Pak. J. Bot., 49(6): 2095-2101, 2017.

MODULATION EFFECT OF INOCULATED *RAOULTELLA PLANTICOLA* ON GLYCINEBETAINE METABOLISM IN TWO MAIZE (*ZEA MAYS* L.) CULTIVARS DIFFERING IN DROUGHT TOLERANCE

GAILI NIU[1], NAHEEDA BEGUM[1], WEI GOU[1], PENG ZHENG[1], CHENG QIN[1], LIXIN ZHANG[1*] AND ABD EI-FATAH ABOMOHRA[2]

[1]College of Life Sciences, Northwest A&F University, Yangling 712100, P. R. China
[2]Botany Department, Faculty of Science, Tanta University, 31527 Tanta, Egypt
[*]Correspondence author's email: zhanglixin@nwsuaf.edu.cn; Ph: +0086-29-87092262

Abstract

A comprehensive investigation was carried out to evaluate the changes in seed germination and glycine betaine (GB) synthesis in drought stressed maize (*Zea mays* L.) in response to *Raoultella planticola* treatment. Two maize cultivars were used in our experiment, Zheng Dan 958 (drought tolerant) and Jun Dan 20 (drought sensitive). Under drought stress, seed germination of both cultivars was drastically inhibited. However, germination rate increased, and germination period was shortened with with *R. planticola* Radicle length and plumules were improved, while no significant effects on hypocotyl length. Maize seedlings inoculated with *R. planticola* increased the level of biosynthetic pathways of glycine betaine (GB) and choline (Cho). The activities of some key enzymes involved in GB synthesis, such as phosphoethanolamine *N*-methyltransferase (PEAMT), choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH), were enhanced resulting in net increase in GB content. *R. planticola* treatment showed a marked expression of *CMO*, *BADH* and *PEAMT*. In conclusion, *R. planticola* inoculation reduced the negative effects of drought stress by regulating GB synthesis in the seedlings of both maize cultivars under drought stress.

Key word: Drought tolerance, Glycine betaine, Plant-microbe interactions, *Zea mays* L., Rhizobacteria, Seed development.

Introduction

Maize (*Zea mays* L.) is a stable and economical crop in China because of a suitable soil and climatic conditions. Drought, chilling and other abiotic stresses are increasing challenges as they increasingly cause considerable yield loss of many crops (Lesk *et al*., 2016). As an example, the northeast farming area of China is popular for maize production because 30% of maize production is attained from this area. However, as a result of climate change, several regions of China suffer the serious problem of water resource shortage, which has an adverse effect on maize yield (Li and Wang 2003; Xie *et al*., 2009).

Drought stress considerably affects the biological processes at molecular, cellular and entire plant levels, which in turn badly affect plant growth (Rahdari *et al*., 2012). Among various mechanisms of drought resistance, the key is cellular and molecular processes, because they lead to water and metabolism homeostasis at the whole-plant level under environmental stresses. On the other hand, when the stress-sensitive plants are grown in environmental cues, they are vulnerable because of the rapid changes of their cells and tissues (Bohnert *et al*., 1995). Based on the previous investigations on maize, cv. Zheng Dan 958 (ZD) has been categorized as a drought-tolerant cultivar, while as cv. Jun Dan 20 (JD) as drought-sensitive one (Zhang *et al*., 2015). It is essential to develop some effective means to improve water use efficiency of crops to to achieve optimum crop production (Ghanbari *et al*., 2007).

Plant growth-promoting rhizobacteria (PGPRs) have positive effects on plant tolerance to poor growth environment (Han *et al*., 2017; Kataoka *et al*., 2017; Timmusk *et al*., 2014). They are contemplated as environment-friendly organisms for yield improvement (Simova-Stoilova *et al*., 2008; Yang *et al*., 2009). These useful microbes inhabit the rooting zone of plants and promote plant growth by regulating a variety of physiological processes (Grover *et al*., 2011). In plants, several metabolic processes are regulated by PGPRs such as variation in enzymatic activities (Penrose and Glick, 2003), dissolution of mineral matter (Panhwar *et al*., 2014), azotification (Soares *et al*., 2006), and decreased ethylene concentration (Cheng *et al*., 2007). *Raoultella planticola*, one of potential PGPRs, is likely to enhance the accumulation of osmoprotectants in plants under stressful cues (Lam & Salit, 2014; Gou *et al*., 2015).

Plants accumulate/synthesize different types of compatible solutes/osmolytes like betaines, proline, and sugars under stressful conditions (Kaya *et al*., 2007; Hoseini, 2010; Osakabe *et al*., 2014). Choline has an energetic part in plant tolerance to in plant tolerance to an abiotic stress particularly by elevating the synthesis of glycine betaine (GB) (Zhu & Zeisel, 2009; Zhang *et al*., 2010). In plants, GB synthesis pathways includes two oxidation reactions catalyzed by choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH), and the intermediate is GB aldehyde. CMO plays a vital role in the first step in which choline is translated into GB aldehyde, and the second oxidation step is catalyzed by BADH, which further gives rise GB (Yu *et al*., 2014). When plants grow at stressful environment, their cells can accumulate a large amount of GB (Ahmad *et al*., 2013).

