shoot FW and shoot DW in cucumber; L-56 +
321 SAP treatment significantly promoted the shoot FW, root FW and shoot DW in wheat compared
322 with the SAP treatment, and also increased shoot FW and shoot DW in cucumber. Considering the
323 water-holding capacity of SAP, it was hypothesized that SAP is conductive to creating a more
324 suitable environment for the reproduction of introduced inoculants in soil under drought stress.
325 Such hypothesis was proven by the fact that survival of *P. beijingensis* BJ-18 and *Bacillus* sp. L-56
326 was greater in SAP treated soil as compared to the un-amended soil.
327 After application of biofertilizer amended with SAP in wheat and cucumber, the activities of
328 urease, sucrose and dehydrogenase were significantly increased as compared the control. Urease
329 increased utilization of N fertilizer by catalysing urea hydrolysis into ammonia (Bowles et al.,
330 2014). No matter for wheat or cucumber, the BJ-18 + SAP group showed the strongest urease
331 activity in all treatments, which might be related to the N-fixing ability of *P. beijingensis* BJ-18
332 (Wang et al., 2013). The activity of dehydrogenase, a vital indicator of microorganism activity,
333 was increased after the application of inoculation to soil (Siddikee et al., 2016). Here, the highest
334 activity of dehydrogenase and sucrose was detected in the BJ-18 + SAP group of wheat
335 rhizosphere soil and L-56 + SAP group of cucumber rhizosphere soil, which was consistent with
336 the survival of *P. beijingensis* BJ-18 and *Bacillus* sp. L-56 in the wheat or cucumber rhizosphere
337 soil. These findings were also consistent with previous report (Xun et al., 2015), which
338 demonstrates that inoculation with PGPR could significantly enhance the activities of soil

339 enzymes (urease, dehydrogenase, and sucrose). However, acid phosphatase (alkaline phosphatase)
340 activity showed no significant difference among different treatments, indicating that these two
341 inoculants have little effects on this enzyme. In addition, the activity of alkaline phosphatase in
342 different treatments was higher than that of acid phosphatase, which might be related to the
343 influence of alkaline soil. Similar results have been reported in rice inoculated with *Phomopsis*
344 *liquidambari* under LN level (Siddikee et al., 2016).

345 Under drought stress, the accumulation of proline and total soluble sugar can protect plant cells
346 from osmotic damage (Delauney & Verma, 1993;Gontia-Mishra et al., 2016;Tiwari et al., 2016).
347 Accordingly, a reduction in proline and total soluble sugar content was observed in both wheat and
348 cucumber seedlings in the SAP treatment group compared with control, BJ-18 and L-56
349 treatments. However, wheat and cucumber showed a further decrease in proline and total soluble
350 sugar in the BJ-18 + SAP and L-56 + SAP treatments as compared with SAP treatment group. It
351 was obvious that SAP amendment could help the biofertilizer to perform better by providing a
352 suitable habitat for inoculants in soil, and therefore, less proline and total soluble sugar were
353 accumulated by synergistic use of biofertilizer and SAP. Under drought stress, less proline
354 accumulation of *Caragana korshinskii* grown in SAP amended soil was also reported by (Su et al.,
355 2017). Chlorophyll content is also significant biochemical indicator of stress tolerance in plants
356 (Percival, Fraser & Oxenham 2003). In this study, the BJ-18 + SAP and L-56 + SAP treatments
357 significantly improved the chlorophyll content in the wheat and cucumber seedlings. The
358 application of biochar could also increase the chlorophyll content of *Chenopodium quinoa* by

359 improving soil moisture content (Gorim & Asch, 2017). PGPR-inoculated plants also showed a
360 significant increase in chlorophyll content (Kakar et al., 2016; Abdelkrim et al. 2018; El-Esawi et
361 al. 2018).

362 To better understand how the plants (wheat and cucumber) respond to the different treatments at
363 the transcriptional level, some representative drought-responsive genes were selected. Both *CAT*
364 and *APX* genes were involved in reactive oxygen species (ROS) scavenging, and ROS
365 overproduction resulted in a negative oxidative stress on plant growth. (Lata et al., 2011; Tiwari et
366 al., 2016). qRT-PCR analysis showed that under drought stress, the expression levels of *APX* and
367 *CAT* in seedlings of the biofertilizer + SAP groups were lower than those of other treatment groups,
368 suggesting that biofertilizer + SAP treatments could relieve drought stress and ensure normal
369 growth of seedlings. It was also reported chickpea inoculated with *Pseudomonas* showed
370 significantly lower expression of *CAT* and *APX* during the stress period (Tiwari et al., 2016;Saif &
371 Khan 2018). Ethylene has been reported to regulate some different aspects of plant growth and
372 development, particularly abiotic stressed such as drought stress (Yang, Kloepper & Ryu 2009;
373 Habben et al. 2014). Both *ACO* and *ACS* are involved in ethylene metabolism. In this study, the
374 biofertilizer + SAP treatments significantly repressed the expression levels of *CAT* and *APX*,
375 indicating less ethylene accumulation in plants treated with biofertilizer + SAP. Dehydrin and late
376 embryogenesis abundant protein encoded by *DHN* and *LEA* genes play great roles in adaptive
377 responses of plants to drought stress (Gao et al., 2008). The biofertilizer + SAP groups showed
378 relatively low expression level of *DHN* and *LEA*. There were plenty of evidence for the

379 involvement of salicylic acid in plant drought stress (Fujita et al., 2006). *PR1* participate in
380 salicylic acid metabolism. In this study, the biofertilizer + SAP treatments significantly inhibited
381 the expression of *PR1*. NAC transcription factors have been reported to participate in biotic and
382 abiotic stress tolerance in plants (Delessert et al. 2005; Nakashima, Ito & Yamaguchi-Shinozaki
383 2009;Wu et al., 2009). *TaNAC2D* was induced by abiotic stress (Huang & Wang, 2016) and
384 *CsNAC22* responded to drought stress (Zhang et al., 2017). In this study, the biofertilizer + SAP
385 groups inhibited the expression of *NAC*. Taken together, our results suggest that the biofertilizer +
386 SAP treatments significantly ameliorate drought stress.

387 **CONCLUSIONS**

388 This study showed that the addition of SAP significantly enhanced survival rate of inoculants (*P.*
389 *beijingensis* BJ-18 and *Bacillus* sp. L-56), and then promoted seed germination of wheat and
390 cucumber, plant growth, soil fertility (urease, sucrose and dehydrogenase). qRT-PCR analysis also
391 showed that the transcript levels of some stress-related genes were down-regulated in wheat and
392 cucumber treated with biofertilizer + SAP, respectively, which imply that the biofertilizer + SAP
393 treatments contribute to drought tolerance of wheat and cucumber. Our results indicate that SAP
394 addition in biofertilizer is a good strategy for improving the efficiency of biofertilizer, especially in
395 the areas suffered from long-term drought stress. This study was carried out in the greenhouse,
396 therefore, further experiments are still needed to confirm the effect of biofertilizer amended with
397 SAP in the field.

398

Acknowledgements

We would like to thank Caixia Wang for helping to improve manuscript.

Figure captions

Figure 1: Effects of super absorbent polymer supply on shoot length (A), root length (B), shoot fresh weight (C), root fresh weight (D), shoot dry weight (E) and root dry weight (F) of wheat and cucumber seedlings.

Values are given as mean of three independent biological replicates, and bearing different letters (a, b, c) are significantly different from each other according to the least significant difference (LSD) test ($p<0.05$). The bars represent the standard error. SAP: Super Absorbent Polymer, BJ-18: *P. beijingensis* strain BJ-18, L-56: *Bacillus* sp. strain L-56.

Figure 2： Effects of super absorbent polymer supply on proline concentration (A), total soluble sugar concentration (B), chlorophyll content (C) of wheat and cucumber seedlings.

Values are given as mean of three independent biological replicates, and bearing different letters (a, b, c) are significantly different from each other according to the least significant difference (LSD) test ($p<0.05$). The bars represent the standard error. SAP: Super Absorbent Polymer, BJ-18: P. beijingensis strain BJ-18, L-56: Bacillus sp. strain L-56.

Figure 3: Effects of super absorbent polymer supply on enzyme activities of urease (A), sucrose (B), alkaline phosphatase (C), acid phosphatase (D) and dehydrogenase (E) of rhizosphere soil.