In our previous study, *Raoultella planticola* was isolated (Zheng *et al*., 2014), and it was suggested as a PGPR. Most of PGPRs may be able to alleviate abiotic stresses (Lam & Salit, 2014; Gou *et al*., 2015). Therefore, we presume that this microorganism may have the function of regulating the GB synthesis for improving plant resistance to stress. To prove the speculation, the relationship between GB synthesis and *R. planticola* was investigated at transcriptional, phenotypic and physiological levels under drought stress.

Materials and Methods

Plant and bacterial material: *R. planticola* (Genebank ID: KJ465991) cells were suspended in 5 ml of sterilized Dworkin-Foster (DF) liquid medium, and incubated at shaking condition (200 r/min, 28°C). Then bacterial suspension (1.0 ml) was added to 50 mL fresh DF medium. The mixture was placed in a shaking incubator (28 °C) until the absorbance of it was 0.5 at 600 nm. The bacteria were collected by centrifugation at 4°C (8000 *g*, 10 min). After that, the cells (precipitate part) were suspended using ultrapure water. The suspension (109 CFU ml^{-1}) was then ready for inoculation (Dworkin & Foster, 1958; Zheng *et al*., 2014).

Uniform healthy seeds of cv. ZD 958 and cv. JD 20 were sterilized by 75% ethanol solution (3 min) and then transferred to the solution of 2% sodium hypochlorite (NaOCl) for 2 min (Zhang *et al*., 2015). After rinsing, the seeds were inoculated with the bacterial suspension (109 CFU ml^{-1}) as the experimental group and non-inoculated as the control. Both groups were replicated 3 times.

Seed germination and seedling growth: Seeds of cv. ZD 958 and cv. JD 20 were sown in a germination box. Seeds showing the radical emergence of 2 mm were considered as germinated ones. Polyethylene glycol-6000 (10 mL per box, 15%) was used to impart drought stress and control was treated with equal amount of DW. Following Gholami *et al.,* (2009) the lengths of radicle, hypocotyl and plumule were measured at the first leaf stage. Thereafter, the seeds were selected randomly from each box and grown in half strength of Hoagland's nutrient solution (Hoagland & Arnon, 1950), which were placed in a controlled growth chamber at 25°C/18°C and 9/15 h (night/day). The seedlings were grown in 15% PEG-6000 solution, simulating drought stress, at the stage of three-leaf. The leaves were harvested after 4 h for the next experiment.

Choline and GB content: The contents of GB and choline were measured following the protocol of Gou *et al.* (2015). GB and choline were extracted from plant samples by water-methanol-chloroform-(3:12:5, v/v/v) and placed into a water bath for 20 min at 60°C. The upper methanol phase was collected by centrifugation (5000 *g*, 10 min), which was concentrated to 0.2 ml by vacuum centrifugal concentration meter and then diluted with water to 1.5mL. It was than purified using an ion exchange resin (Bio-Rad AG1-X8). After filtration through a membrane filter (0.45μm), the supernatant was analyzed by a HPLC equipped with a UV detector and the mobile phase was acetonitrile/water (95:5, v/v). Choline and GB contents were determined at 230 nm, which was quantified by comparing every peak surface areas (2.5 min and 3.5 min).

Phosphoethanolamine *N*-methyltransferase (PEAMT) activity: It was detected by the Methyltransferase Colorimetric Assay Kit (Cayman Chemical Company, USA) following the specifications of the Company.

Choline monooxygenase (CMO) activity: The crude protein was extracted by precipitation with ammonium sulfate. The CMO activity was assayed following the protocol described elsewhere (Burnet *et al.,* 1995; Landfald *et al.*, 1986). 3 ml reaction volume contained contained 0.125 mol L^{-1} sucrose, 0.05 mol.L^{-1} EDTA-Na_2, 40 mmol.L^{-1} PBS (pH 7.4), 10 mmol.L^{-1} choline chloride, 10 mg phenazine dimethyl sulfate (PMS) and 0.1 mL of crude enyme extract. The reaction was initiated by cytochrome C (100 mg) at 25°C. The CMO activity was determined by absorption spectroscopy (WFZ UV-2800A, China) at 549 nm. One unit of enzyme activity was expressed as an increase by 1.0 of OD549.

Betaine aldehyde dehydrogenase (BADH) activity: Leaf samples (0.5 g) were homogenized with extraction buffer (10 mL), which contained tricine-KOH (100 mmol L^{-1}, pH 8.5), EDTA (2 mmol L^{-1}), sucrose (0.6 mol L^{-1}), and DTT (2.0 mmol L^{-1}). Then it was centrifuged for 10 min (10000 *g*, 4°C). The supernatant was used for the BADH activity assay. The reaction mixture comprised 100 mmol L^{-1}Tris-HCl (pH 8.0), 0.5 mmol L^{-1} NAD^+, 5.0 mmol L^{-1} DTT and crude enzyme extract (0.5 mL). Finally, the reaction was initiated by adding 0.05 mL betaine aldehyde (10 mmol L^{-1}). The BADH activity was determined spectrophotometrically at 340 nm based on NADH production. The activity of one-unit enzyme was expressed as the release of 1 mmol of NADH per second (Arakawa *et al.*, 1990).

RNA extraction and cDNA synthesis: Total RNA of plant samples were extracted by RNease mini-kit (Qiagen, Valencia, USA). The absorbance at 260/280 nm was measured by NanoDrop 2000 Ultraviolet-Visible Spectrophotometer (Thermo, USA) for detecting the total RNA concentration. cDNAs were synthesized by MuMLV-RT (Fisher Scientific, Houston, USA) according to the prescribed specifications. The quality of cDNA was examined using one percent agarose gel electrophoresis.

Primer designing and real-time PCR analysis: All primers were designed by Primer Premier 5.0 (Table 1). Analysis of relative gene expression was conducted by Bio-Rad iQ5 Sequence Detection System (Bio-Rad Company, USA) using SYBR green PCR master mix (Applied Biosystems, Foster City, CA, USA). PCR reactions using 50 μl were started at 95 °C (5 min) and then entered into 40 cycles of 95°C (10 s), 56°C (30s), 72°C (20 s). The relative expression of target gene was evaluated and analyzed using the method of $2^{-\Delta\Delta Ct}$ (Livak *et al*., 2001).

Statistical analysis: The experimental data were analyzed with SPSS 20.0 and the significant differences between all of treatments determined with variance (ANOVA) at probability level (P)<0.05. The results were presented as means ± standard errors (SE).

Table 1. Primer sequences of real-time quantitative PCR.

GenBank ID	Gene	PCR product size	Primer sequence (5´-3´)
NM_001154731	*β-Actin*	264bp	F: AACTGCCCAGCAATGTATG R: CATCAGGTTGTCGGTAAGGT
EU957102	*BADH*	168bp	F: GCCTTGGCTGCTGGGTGTA R: ATGGGAATGTGAGGATAATGGAG
DQ864498.1	*CMO*	231bp	F: GATTGGATGGCACCCTT R: TATGCGGCAGTGAAGTGT
AY626156.3	*PEAMT*	228bp	F: CAAAGACATACCCAGACCA R: ATCTGTCCATAAGCCTCC

Table 2. The germination of two maize cultivars inoculated with *Raoultella planticola.*

Treatment		Germination rate (Day)	Germination percentage (%)	Radicle Length (cm)	Hypocotyl length (cm)	Plumule length (cm)
ZD	CK	2.75 ± 0.03c	87.5 ± 0.42a	9.35 ± 0.04b	0.55 ± 0.02a	8.48 ± 0.07b
	CK+I	2.50 ± 0.06d	87.0 ± 0.50a	11.4 ± 0.37a	0.63 ± 0.03a	9.60 ± 0.08a
	PEG	3.75 ± 0.04a	82.0 ± 0.60b	7.75 ± 0.21c	0.45 ± 0.02b	6.90 ± 0.16d
	PEG+I	3.25 ± 0.03b	86.0 ± 0.23a	10.5 ± 0.37a	0.58 ± 0.03a	8.13 ± 0.09c
JD	CK	2.75 ± 0.05c	87.0 ± 0.16a	9.23 ± 0.06c	0.53 ± 0.02a	8.40 ± 0.08b
	CK+I	2.75 ± 0.06c	85.0 ± 0.22b	11.3 ± 0.34a	0.60 ± 0.02a	9.25 ± 0.04a
	PEG	4.25 ± 0.02a	73.5 ± 0.13d	7.05 ± 0.07d	0.43 ± 0.03b	5.53 ± 0.05d
	PEG+I	3.50 ± 0.06b	82.5 ± 0.32c	10.5 ± 0.06b	0.55 ± 0.02a	6.88 ± 0.16c

PEG and CK represent the drought and control treatments, respectively, while I represents inoculation. Different letters in the table above indicate significant difference between all the treatments at $p<0.05$. Data were presented as treatment means ± SE ($n=3$)

Results

Germination responses: It was evident that both maize cultivars showed drastic inhibition in seed germination under dry condition induced by PEG. However, *R. planticola* inoculation increased the germination rate under drought stress, which showed insignificant difference with that of the control. In addition, seeds treated with inoculum showed shorter germination period in comparison with that of non-inoculated ones, particularly for cv. JD. On the other hand, inoculum treatments increased the lengths of radicles and plumules of both cultivars under stress conditions. For the hypocotyl length of both maize cultivars with *R. planticola*, did not show a significant effect (Table 2).

Contents of choline and GB: *R. planticola* inoculation significantly influenced the contents of choline and GB under drought stress (Fig. 1). Drought stress significantly increased the choline content in cv. ZD and JD (Fig. 1A). In addition, a significant increase in GB content was recorded in both cv. ZD and cv. JD under drought stress (Fig. 1B). The inoculum treatment showed a greater increase in choline and GB levels in both cultivars, especially for choline content. The corresponding data for cv. JD were greater for choline accumulation (1.32 times) and GB accumulation (1.0 time). Under drought stress, cv. ZD accumulated more choline and GB than did cv. JD after the same inoculation conditions.