The values are given as mean of three independent biological replicates, and bearing different letters (a, b, c) are significantly different from each other according to the LSD test ($p<0.05$). The

420 bars represent the standard error. SAP: Super Absorbent Polymer, BJ-18: *P. beijingensis* strain BJ-
421 18, L-56: *Bacillus* sp. strain L-56, pNP: *p*-Nitrophenol, TPF: Triphenylformazan.

422 **Figure 4:** Effects of super absorbent polymer supply on relative gene expression level of stress-
423 genes in wheat and cucumber seedlings.
424 The values are given as mean of three independent biological replicates, and bearing different
425 letters (a, b, c) are significantly different from each other according to the least significant
426 difference (LSD) test ($p<0.05$). The bars represent the standard error. SAP: Super Absorbent
427 Polymer, BJ-18: *P. beijingensis* strain BJ-18, L-56: *Bacillus* sp. strain L-56. a: *DHN* for wheat,
428 *ACS* for cucumber.

429 **Table caption**

430 **Table 1:** Bacterial cultures and genomes used in this study.

431 **Table 2:** Description of treatments used for pot experiment.

432 **Table 3:** Primers sequence and accession number in NCBI.

433 **Table 4:** Effects of super absorbent polymer supply on germination of wheat and cucumber seeds.
434 Values are given as mean ± SE of three independent biological replicates, and bearing different
435 letters (a, b, c) at one specific time (e.g. 5 or 6 days) are significantly different from each other
436 according to the least significant difference (LSD) test ($p<0.05$); D: days.

437 **Table 5:** Survival of different bacterial strains inoculated to plants (wheat and cucumber) and
438 population dynamics in the rhizosphere after thinning.

439 Values are given as mean ± SE of three independent biological replicates, and bearing different
440 letters (a, b, c) at one specific time (e.g. 14 or 19 days) are significantly different from each other
441 according to the LSD test ($p<0.05$); D: days; ND: not detected.
442

REFERENCE

Abdelkrim S, Jebara SH, Saadani O, and Jebara M. 2018. Potential of efficient and resistant plant growth-promoting rhizobacteria in lead uptake and plant defence stimulation in *Lathyrus sativus* under lead stress. *Plant Biology* **20:**857-869 DOI 10.1111/plb.12863.

Ankita S, Singh PJ, Sudheer Y, Sarma BK, Singh SJ, Singh HB. 2014. Studies on rhizosphere-bacteria mediated biotic and abiotic stress tolerance in chickpea (*Cicer arietinum* L.). *Vegetos* **27(1)**:158-169 DOI 10.5958/j.2229-4473.27.1.025.

Avrahami S, Bohannan BJ. 2007. Response of *Nitrosospira* sp. strain AF-like ammonia oxidizers to changes in temperature, soil moisture content, and fertilizer concentration. *Applied and Environmental Microbiology* **73(4)**:1166-1173 DOI 10.1128/AEM.01803-06.

Bashan Y, de-Bashan LE, Prabhu SR, Hernandez J-P. 2013. Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998-2013). *Plant and Soil* **378(1-2)**:1-33 DOI 10.1007/s11104-013-1956-x.

Bowles TM, Acosta-Martínez V, Calderón F, Jackson LE. 2014. Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. *Soil Biology and Biochemistry* **68:**252-262 DOI 10.1016/j.soilbio.2013.10.004.

Calvo P, Ormeno-Orrillo E, Martinez-Romero E, Zuniga D. 2010. Characterization of *Bacillus* isolates of potato rhizosphere from andean soils of peru and their potential PGPR characteristics. *Brazilian Journal of Microbiology* **41:**899-906 DOI 10.1590/S1517-83822010000400008.

Chen J, Liu X, Zheng J, Zhang B, Lu H, Chi Z, Pan G, Li L, Zheng J, Zhang X, Wang J, Yu X. 2013a. Biochar soil amendment increased bacterial but decreased fungal gene abundance with shifts in community structure in a slightly acid rice paddy from Southwest China. *Applied Soil Ecology* **71:**33-44 DOI 10.1016/j.apsoil.2013.05.003.

Chen ZJ, Sheng XF, He LY, Huang Z, Zhang WH. 2013b. Effects of root inoculation with bacteria on the growth, Cd uptake and bacterial communities associated with rape grown in Cd-contaminated soil. *Journal of Hazardous Materials* **244-245:**709-717 DOI 10.1016/j.jhazmat.2012.10.063.

Choudhury ATMA, Kecskes ML, Kennedy lR. 2014. Utilization of BNF technology supplementing urea N for sustainable rice production. *Journal of Plant Nutrition* **37:**1627-1647 DOI 10.1080/01904167.2014.888746.

Delauney A, Verma D. 1993. Proline biosynthesis and osmoregulation in plants. *Plant Journal* **4(2)**:215-223.

Delessert C, Kazan K, Wilson IW, Van Der Straeten D, Manners J, Dennis ES, Dolferus R. 2005. The transcription factor *ATAF2* represses the expression of pathogenesis-related genes in *Arabidopsis*. *Plant Journal* **43(5)**:745-757 DOI 10.1111/j.1365-313X.2005.02488.x.

Dong C-J, Li L, Shang Q-M, Liu X-Y, Zhang Z-G. 2014. Endogenous salicylic acid accumulation is required for chilling tolerance in cucumber (*Cucumis sativus* L.) seedlings. *Planta* **240(4)**:687-700 DOI 10.1007/s00425-014-2115-1.

Dorraji SS, Golchin A, Ahmadi S. 2010. The effects of hydrophilic polymer and soil salinity on corn growth in sandy and loamy soils. *CLEAN - Soil, Air, Water*:**38(7):**584-591 DOI 10.1002/clen.201000017.

Du N, Shi L, Yuan Y, Li B, Shu S, Sun J, Guo S. 2016. Proteomic analysis reveals the positive roles of the plant-growth-promoting *Rhizobacterium* NSY50 in the response of cucumber roots to *Fusarium oxysporum* f. sp. cucumerinum inoculation. *Frontiers in Plant Science* **7:**1859 DOI 10.3389/fpls.2016.01859.

El-Esawi MA, Alaraidh IA, Alsahli AA, Alamri SA, Ali HM, and Alayafi AA. 2018. *Bacillus firmus* (SW5) augments salt tolerance in soybean (*Glycine max* L.) by modulating root system architecture, antioxidant defense systems and stress-responsive genes expression. *Plant physiology and biochemistry* **132:**375-384. DOI 10.1016/j.plaphy.2018.09.026.

Feng F, Ge J, Li Y, He S, Zhong J, Liu X, Yu X. 2017. Enhanced degradation of chlorpyrifos in rice (*Oryza sativa* L.) by five strains of endophytic bacteria and their plant growth promotional ability. *Chemosphere* **184:**505-513 DOI 10.1016/j.chemosphere.2017.05.178.

Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki K. 2006. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. *Current Opinion in Plant Biology* **9(4)**:436-442 DOI 10.1016/j.pbi.2006.05.014.

Gao WR, Wang XS, Liu QY, Peng H, Chen C, Li JG, Zhang JS, Hu SN, Ma H. 2008. Comparative analysis of ESTs in response to drought stress in chickpea (*C. arietinum* L.). *Biochemical and Biophysical Research Communications* **376(3)**:578-583 DOI 10.1016/j.bbrc.2008.09.030.

505 **Gholami A, Shahsavani S, Nezarat S. 2009.** The effect of plant growth promoting rhizobacteria
506 (PGPR) on germination, seedling growth and yield of maize. *Proceedings of World*
507 *Academy of Science Engineering & Technolog* **49:**19**.**

508 **Gontia-Mishra I, Sapre S, Sharma A, Tiwari S. 2016.** Amelioration of drought tolerance in
509 wheat by the interaction of plant growth-promoting rhizobacteria. *Plant Biology (Stuttg)*
510 **18(6)**:992-1000 DOI 10.1111/plb.12505.

511 **Gorim L, Asch F. 2017.** Seed coating increases seed moisture uptake and restricts embryonic
512 oxygen availability in germinating cereal seeds. *Biology (Basel)* **6(2)** DOI
513 10.3390/biology6020031.

514 **Gray D. 2011.** Fluid drilling of vegetable seeds. *Horticultural Reviews* **3:**1-27 DOI
515 10.1099/ijs.0.013649-0.

516 **Grady EN, MacDonald J, Liu L, Richman A, Yuan ZC, 2016.** Current knowledge and
517 perspectives of *Paenibacillus:* a review. *Microbial Cell Factories* **15(1)**: 203 DOI:
518 10.1186/s12934-016-0603-7.