Enzymes involved in GB metabolism: In the leaves of both cultivars, the activities of PEAMT, CMO and BADH were found to be increased due to the inoculation of plant roots under drought stress conditions, particularly for the activities of CMO and BADH (Fig. 2). As for PEAMT activity, cv. ZD treated with *R. planticola* showed greater increase by 22.9% over the control at day 4 (Fig. 2A). In addition, inoculation enhanced CMO activity in cv. ZD by 22% and 29% over the control at days 2 and 4, respectively. Likewise, inoculation of cv. JD enhanced CMO by 31% and 47% over the control at days 2 and 4, respectively (Fig. 2B). Moreover, inoculation significantly enhanced BADH activity in cv. ZD and cv. JD, however, insignificant difference was recorded in BADH activity in inoculated and non-inoculated cv. ZD at day 4 (Fig. 2C).

Relative Expression of PEAMT, CMO and BADH: *R. planticola* inoculation under drought stress conditions significantly increased the expression levels of *PEAMT*, *CMO* and *BADH* (Fig. 3). Inoculation treatment increased *PEAMT* transcript level in cv. ZD and cv. JD by 39% and 47%, respectively, over the control after 4 days (Fig. 3A). In addition, *CMO* expression was increased by 23% and 61% over the control in cv. ZD and cv. JD, respectively, after 4 days of inoculation (Fig. 3B). In addition, *BADH* transcript levels in the inoculated cv. ZD and cv. JD were enhanced by 48% and 32%, respectively, over the control (Fig. 3C).

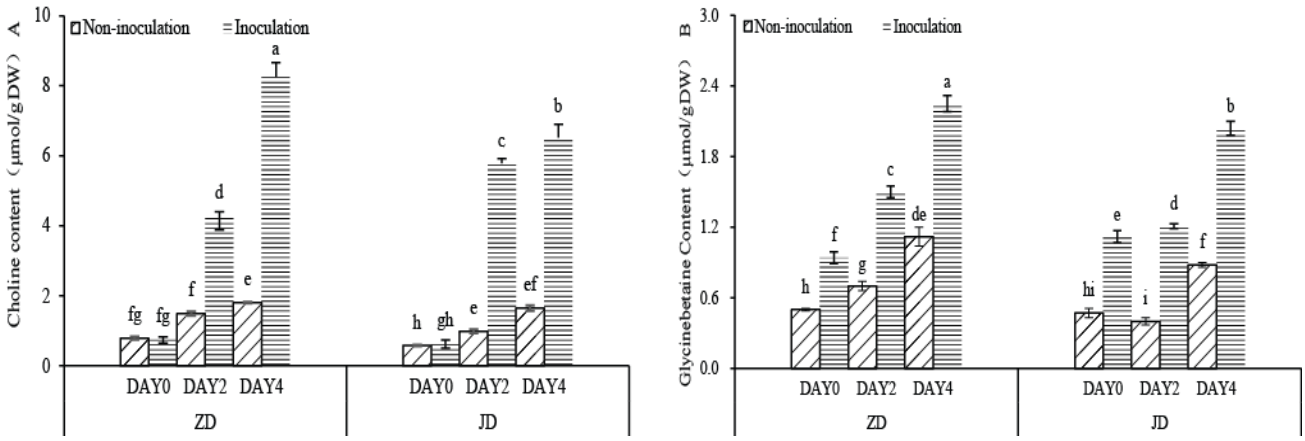

Fig. 1. Contents of choline (A) and GB (B) in ZD and JD inoculated with *R. planticola* under drought stress.
The drought stress lasted for 4 days and each cultivar was observed on day 2 and day 4. Different letters in the figure above indicate significant difference between all the treatments at $p<0.05$ and the differences between the inoculation treatments under drought stress were compared by ANOVA. Data presented as treatment means ± SE ($n = 3$), similarly hereinafter. Ni