519 **Guerrero-Molina MF, Lovaisa NC, Salazar SM, Martinez-Zamora MG, Diaz-Ricci JC,**
520 **Pedraza RO. 2015.** Physiological, structural and molecular traits activated in strawberry
521 plants after inoculation with the plant growth-promoting bacterium *Azospirillum brasilense*
522 REC3. *Plant Biology (Stuttg)* **17(3)**:766-773 DOI 10.1111/plb.12270.

523 **Habben JE, Bao X, Bate NJ, DeBruin JL, Dolan D, Hasegawa D, Helentjaris TG, Lafitte RH,**
524 **Lovan N, Mo H, Reimann K, and Schussler JR. 2014.** Transgenic alteration of ethylene
525 biosynthesis increases grain yield in maize under field drought-stress conditions. *Plant*
526 *Biotechnology Journal* DOI 12:685-693. 10.1111/pbi.12172.

527 **Hao T, Chen S. 2017.** Colonization of wheat, maize and cucumber by *Paenibacillus polymyxa*
528 WLY78. *PLoS One* **12(1)**:e0169980 DOI 10.1371/journal.pone.0169980.

529 **Hestrin S, Goldblum J. 1953.** Lævanpolyas. *Nature* **172:**1046-1047.

530 **Huang Q, Wang Y. 2016.** Overexpression of TaNAC2D Displays opposite responses to abiotic
531 stresses between seedling and mature stage of transgenic *Arabidopsis*. *Frontiers in Plant*
532 *Science* **7** DOI 10.3389/fpls.2016.01754.

533 **Islam MR, Eneji AE, Changzhong R, Yuegao H, Gong C, Xuzhang X. 2010.** Oat-
534 basedcropping system for sustainable agricultural development in aridregions of northern
535 China. *Journal of Agriculture, Biotechnology & Ecology* **3(3)**:1-8.

Islam MR, Sultana T, Joe MM, Yim W, Cho JC, Sa T. 2013. Nitrogen-fixing bacteria with multiple plant growth-promoting activities enhance growth of tomato and red pepper. *Journal of Basic Microbiology* **53(12)**:1004-1015 DOI 10.1002/jobm.201200141.

Johnson MS. 1984. The effects of gel-forming polyacrylamides on moisture storage in sandy soils. *Journal Science Food Agriculture* **35(11)**:1196-1200.

Johnson MS, Veltkamp CJ. 1985. Structure and functioning of water-storing agricultural polyacrylamides. *Journal Science Food Agriculture* **36:**789-793.

Kakar KU, Ren XL, Nawaz Z, Cui ZQ, Li B, Xie GL, Hassan MA, Ali E, Sun GC. 2016. A consortium of rhizobacterial strains and biochemical growth elicitors improve cold and drought stress tolerance in rice (*Oryza sativa* L.). *Plant Biology (Stuttg)* **18(3)**:471-483 DOI 10.1111/plb.12427.

Kandeler E, Kampichler C, Horak O. 1996. Influence of heavy metals on the functional diversity of soil microbial communities. *Biology and Fertility of Soils* **23(3)**:299-306 DOI 10.1007/BF00335958.

Kaur T, Brar BS, Dhillon NS. 2007. Soil organic matter dynamics as affected by long-term use of organic and inorganic fertilizers under maize–wheat cropping system. *Nutrient Cycling in Agroecosystems* **81(1)**:59-69 DOI 10.1007/s10705-007-9152-0.

Khan MS, Zaidi A, Ahemad M, Oves M, Wani PA. 2010. Plant growth promotion by phosphate solubilizing fungi-current perspective. *Archives of Agronomy and Soil Science* **56(1)**:73-98 DOI 10.1080/03650340902806469.

Kumar B, Trivedi P, Pandey A. 2007. *Pseudomonas corrugata*: A suitable bacterial inoculant for maize grown under rainfed conditions of Himalayan region. *Soil Biology and Biochemistry* **39(12)**:3093-3100 DOI 10.1016/j.soilbio.2007.07.003.

Lata C, Jha S, Dixit V, Sreenivasulu N, Prasad M. 2011. Differential antioxidative responses to dehydration-induced oxidative stress in core set of foxtail millet cultivars [*Setaria italica* (L.)]. *Protoplasma* **248:**817-828 DOI 1007/s00709-010-0257-y).

Lebrun JD, Trinsoutrot-Gattin I, Vinceslas-Akpa M, Bailleul C, Brault A, Mougin C, Laval K. 2012. Assessing impacts of copper on soil enzyme activities in regard to their natural spatiotemporal variation under long-term different land uses. *Soil Biology and Biochemistry* **49:**150-156 DOI 10.1016/j.soilbio.2012.02.027.

Li N, Zhang S, Liang YJ, Qi YH, Chen J, Zhu WN, Zhang LS. 2018. Label-free quantitative proteomic analysis of drought stress-responsive late embryogenesis abundant proteins in the

seedling leaves of two wheat (*Triticum aestivum* L.) genotypes. *Journal of Proteomics* **172:**122-142 DOI 10.1016/j.jprot.2017.09.016.

Li Y, Liu X, Hao T, Chen S. 2017. Colonization and maize growth promotion induced by phosphate solubilizing bacterial isolates. *International Journal of Molecular Sciences* **18(7)** DOI 10.3390/ijms18071253.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{(-\text{Delta Delta C(T)})}$ Method. *Methods* **25(4)**:402-408 DOI 10.1006/meth.2001.1262.

Longa CM, Savazzini F, Tosi S, Elad Y, Pertot I. 2009. Evaluating the survival and environmental fate of the biocontrol agent *Trichoderma atroviride* SC1 in vineyards in northern Italy. *Journal of Applied Microbiology* **106(5)**:1549-1557 DOI 10.1111/j.1365-2672.2008.04117.x.

Meyer JA, Garber ED, Shaeffer SG. 1964. Genetics of phytopathogenic fungi. XII. Detection of esterases and phosphatases in culture filtrates of *Fusarium oxysporum* and *F. xylarioides* by starch-gel zone electrophoresis. *International Journal of Plant Sciences* **125(4)**:298-300

Mikkelsen RL. 1994. Using hydrophilic polymers to control nutrient release. *Nutrient Cycling in Agroecosystems* **38(1)**:53-59.

Moloudi F, Navabpour S, S H, R SS, S H. 2013. *Catalase* and *Metallothionein* genes expression analysis in wheat cultivars under drought stress condition. *JPMB* **1:**54-68 DOI 10.22058/JPMB.2013.3262.

Muhammad S, Sumera Y, Maria R, Mahreen Y, Babar MA, Muhammad SM. 2018. Phosphate solubilizing bacteria with glucose dehydrogenase gene for phosphorus uptake and beneficial effects on wheat *PloS One* **13(9):**e0204408 DOI10.1371/journal.pone.0204408

Nadeem SM, Imran M, Naveed M, Khan MY, Ahmad M, Zahir ZA, Crowley DE. 2017. Synergistic use of biochar, compost and plant growth-promoting rhizobacteria for enhancing cucumber growth under water deficit conditions. *Journal of the Science of Food and Agriculture* DOI 10.1002/jsfa.8393.

Nakashima K, Ito Y, Yamaguchi-Shinozaki K. 2009. Transcriptional regulatory networks in response to abiotic stresses in *Arabidopsis* and Grasses. *Plant Physiology* **149(1)**:88-95 DOI 10.1104/pp.108.129791.

Naqqash T, Hameed S, Imran A, Hanif MK, Majeed A, van Elsas JD. 2016. Differential response of potato toward inoculation with taxonomically diverse plant growth promoting rhizobacteria. *Frontiers of Plant Science* **7:**144 DOI 10.3389/fpls.2016.00144.

Naresh PS, Pramod KP, Sandeep KV. 2014. Morpho-physiological characterization of Indian wheat genotypes and their evaluation under drought condition. *African journal of Biotechnology* **13(20)**:2022-2027 DOI 10.5897/ajb2013.13486.

Naureen Z, Rehman NU, Hussain H, Hussain J, Gilani SA, Al Housni SK, Mabood F, Khan AL, Farooq S, Abbas G, Harrasi AA. 2017. Exploring the potentials of *Lysinibacillus sphaericus* ZA9 for plant growth promotion and biocontrol activities against phytopathogenic fungi. *Frontiers in Microbiology* **8:**1477 DOI 10.3389/fmicb.2017.01477.

Naveed M, Hussain MB, Zahir ZA, Mitter B, Sessitsch A. 2013. Drought stress amelioration in wheat through inoculation with *Burkholderia phytofirmans* strain PsJN. *Plant Growth Regulation* **73(2)**:121-131 DOI 10.1007/s10725-013-9874-8.

Niu XG, Song LC, Xiao YN, and Ge WD. 2018. Drought-tolerant plant growth-promoting rhizobacteria associated with foxtail millet in a semi-arid and their potential in alleviating drought stress. *Frontiers in Microbiology* **8:**11 DOI 10.3389/fmicb.2017.02580

Oteino N, Lally RD, Kiwanuka S, Lloyd A, Ryan D, Germaine KJ, Dowling DN. 2015. Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Frontiers in Microbiology* **6:**745 DOI 10.3389/fmicb.2015.00745.

Pandey PK, Yadav SK, Singh A, Sarma BK, Mishra A, Singh HB. 2012. Cross-species alleviation of biotic and abiotic stresses by the endophyte *Pseudomonas aeruginosa* PW09. *Journal of Phytopathology* **160(10)**:532-539 DOI 10.1111/j.1439-0434.2012.01941.x.

Percival GC, Fraser GA, Oxenham G. 2003. Foliar salt tolerance of *Acer* genotypes using chlorophyll fluorescence. *Journal of Arboriculture* **29(3)**.

Pujol M, Badosa E, Manceau C, Montesinos E. 2006. Assessment of the environmental fate of the biological control agent of fire blight, *Pseudomonas fluorescens* EPS62e, on apple by culture and real-time PCR methods. *Applied and Environmental Microbiology* **72(4)**:2421-2427 DOI 10.1128/AEM.72.4.2421-2427.2006.

Qi PF, Johnston A, Balcerzak M, Rocheleau H, Harris LJ, Long XY, Wei YM, Zheng YL, Ouellet T. 2012. Effect of salicylic acid on *Fusarium graminearum*, the major causal agent of fusarium head blight in wheat. *Fungal Biology* **116(3)**:413-426 DOI 10.1016/j.funbio.2012.01.001.

Rajkumar M, Ae N, Prasad MNV, Freitas H. 2010. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends in Biotechnol* **28:**142-149 DOI 10.1016/j.tibtech.2009.12.002.

Rehman A, Ahmad R, Safdar M. 2011. Effect of hydrogel on the performance of aerobic rice sown under different techniques. *Plant Soil and Environment* **57(7)**:321-325.

Saif S, and Khan MS. 2018. Assessment of toxic impact of metals on proline, antioxidant enzymes, and biological characteristics of *Pseudomonas aeruginosa* inoculated *Cicer arietinum* grown in chromium and nickel-stressed sandy clay loam soils. *Environmental Monitoring and Assessment* **190** DOI 10.1007/s10661-018-6652-0

Sandhya V, Ali SZ, Venkateswarlu B, Reddy G, and Grover M. 2010. Effect of osmotic stress on plant growth promoting *Pseudomonas* spp. *Archives of Microbiology* **192:**867-876 DOI 10.1007/s00203-010-0613-5

Savazzini F, Longa CM, Pertot I, Gessler C. 2008. Real-time PCR for detection and quantification of the biocontrol agent *Trichoderma atroviride* strain SC1 in soil. *Journal of Microbiological Methods* **73(2)**:185-194 DOI 10.1016/j.mimet.2008.02.004.

Schinner F, Ohlinger R, Kandeler E, Margesin R. 1996. *Mtthods in soil biology.* Berlin: Springer-Verlag.

Shakeel M, Rais A, Hassan MN, Hafeez FY. 2015. Root associated *Bacillus* sp. improves growth, yield and zinc translocation for basmati rice (*Oryza sativa*) varieties. *Frontiers in Microbiology* **6:**1286 DOI 10.3389/fmicb.2015.01286.

Sharma A, Gontia I, Agarwal PK, Jha B. 2010. Accumulation of heavy metals and its biochemical responses in*Salicornia brachiata*, an extreme halophyte. *Marine Biology Research* **6(5)**:511-518 DOI 10.1080/17451000903434064.

Sharma S, Kulkarni J, Jha B. 2016. Halotolerant rhizobacteria promote growth and enhance salinity tolerance in peanut. *Frontiers in Microbiology* **7:**1600 DOI 10.3389/fmicb.2016.01600.

Shukla PS, Agarwal PK, Jha B. 2012. Improved salinity tolerance of *Arachis hypogaea* (L.) by the interaction of halotolerant plant-growth-promoting rhizobacteria. *Journal of Plant Growth Regulation* **31(2)**:195-206 DOI 10.1007/s00344-011-9231-y.

Siddikee MA, ereen MIZ, LI C, Dai C. 2016. Endophytic fungus *Phomopsis liquidambari* and different doses of N-fertilizer alter microbial community structure and function in rhizosphere of rice. *Scientific Reports* **6:**32270 DOI 0.1038/srep32270.

662 **Simonsen AK, Han S, Rekret P, Rentschler CS, Heath KD, Stinchcombe JR. 2015.** Short-term
663 fertilizer application alters phenotypic traits of symbiotic nitrogen fixing bacteria. *PeerJ*
664 **3:**e1291 DOI 10.7717/peerj.1291.

665 **Sleimi N, Bankaji I, Touchan H, Corbineau F. 2013.** Effects of temperature and water stresses
666 on germination of some varieties of chickpea (*Cicer arietinum*). *African Journal of*
667 *Biotechnology* **12:**2201-2206

668 **Sojka RE, Entry JA, Fuhrmann JJ. 2006.** The influence of high application rates of
669 polyacrylamide on microbial metabolic potential in an agricultural soil. *Applied Soil*
670 *Ecology* **32(2)**:243-252 DOI 10.1016/j.apsoil.2005.06.007.

671 **Su LQ, Li JG, Xue H, Wang XF. 2017.** Super absorbent polymer seed coatings promote seed
672 germination and seedling growth of *Caragana korshinskii* in drought. *Journal of Zhejiang*
673 *University Science B (Biomedicine & Biotechnology)* **18(8)**:696-706 DOI
674 10.1631/jzus.B1600350.

675 **Sudisha J, Niranjana SR, Sukanya SL, Girijamba R, Lakshmi Devi N, Shekar Shetty H.**
676 **2010.** Relative efficacy of strobilurin formulations in the control of downy mildew of
677 sunflower. *Journal of Pest Science* **83(4)**:461-470 DOI 10.1007/s10340-010-0316-3.

678 **Tabatabai M, Bremner J. 1969.** Use of p-nitrophenyl phosphate for assay of soil phosphatase
679 activity. *Soil Biology and Biochemistry* **1(4)**:201-207 DOI 10.1016/0038-0717(69)90012-1.

680 **Terry, Richard E, Nelson, Sheldon D. 1986.** Efficts of polyacrylamide and irrigation method on
681 soil physical properties. *Soil Science* **141(5)**:317-320 DOI 10.1007/bf00008338.

682 **Tiwari S, Lata C, Chauhan PS, Nautiyal CS. 2016.** *Pseudomonas putida* attunes
683 morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during
684 drought stress and recovery. *Plant Physiology and Biochemistry* **99:**108-117 DOI
685 10.1016/j.plaphy.2015.11.001.

686 **Tripti, Kumar A, Usmani Z, Kumar V, Anshumali. 2017.** Biochar and flyash inoculated with
687 plant growth promoting rhizobacteria act as potential biofertilizer for luxuriant growth and
688 yield of tomato plant. *Journal of Environmental Management* **190:**20-27 DOI
689 10.1016/j.jenvman.2016.11.060.

690 **Vrieze MD, Pandey P, Bucheli TD, Varadarajan AR, Ahrens CH, Weisskopf L, Bailly A.**
691 **2015.** Volatile organic compounds from native potato-associated *Pseudomonasas* potential
692 anti-oomycete agents. *Frontiers in Microbiology* **6(e1003311)** DOI
693 10.3389/fmicb.2015.01295.

694 **Wan H, Zhao Z, Qian C, Sui Y, Malik AA, Chen J. 2010.** Selection of appropriate reference
695 genes for gene expression studies by quantitative real-time polymerase chain reaction in
696 cucumber. *Analytical Biochemistry* **399(2)**:257-261 DOI 10.1016/j.ab.2009.12.008.

697 **Wang HW, Dai CC, Zhu H, Wang XX. 2014.** Survival of a novel endophytic fungus *Phomopsis*
698 *liquidambari* B3 in the indole-contaminated soil detected by real-time PCR and its effects on
699 the indigenous microbial community. *Microbiology Research* **169(12)**:881-887 DOI
700 10.1016/j.micres.2014.05.006.