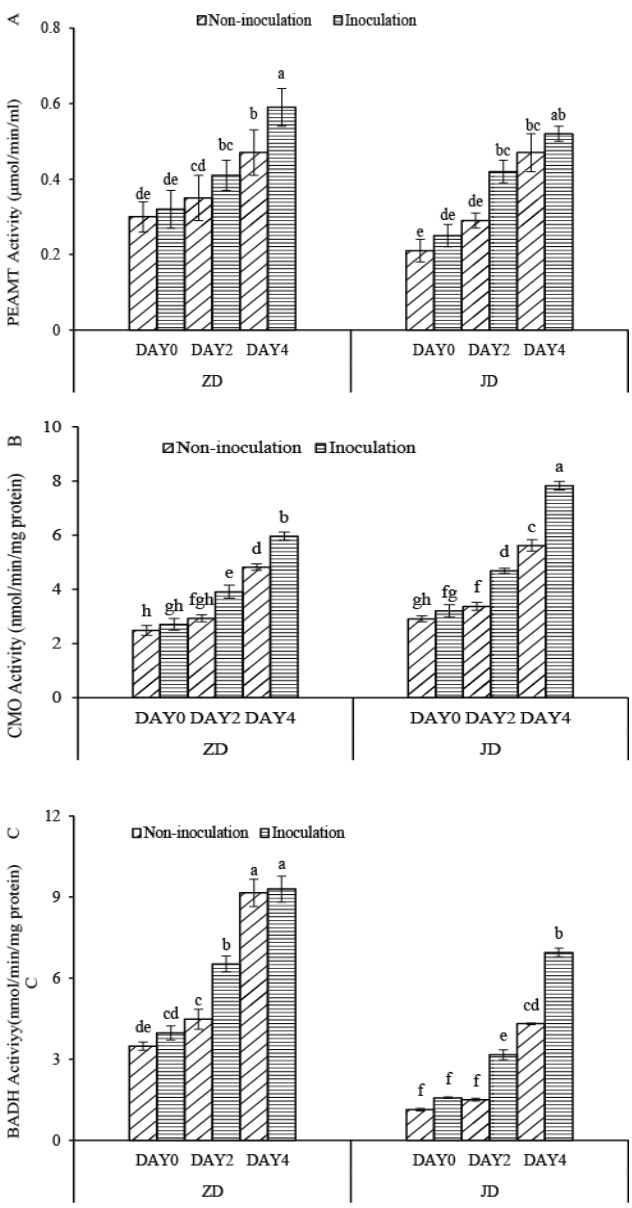

Fig. 2. Activities of PEAMT (A), CMO (B) and BADH (C) in ZD and JD inoculated with *R. planticola* under drought stress.

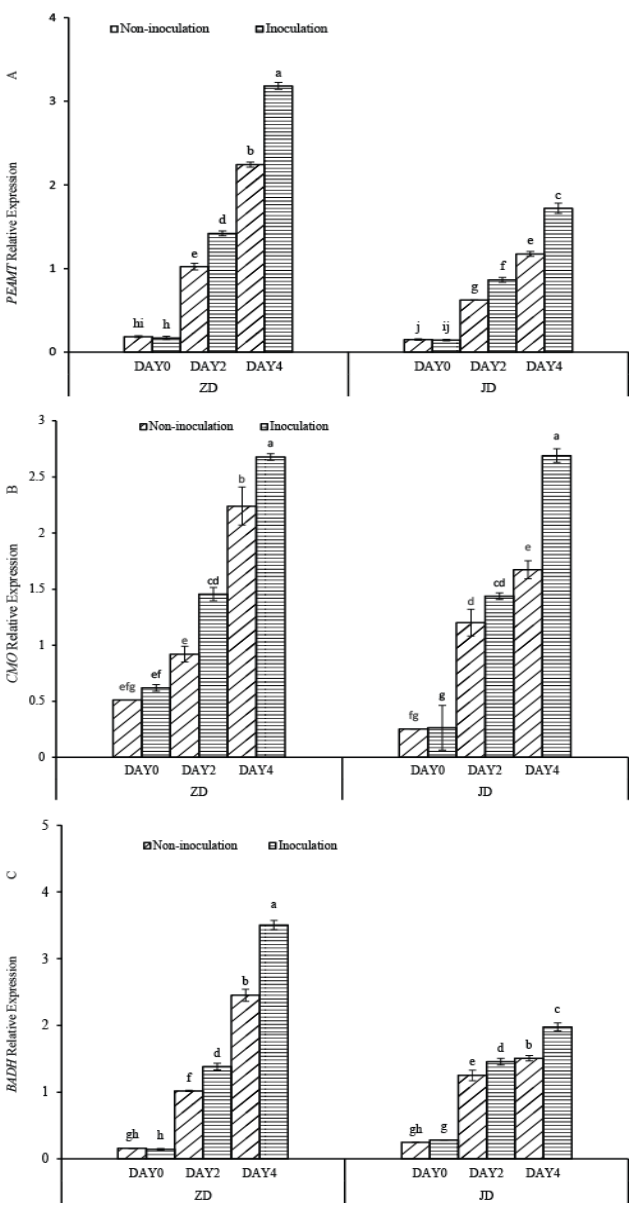

Fig. 3. Relative transcript levels of *PEAMT* (A), *CMO* (B) and *BADH* (C) in ZD and JD inoculated with *R. planticola* under drought stress.

Discussion

It is widely reported that bacterial inoculation could promote seed germination and plant growth under adverse environmental conditions (Sreedhar *et al.*, 2014). The present results showed that drought stress drastically suppressed seed germination of both maize cultivars, which was alleviated by *R. planticola* inoculation. In plants, choline and GB, act as key osmoprotectants resulting in alleviation of adverse effects of drought stress by osmotic adjustment (Fan *et al.*, 2016). Hanson & Nelsen (1978) showed that modification in membrane function may result from the flux of sodium from cytoplasm to vacuole induced by the increase in GB contents under water stress. However, some of the plants cannot naturally accumulate GB under water or other abiotic stresses (Chen & Murata, 2015). This limitation can be mitigated by transgene technology (Li *et al.*, 2014), but the level of GB is still lower than that by naturally synthesis (Ashraf & Foolad, 2007). In the present study, it was found that cells could accumulate higher GB if the plants were inoculated with *R. planticola* in roots under poor conditions. The choline content, as the key precursor of GB, also increased significantly at the same condition during the process of GB biosynthesis. This may have been due to the fact that *R. planticola* enhanced the ability of drought resistance by regulating GB synthesis, which was an important substance for the osmotic-regulation mechanism. Besides, other researchers also reported that PGPR such as *Pseudomonas* sp. and GB03 could improve the accumulation of GB in *Oryza sativa* (Jha *et al.*, 2011), and *Arabidopsis* (Zhang *et al.*, 2010) in dry environment, respectively.