701 **Wang LY, Li J, Li QX, Chen SF. 2013.** *Paenibacillus beijingensis* sp. nov., a nitrogen-fixing
702 species isolated from wheat rhizosphere soil. *Antonie Van Leeuwenhoek* **104(5)**:675-683
703 DOI 10.1007/s10482-013-9974-5.

704 **Wang Z, Xu G, Ma P, Lin Y, Yang X, Cao C. 2017.** Isolation and characterization of a
705 phosphorus-solubilizing bacterium from rhizosphere soils and its colonization of chinese
706 cabbage (*Brassica campestris* ssp. chinensis). *Frontiers in Microbiology* **8:**1270 DOI
707 10.3389/fmicb.2017.01270.

708 **Webb J, Harrison R, Ellis S. 2000.** Nitrogen fluxes in three arablesoils in the UK. *European*
709 *Journal of Agronomy* **13:**207-223.

710 **Wei L, Deng X-G, Zhu T, Zheng T, Li P-X, Wu J-Q, Zhang D-W, Lin H-H. 2015.** Ethylene is
711 involved in brassinosteroids induced alternative respiratory pathway in cucumber (*Cucumis*
712 *sativus* L.) seedlings response to abiotic stress. *Frontiers in Plant Science* **6(982)** DOI
713 10.3389/fpls.2015.00982.

714 **Wu YR, Deng ZY, Lai JB, Zhang YY, Yang CP, Yin BJ, Zhao QZ, Zhang L, Li Y, Yang CW,**
715 **Xie Q. 2009.** Dual function of Arabidopsis ATAF1 in abiotic and biotic stress responses.
716 *Cell Research* **19(11)**:1279-1290 DOI 10.1038/cr.2009.108.

717 **Xun F, Xie B, Liu S, Guo C. 2015.** Effect of plant growth-promoting bacteria (PGPR) and
718 arbuscular mycorrhizal fungi (AMF) inoculation on oats in saline-alkali soil contaminated
719 by petroleum to enhance phytoremediation. *Environmental Science & Pollution Research*
720 **22(1)**:598-608 DOI 10.1007/s11356-014-3396-4.

721 **Yang B, Wang X-M, Ma H-Y, Jia Y, Li X, Dai C-C. 2013.** Effects of the fungal endophyte
722 *Phomopsis liquidambari* on nitrogen uptake and metabolism in rice. *Plant Growth*
723 *Regulation* **73(2)**:165-179 DOI 10.1007/s10725-013-9878-4.

724 **Yang J, Kloepper JW, Ryu CM. 2009.** Rhizosphere bacteria help plants tolerate abiotic stress.
725 *Trends in Plant Science* **14(1)**:1-4 DOI 10.1016/j.tplants.2008.10.004.

Zhang J, Liu J, Meng L, Ma Z, Tang X, Cao Y, Sun L. 2012. Isolation and characterization of plant growth-promoting rhizobacteria from wheat roots by wheat germ agglutinin labeled with fluorescein isothiocyanate. *Journal of Microbiology* **50(2)**:191-198 DOI 10.1007/s12275-012-1472-3.

Zhang XM, Yu HJ, Sun C, Deng J, Zhang X, Liu P, Li YY, Li Q, Jiang WJ. 2017. Genome-wide characterization and expression profiling of the NAC genes under abiotic stresses in Cucumis sativus. *Plant Physiology and Biochemistry* **113(**98-109 DOI 10.1016/j.plaphy.2017.01.023.

Zhou Y, He P, Xu Y, Liu Q, Yang Y, Liu S. 2017. Overexpression of *CsLEA11*, a Y3SK2-type dehydrin gene from cucumber (*Cucumis sativus*), enhances tolerance to heat and cold in *Escherichia coli*. *Amb Express* **7** DOI 10.1186/s13568-017-0483-1.

Zhu X, Qi L, Liu X, Cai S, Xu H, Huang R, Li J, Wei X, Zhang Z. 2014. The wheat ethylene response factor transcription factor pathogen-induced *ERF1* mediates host responses to both the necrotrophic pathogen rhizoctonia cerealis and freezing stresses. *Plant Physiology* **164(3)**:1499-1514.

Figure 1

Effects of super absorbent polymer supply on shoot length (A), root length (B), shoot fresh weight (C), root fresh weight (D), shoot dry weight (E) and root dry weight (F) of wheat and cucumber seedlings.

Values are given as mean of three independent biological replicates, and bearing different letters (a, b, c) are significantly different from each other according to the least significant difference (LSD) test ($p<0.05$). The bars represent the standard error. SAP: Super Absorbent Polymer, BJ-18: *P. beijingensis* strain BJ-18, L-56: *Bacillus* sp. strain L-56.

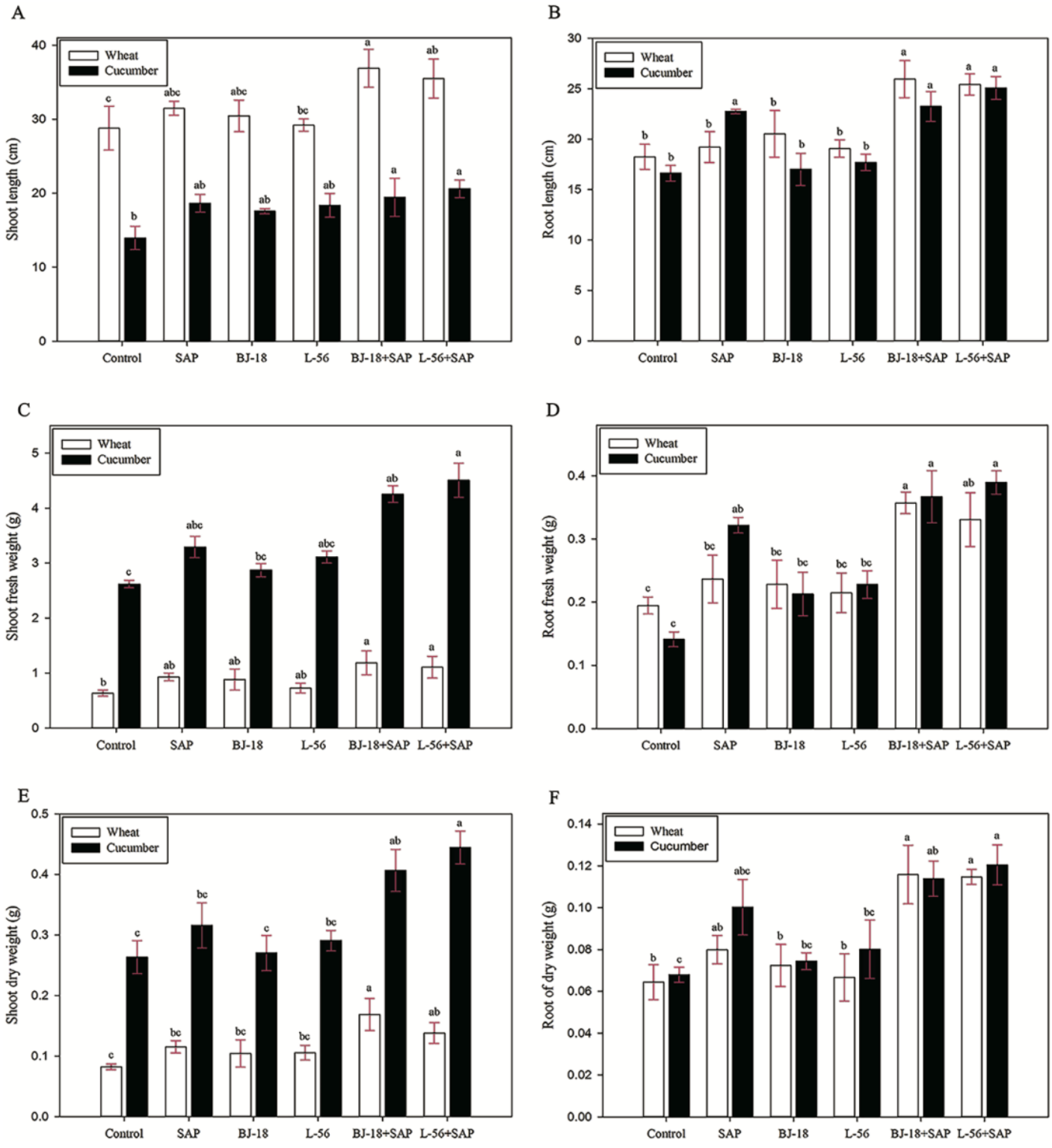
A
Shoot length (cm)
Wheat
Cucumber
Control
SAP
BJ-18
L-56
BJ-18+SAP
L-56+SAP
B
Root length (cm)
C
Shoot fresh weight (g)
D
Root fresh weight (g)
E
Shoot dry weight (g)
F
Root of dry weight (g)

Figure 2

Effects of super absorbent polymer supply on proline concentration (A), total soluble sugar concentration (B), chlorophyll content (C) of wheat and cucumber seedlings.

Values are given as mean of three independent biological replicates, and bearing different letters (a, b, c) are significantly different from each other according to the least significant difference (LSD) test ($p<0.05$). The bars represent the standard error. SAP: Super Absorbent Polymer, BJ-18: P. beijingensis strain BJ-18, L-56: Bacillus sp. strain L-56.

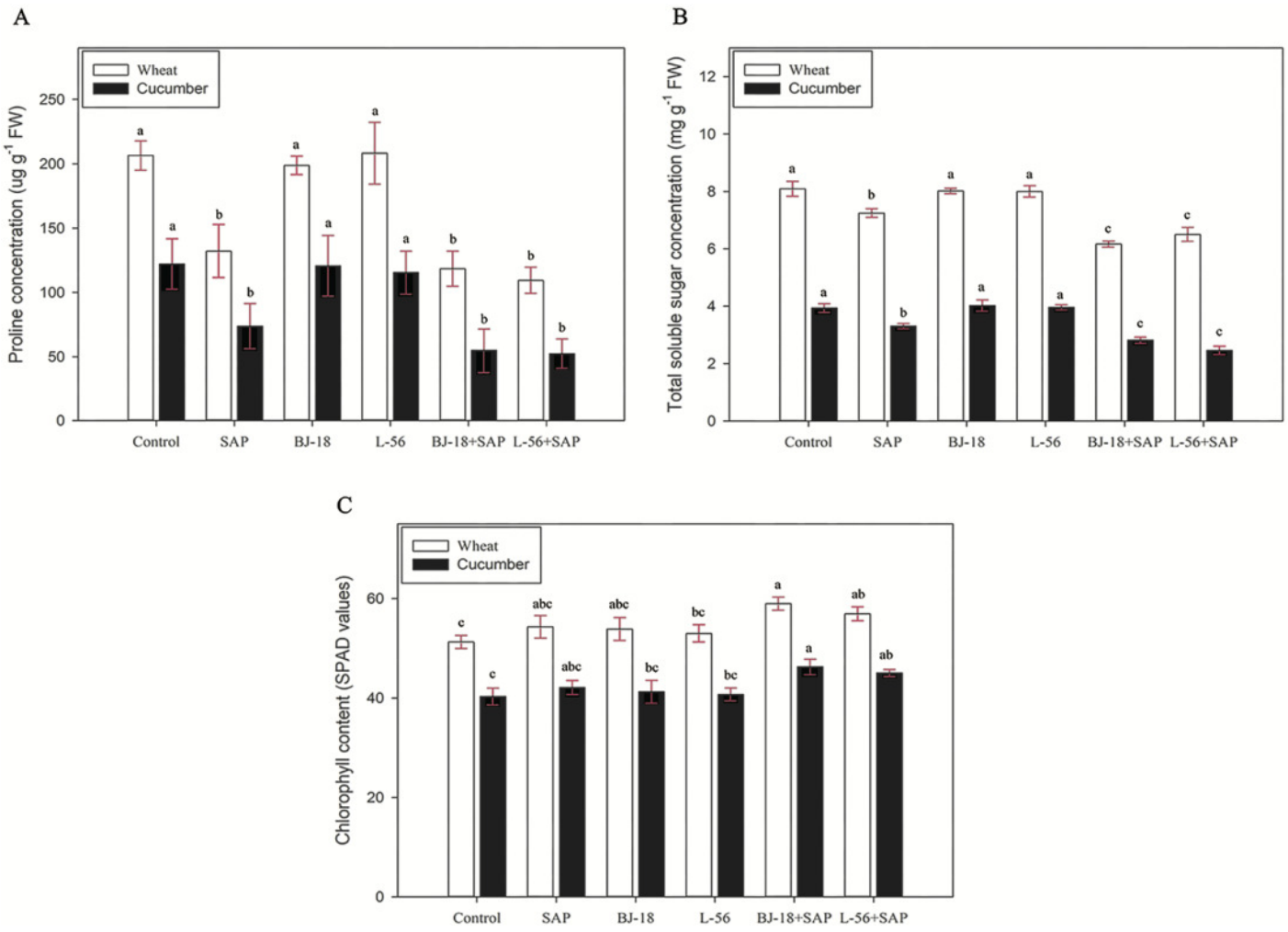

Figure 3

Effects of super absorbent polymer supply on enzyme activities of urease (A), sucrose (B), alkaline phosphatase (C), acid phosphatase (D) and dehydrogenase (E) of rhizosphere soil.

The values are given as mean of three independent biological replicates, and bearing different letters (a, b, c) are significantly different from each other according to the LSD test ($p<0.05$). The bars represent the standard error. SAP: Super Absorbent Polymer, BJ-18: *P. beijingensis* strain BJ-18, L-56: *Bacillus* sp. strain L-56, pNP: *p*-Nitrophenol, TPF: Triphenylformazan.

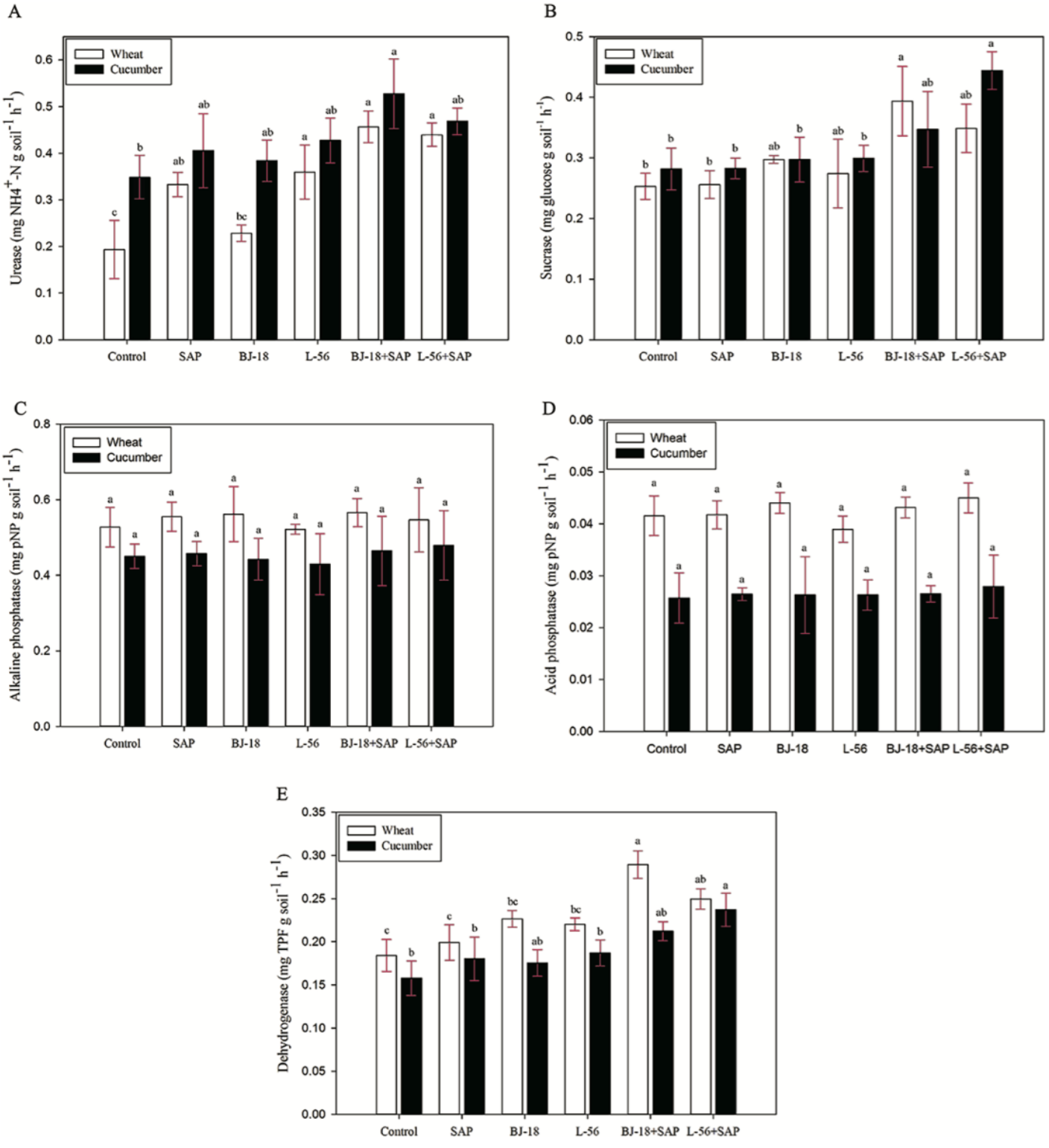
A
Urease (mg NH4+-N g soil-1 h-1)
Wheat
Cucumber
Control
SAP
BJ-18
L-56
BJ-18+SAP
L-56+SAP
B
Sucrase (mg glucose g soil-1 h-1)
C
Alkaline phosphatase (mg pNP g soil-1 h-1)
D
Acid phosphatase (mg pNP g soil-1 h-1)
E
Dehydrogenase (mg TPF g soil-1 h-1)

Figure 4

Effects of super absorbent polymer supply on relative gene expression level of stress-genes in wheat and cucumber seedlings.