The key enzymes such as, PEAMT, CMO, and BADH, are involved in the synthesis of GB in chloroplast (Chen & Murata, 2011). Choline and GB synthesis is a complicated process because PEAMT, CMO and BADH are regulated by different patterns (Peel *et al.*, 2010). This study also demonstrated that these enzyme activities increased in both maize cultivars due to PGPR inoculation *R. planticola* inoculation under drought stress condition. McNeil *et al.* (2001) reported that the content of free choline is stimulated in transgenic tobacco with overexpression of *PEAMT*. Meanwhile *PEAMT* expresses in plants subjected to drought or saline conditions, which catalyzes the synthesis of choline (Nuccio *et al*., 2000). Under drought stress conditions, the expression of *PEAMT* was found to be reasonably high when the plant was inoculated with GB03 (Zhang *et al.*, 2010). On the other hand, in barley (*Hordeum vulgare* L.) grown on salt stress, the proteins associated with GB, CMO and BADH, kept high expression level and correlated with this phenomenon. The accumulation of GB also increased significantly in young leaves. Perhaps it shows that GB is inclined to keep young organs safe from salt-induced injury by regulating GB synthesis (Hattori *et al.*, 2009). Further, the expression of *BADH* as well as *CMO* varies with different stresses and cultivars. Zingaretti *et al.* (2014) showedthat the gene is designed for the sensitive cultivar cultivated under moderate and severe stress, but for the tolerant cultivar, *BADH* and *CMO* begin to specifically express at the start of stress. In this research, a significant increase in *CMO* and *BADH* transcriptional levels was observed when the stress was imposed and in two maize varieties treated with *R. planticola*, the expression of *CMO* and *BADH* wwas found to be enhanced obviously, particularly for cv. ZD. The content of choline was lower in the drought sensitive cultivar (cv. JD) than that in the tolerant one (cv. ZD). These findings suggested that *R. planticola* could enhance the synthesis and accumulation of choline and GB in stressed maize plants through promoting gene expression levels and the activities of the three key enzymes involved in GB synthesis.

Conclusion

In the present study, bacterial inoculation effectively promoted seed germination and improved GB metabolism under drought stress through regulating the activities of PEAMT, CMO and BADH as well as their gene expression. The main restricting factor of GB metabolism was choline shortage, which can be effectively solved by regulating *PEAMT* expression. The above effects were cultivar-dependent. Although the present study confirmed that *R. planticola* provides a safe and economical approach to enhance drought tolerance in maize, the mechanism of the increased osmoprotectant levels induced by bacterial inoculation needs further investigation.

Acknowledgement

We are grateful to State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau (A314021402-1514) and Sci-tech Development Foundation of NWSUAF (A2990215264) for their financial support. GAILI NIU, NAHEEDA BEGUM, WEI GOU and PENG ZHENG contributed equally to this work.

References

Ahmad, R., C.J. Lim and S.Y. Kwon. 2013. Glycine betaine: a versatile compound with great potential for gene pyramiding to improve crop plant performance against environmental stresses. *Plant Biotechnol. Rep.*, 7(1): 49-57.

Arakawa, K., M. Katayama and T. Takabe. 1990. Levels of betaine and betaine aldehyde dehydrogenase activity in the green leaves, and etiolated leaves and roots of barley. *Plant Cell Physiol.*, 31(6): 797-803.

Ashraf, M. and M.R. Foolad. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.*, 59(2):206-216.

Bohnert, H.J., D.E. Nelson and R.G. Jensen. 1995. Adaptations to environmental stresses. *Plant Cell*, 7(7): 1099-1111.

Burnet, M., P.J. Lafontaine and A.D Hanson. 1995. Assay, purification, and partial characterization of choline monooxygenase from spinach. *Plant Physiol.*, 108(2): 581-588.

Chen, T. H. and N. Murata. 2015. Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. *Plant Cell Environ.*, 34(1):1-20.

Chen, T.H.H. and N. Murata. 2011. Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. *Plant Cell Environ.*, 34(1): 1-20.

Cheng, Z., E. Park and B.R. Glick. 2007. 1-aminocyclopropane-1-carboxylate deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. *Can. J. Microbiol.*, 53(7): 912-918.

Dworkin, M. and J.W. Foster. 1958. Experiments with some microorganisms which utilize ethane and hydrogen. *J. Bacteriol.*, 75(5): 592-603.

Fan, W., H. Wang and P. Zhang. 2016. Engineering Glycinebetaine Metabolism for Enhanced Drought Stress Tolerance in Plants. *Springer International Publishing*.