The values are given as mean of three independent biological replicates, and bearing different letters (a, b, c) are significantly different from each other according to the least significant difference (LSD) test ($p<0.05$). The bars represent the standard error. SAP: Super Absorbent Polymer, BJ-18: *P. beijingensis* strain BJ-18, L-56: *Bacillus* sp. strain L-56.. a: *DHN* for wheat, *ACS* for cucumber.

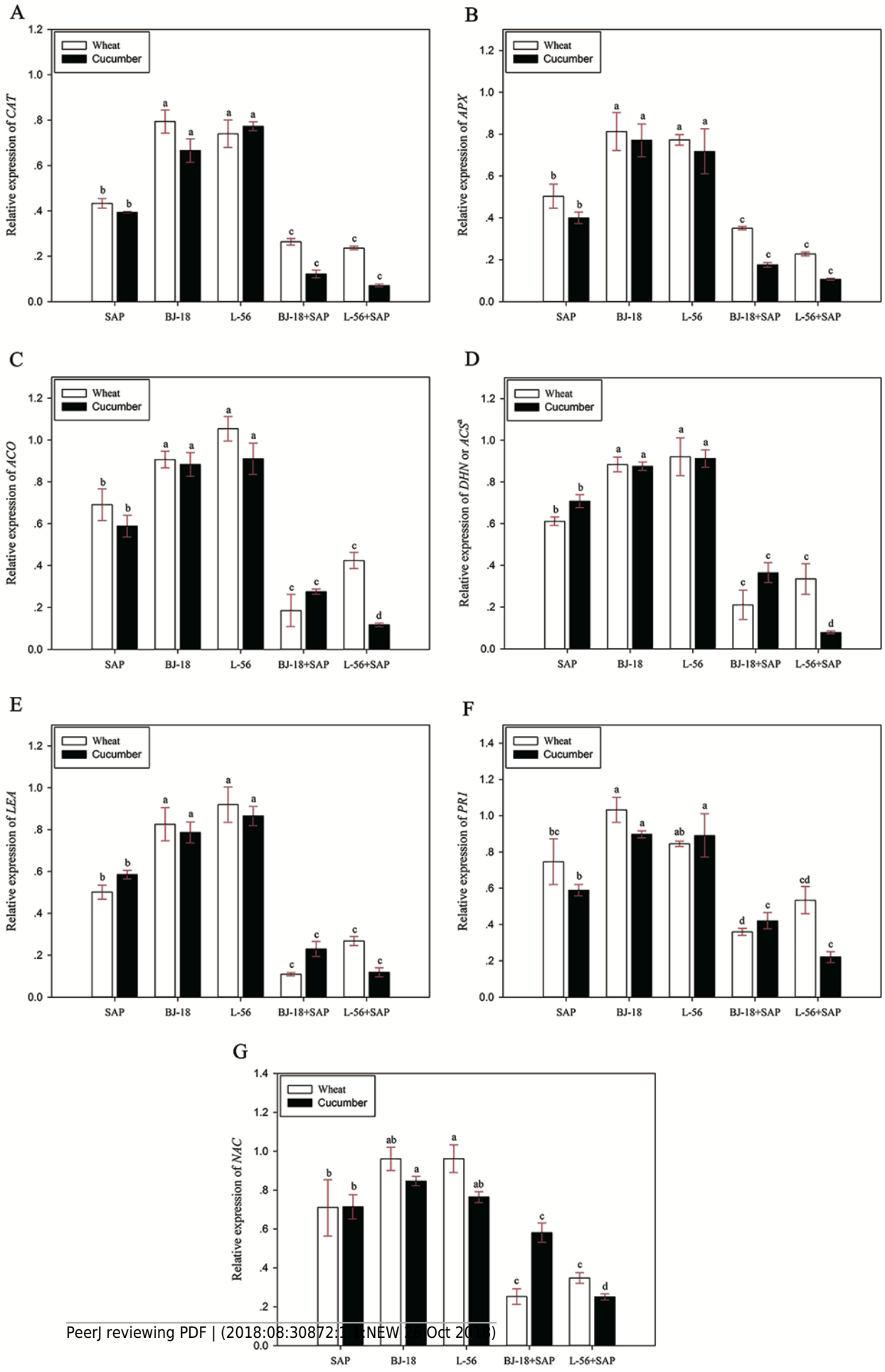
A
Relative expression of CAT
B
Relative expression of APX
C
Relative expression of ACO
D
Relative expression of DHN or ACS
E
Relative expression of LEA
F
Relative expression of PR1
G
Relative expression of NAC
Wheat
Cucumber
SAP
BJ-18
L-56
BJ-18+SAP
L-56+SAP

Table 1(on next page)

Bacterial cultures and genomes used in this study.

1 **Table 1 Bacterial cultures and genomes used in this study.**

Strain No.	Original Source	ID	References/Accession No.
BJ-18	Wheat (rhizosphere isolate), China.	*Paenibacillus beijingensis*	(Wang et al., 2013) JN873136
L-56	Maize (rhizosphere isolate), China	*Bacillus* sp.	MF988362

2
3

Table 2(on next page)

Description of treatments used for pot experiment.

1 **Table 2 Description of treatments used for pot experiment.**

Treatments	Description
Control	Rice hull powder without biofertilizer and SAP
SAP	Rice hull powder + SAP (100:1, w/w)
BJ-18	Biofertilizer of *P. beijingensis* BJ-18
L-56	Biofertilizer of *Bacillus* sp. L-56
BJ-18 +SAP	Biofertilizer of *P. beijingensis* BJ-18 + SAP (100:1, w/w)
L-56+SAP	Biofertilizer of *Bacillus* sp. L-56 + SAP (100:1, w/w)

2

3

Table 3(on next page)

Primers sequence and accession number in NCBI.

1 **Table 3 Primers sequence and accession number in NCBI.**

Primer	Primer sequence 5'-3'	NCBI Accession No.	Reference
nifB	GAAGGTGAGAGTGAGGATGG TTGCTTCAGGCTCATCTCC	MH202771	This study
amye	CTTCTCGTTCAGGCAGTAC TATTGACCGCAGTGATAGC	MH202772	This study
TaCAT	CCATCTGGCTCTCCTACTGG AGAACTTGGACGACGGCCCTGA	E16461	(Moloudi et al., 2013)
CsCAT	TTCGGCGTACAAGCATATTCC GTGGCGTGACTGTGATTCG	AY274258	This study
TaAPX	CACCACATCTAAGGGACATC TTCAGAGGGTCACGAGTC	EF555121	This study
CsAPX	ATGATTGTTGACTTGTTATGTG GATGAGGCAGAACGAACC	EU798448	This study
TaNAC2D	ACCTCAGCTACGACGACATCCAG GCGGCGAAGAAGTCATCCGTTCC	GQ231954.1	(Huang and Wang, 2016)
CsNAC22	GACCTCGGTCTCGTCTGAAG CTACAATGTTGTTGGACTTCGG	---	(Zhang et al., 2017)
TaLEA	AGATCGACGGTGACGTGAAG GTCCATGATCTTGCCCAGTAG	---	(Li et al., 2018)
CsLEA11	CGAGCAGTTCCAGCTCTA CTTCCGGTTAACTTCTCCTT	---	(Zhou et al., 2017)
TaACO2	GAGGAACGAGGGCGAGGAG TCAGTTATCAGGCGGTGGC	---	(Zhu et al., 2014)
CsACO1	ACCTTCTTCTTACGCCATC GCCACCTACCTTGTCATC	AB006806	(Wei et al., 2015)
CsACS1	CTTCAGGCGTTATTCAGATG GTGCTCGTGTTCTCCATT	U59813.1	(Wei et al., 2015)
TaDHN3	TGGGACGGGCTCAGTGCT ATGGGCGGGAGGAGGAAG	---	(Zhu et al., 2014)
TaPR1-1a	TTCATCATCTGCAGCTACAACC CGGTACATATATACAGCCGGTCTAA	---	(Qi et al., 2012)
CsPR1-1a	AACTCTGGCGGACCTTAC TCAATATGGCCTTTGGTATAAG	DQ641122	(Dong et al., 2014)
TaACTIN	GTCGGTGAAGGGGACTTACA TTCATACAGCAGGCAAGCAC	AB181991.1	(Huang and Wang, 2016)
CsACTIN	AGAGATGGCTGGAATAGAAC CTGGTGATGGTGTGAGTC	DQ115883	(Wan et al., 2010)

2

Table 4(on next page)

Effects of super absorbent polymer supply on germination of wheat and cucumber seeds.