Ghanbari, A., F. Nadjafi and J. Shabahang. 2007. Effect of irrigation regimes and row arrangement on yield, yield components and seed quality of pumpkin (*Cucurbita pepo* L.). *Asian. J. Plant Sci.*, 6: 1072-1079.

Gholami, A., S. Shahsavani and S. Nezarat. 2009. The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *Int. J. Biol. Sci.*, 1(1): 35-40.

Gou, W., L. Tian, Z. Ruan, P. Zheng, F.C. Chen and L.X. Zhang. 2015. Accumulation of choline and glycinebetaine and drought stress tolerance induced in maize (*Zea mays*) by three plant growth promoting rhizobacteria (PGPR) strains. *Pak. J. Bot.*, 47(2): 581-586.

Grover, M., S.Z. Ali, V. Sandhya, A. Rasul and B. Venkateswarlu. 2011. Role of microorganisms in adaptation of agriculture crops to abiotic stresses. *World J. Microb. Biot.*, 27(5): 1231-1240.

Han, Q. Q., Y. N., Wu, H. J., Gao, R., Xu, , P. W., Paré and H., Shi. 2017. Improved salt tolerance of medicinal plant *Codonopsis pilosula*, by *Bacillus amyloliquefaciens*, GB03. *Acta Physiol. Plant.*, 39(1): 35.

Hanson, A.D. and C.E., Nelsen. 1978. Betaine accumulation and [^{14}C] formate metabolism in water-stressed barley leaves. *J. Plant Physiol.*, 62(2): 305-312.

Hattori, T., S. Mitsuya, T. Fujiwara, A.T. Jagendorf and T. Takabe. 2009. Tissue specificity of glycinebetaine synthesis in barley. *Plant Scie*., 176(1): 112-118

Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. *Calif. Agri. Exp. Stn. Circ.*, 347: 32.

Jha, Y., R.B. Subramanian and S. Patel. 2011. Combination of endophytic and rhizospheric plant growth promoting rhizobacteria in *Oryza sativa* shows higher accumulation of osmoprotectant against saline stress. *Acta. Physiol. Plant.*, 33(3): 797-802.

Kataoka, R., E. Güneri, O.C. Turgay, A.E. Yaprak, B. Sevilir and I. Başköse. 2017. Sodium-resistant plant growth-promoting rhizobacteria isolated from a halophyte, *Salsola grandis*, in saline-alkaline soils of Turkey. *Eurasian Soil Sci.*, 63: 216-225.

Kaya, C., A.L. Tuna and M. Ashraf. 2007. Improved salt tolerance of melon (*Cucumis melo* L.) by the addition of proline and potassium nitrate. *Env. Exp. Bot*., 60: 397-403.

Khayatnezhad, M., R. Gholamin, S. Jamaati-e-Somarin and R. Zabihi-e-Mahmoodabad. 2010. Effects of PEG stress on corn cultivars (*Zea mays* L.) at germination stage. *World Appl. Sci. J.*, 11(5): 504-506.

Lesk, C., P. Rowhani and N. Ramankutty. 2016. Influence of extreme weather disasters on global crop production. *Nature*, 529(7584): 84-87.

Li, M., Z. Li, S. Li, S. Guo, Q. Meng, G. Li and X. Yang. 2014. Genetic engineering of glycine betaine biosynthesis reduces heat-enhanced photo-inhibition by enhancing antioxidative defense and alleviating lipid peroxidation in tomato. *Plant Mol. Biol. Rep.*, 32(1): 42-51.

Li, X. and X. Wang. 2003. Changes in agricultural land use in China: 1981-2000. *Asian Geographer* 22(1-2): 27-42.

Livak, K.J. and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods*, 25(4): 402-408.

McNeil, S.D., M.L. Nuccio, M.J. Ziemak and A.D. Hanson. 2001. Enhanced synthesis of choline and glycine betaine in transgenic tobacco plants that overexpress phosphoethanolamine N-methyltransferase. *P. National Acad. Sci.* 98(17): 10001-10005.

Nuccio, M.L., M.J. Ziemak, S.A. Henry, E.A. Weretilnyk and A.D. Hanson. 2000. cDNA cloning of phosphoethanolamine-N-methyl transferase from spinach by complementation in *Schizosaccharomyces pombe* and characterization of the recombinant enzyme. *J. Biol. Chem.*, 275(19): 14095-14101.

Nuccio, M.L., S.D. McNeil, M.J. Ziemak, A.D. Hanson, R.K. Jain and G. Selvaraj. 2000. Choline import into chloroplasts limits glycine betaine synthesis in tobacco: analysis of plants engineered with a chloroplastic or a cytosolic pathway. *Metab. Eng.*, 2(4): 300-311.

Osakabe, Y., K. Osakabe, K. Shinozaki and L.S.P. Tran. 2014. Response of plants to water stress. *Front. Plant Sci.* 5: 1-8.

Lam, P.W. and I.E. Salit. 2014. *Raoultella planticola* bacterium following consumption of seafood. *Can. J. Infect. Dis. Med. Microbiol*., 25(4): 83-84.