Values are given as mean ± SE of three independent biological replicates, and bearing different letters (a, b, c) at one specific time (e.g. 5 or 6 days) are significantly different from each other according to the least significant difference (LSD) test ($p<0.05$); D: days.

1 **Table 4 Effects of super absorbent polymer supply on germination of wheat and cucumber seeds.**

Plant	Treatments	Seed germination (%) at different day									
		5 D	6 D	7 D	8 D	9 D	10 D	11 D	12 D	13 D	14 D
Wheat	Control	0^{a}	0^{a}	0^{b}	0^{c}	0^{d}	0^{c}	6.3 ± 1.2^{d}	9.7 ± 0.3^{d}	18.0 ± 1.0^{e}	22.7 ± 1.3^{e}
	SAP	0^{a}	0^{a}	0^{b}	0^{c}	5.7 ± 1.2^{c}	12.3 ± 0.9^{b}	24.3 ± 1.5^{b}	37.7 ± 1.8^{b}	48.0 ± 2.3^{c}	52.3 ± 3.0^{c}
	BJ-18	0^{a}	0^{a}	0^{b}	0^{c}	0^{d}	1.0 ± 1.0^{c}	7.7 ± 0.7^{c}	17.7 ± 1.2^{c}	29.0 ± 1.2^{d}	33.0 ± 0.6^{d}
	L-56	0^{a}	0^{a}	0^{b}	0^{c}	0^{d}	1.0 ± 1.0^{c}	7.7 ± 0.7^{cd}	13.0 ± 1.7^{cd}	22.7 ± 1.4^{de}	29.7 ± 1.3^{d}
	BJ-18 +SAP	0^{a}	0^{a}	8.7 ± 1.3^{a}	14.7 ± 2.1^{a}	20.3 ± 2.0^{b}	46.0 ± 2.5^{a}	50.7 ± 1.8^{a}	56.3 ± 2.3^{a}	67.7 ± 1.8^{a}	70.0 ± 1.0^{a}
	L-56+SAP	0^{a}	0^{a}	6.7 ± 2.0^{a}	17.3 ± 2.5^{b}	24.7 ± 1.2^{a}	44.3 ± 2.4^{a}	52.3 ± 2.2^{a}	57.7 ± 7.8^{a}	60.7 ± 1.4^{b}	62.0 ± 2.1^{b}
Cucumber	Control	0^{a}	0^{a}	0^{a}	0^{b}	0^{c}	0^{c}	0^{d}	4.3 ± 0.8^{c}	6.3 ± 0.7^{d}	14.3 ± 0.9^{c}
	SAP	0^{a}	0^{a}	0^{a}	0^{b}	0^{c}	13.3 ± 2.0^{b}	17.3 ± 2.2^{b}	21.7 ± 3.9^{b}	31.3 ± 4.1^{b}	39.3 ± 3.3^{b}
	BJ-18	0^{a}	0^{a}	0^{a}	0^{b}	0^{c}	1.0 ± 1.0^{c}	5.3 ± 1.2^{c}	8.7 ± 1.2^{c}	12.0 ± 1.5^{cd}	19.3 ± 1.2^{c}
	L-56	0^{a}	0^{a}	0^{a}	0^{b}	0^{c}	0^{c}	4.3 ± 0.9^{cd}	7.3 ± 2.0^{c}	13.7 ± 1.2^{c}	20.3 ± 1.3^{c}
	BJ-18 +SAP	0^{a}	0^{a}	0^{a}	8.3 ± 2.1^{a}	10.0 ± 1.0^{b}	27.7 ± 3.8^{a}	35.0 ± 1.5^{a}	37.7 ± 2.2^{a}	44.7 ± 2.0^{a}	51.3 ± 1.8^{a}
	L-56+SAP	0^{a}	0^{a}	0^{a}	8.3 ± 3.1^{a}	13.0 ± 1.0^{a}	24.3 ± 2.3^{a}	32.7 ± 2.0^{a}	41.7 ± 2.6^{a}	49.7 ± 2.8^{a}	56.0 ± 3.2^{a}

2

Values are given as mean ± SE of three independent biological replicates, and bearing different letters (a, b, c) at one specific time (e.g. 5 or 6 day) are significantly different from each other according to the least significant difference (LSD) test ($p<0.05$), D: day, SAP: Super Absorbent Polymer, BJ-18: *P. beijingensis* strain BJ-18, L-56: *Bacillus* sp. Strain L-56.

Table 5(on next page)

Survival of different bacterial strains inoculated to plants (wheat and cucumber) and population dynamics in the rhizosphere after thinning.

Values are given as mean ± SE of three independent biological replicates, and bearing different letters (a, b, c) at one specific time (e.g. 14 or 19 days) are significantly different from each other according to the LSD test ($p<0.05$); D: days; ND: not detected.

Table 5 Survival of different bacterial strains inoculated to wheat and cucumber and population dynamics in the rhizosphere after thinning.

Plant	Treatments	Inoculant concentration in the rhizospher (copies ng^{-1} gDNA)						
		14 D	19 D	24 D	29 D	34 D	39 D	44 D
Wheat	BJ-18	123.9±8.1^{b}	142.0±4.0^{d}	140.0±1.0^{d}	124.2±3.9^{d}	96.1±3.2^{c}	37.1±1.6^{c}	19.3±0.2^{b}
	L-56	198.9±20.0^{a}	214.5±0.9^{b}	200.2±1.4^{b}	180.7±5.8^{b}	130.3±1.4^{b}	48.6±3.7^{c}	17.2±0.2^{b}
	BJ-18 +SAP	121.2±1.6^{b}	157.7±3.7^{c}	173.1±3.2^{c}	264.4±14.7^{c}	184.2±4.6^{a}	109.8±7.9^{a}	68.5±4.3^{a}
	L-56+SAP	210.2±1.9^{a}	236.6±2.4^{a}	254.4±1.0^{a}	237.7±2.0^{a}	182.9±2.1^{a}	140.3±1.4^{a}	61.9±6.7^{a}
Cucumber	BJ-18	110.7±3.3^{c}	129.1±1.5^{d}	127.2±4.9^{c}	116.3±12.1^{d}	83.6±5.4^{c}	53.8±3.9^{c}	9.7±0.6^{d}
	L-56	184.0±12.9^{b}	195.2±1.8^{b}	156.8±5.2^{b}	144.5±5.7^{c}	99.8±10.4^{c}	45.1±2.9^{c}	18.4±0.3^{c}
	BJ-18 +SAP	116.4±2.6^{c}	154.8±8.4^{c}	161.7±3.2^{b}	188.0±6.5^{b}	158.7±10.6^{b}	94.5±2.6^{b}	54.0±2.6^{b}
	L-56+SAP	213.0±3.9^{a}	240.9±3.2^{a}	317.2±2.1^{a}	331.7±2.5^{a}	240.9±11.8^{a}	113.5±6.0^{a}	75.0±2.8a

Values are given as mean ± SE of three independent biological replicates, and bearing different letters (a, b, c) at one specific time (e.g. 14 or 19 days) are significantly different from each other according to the least significant difference (LSD) test ($p<0.05$), D: day, SAP: Super Absorbent Polymer, BJ-18: *P. beijingensis* strain BJ-18, L-56: *Bacillus* sp. strain L-56.