Panhwar, Q.A., R. Othman, Z.A. Rahman, S. Meon and M.R. Ismail. 2014. Isolation and characterization of phosphate-solubilizing bacteria from aerobic rice. *Afr. J. Biotechnol*., 11(11): 2711-2719.

Peel, G.J., M.V. Mickelbart and D. Rhodes. 2010. Choline metabolism in glycinebetaine accumulating and non-accumulating near-isogenic lines of *Zea mays* and *Sorghum bicolor*. *Phytochem.*, 71(4): 404-414.

Penrose, D.M. and B.R. Glick. 2003. Methods for isolating and characterizing ACC-deaminase-containing plant growth-promoting rhizobacteria. *Physiol. Plantarum*, 118(1): 10-15.

Rahdari, P., S.M. Hosseini and S. Tavakoli. 2012. The studying effect of drought stress on germination, proline, sugar, lipid, protein and chlorophyll content in purslane (*Portulaca oleracea* L.) leaves. *J. Medic. Plant Res.*, 6(9): 1539-1547.

Roychoudhury, A. and A. Banerjee. 2016. Endogenous glycine betaine accumulation mediates abiotic stress tolerance in plants. *Tropic Plant Res*, 3: 105-111.

Hoseini, S.M. 2010. Studying effects of salinity stress on germination, proline and carbohydrate content in Thyme (*Thymus vulgaris* L.) seedlings. *Int. J. Agri. Crop Sci.*, 2(2): 34-38.

Siddiqi, E.H., M. Ashraf and N.A. Akram. 2007. Variation in seed germination and seedling growth in some diverse lines of safflower (*Carthamus tinctorius* L.) under salt stress. *Pak. J. Bot.* 39(6): 1937-1944.

Simova-Stoilova, L., K. Demirevska, T. Petrova, N. Tsenov and U. Feller. 2008. Antioxidative protection in wheat varieties under severe recoverable drought at seedling stage. *Plant Soil. Environ.* 54(12): 529-536.

Soares, R.A., L.F.W. Roesch, G. Zanatta and F.A. de Oliveira Camargo, and V. Passaglia. 2006. Occurrence and distribution of nitrogen fixing bacterial community associated with oat (*Avena sativa*) assessed by molecular and microbiological techniques. *Appl. Soil Ecol.* 33(3): 221-234.

Soltani, A., M. Gholipoor and E. Zeinali. 2006. Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. *Environ. Exp. Bot.* 55(1): 195-200.

Sreedhar, S.S. and V. Mohan. 2014. Effect of bio-inoculants on seed germination and disease control of commercially important fast growing native tree species in nursery. *Kavaka*, 43: 41-45.

Timmusk, S., I.A.A. El-Daim, L. Copolovici, T. Tanilas, A. Kännaste, L. Behers, E. Nevo, G. Seisenbaeva, E. Stenström and Ü. Niinemets. 2014. Drought-tolerance of wheat improved by rhizospheric bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. *PlosOne* 9(5): e96086.

Xie, J. 2009. Addressing China's water scarcity: recommendations for selected water resource management issues. *World Bank, Washington*.

Yang, J., J.W. Kloepper and C.M. Ryu. 2009. Rhizospheric bacteria help plants tolerate abiotic stress. *Trends Plant Sci.* 14(1): 1-4.

Yu, J., Y. Li, W. Tang, J. Liu, B.R Lu. and Y. Liu. 2014. The accumulation of glycine betaine is dependent on choline monooxygenase (OsCMO), not on phosphoethanolamine N-methyltransferase (OsPEAMT1), in rice (*Oryza sativa* L. ssp. japonica). *Plant Mol. Biol. Rep.* 32(4): 916-922.

Zhang, H., C. Murzello, Y. Sun, M.S. Kim, X. Xie, R.M. Jeter, J.C. Zak, S.C. Dowd and P.W. Paré. 2010. Choline and osmotic-stress tolerance induced in *Arabidopsis* by the soil microbe *Bacillus subtilis* (GB03). *Mol. Plant Microbe In*, 23(8): 1097-1104.

Zhang, L.X., P. Zheng, Z. Ruan, L. Tian and M. Ashraf. 2015. Nitric oxide accumulation and glycine betaine metabolism in two osmotically stressed maize cultivars supplied with different nitrogen forms. *Biol. Plant.* 59(1): 183-186.

Zheng, P., L.X. Zhang, L. Tian, L. Zhang, F.C. Chen, B.Z. Li and Z.Y. Cui. 2014. Isolation and characterization of novel bacteria containing ACC-deaminase from the rhizosphere resource on dry-farming lands. *Pak. J. Bot.* 46(5): 1905-1910.

Zhu, X. and S.H. Zeisel. 2009. Choline and phosphatidylcholine. Guide to Nutritional Supplements. *Elsevier, Oxford*, UK: 392-396.

Zingaretti, S., P. Demore, T. Morceli and L. Mantovanini. 2014. Glycine betaine biosynthesis genes differentially expressed in sugarcane under water stress. *BMC Proc.*, 8(Suppl. 4): 123.

(Received for publication, 5 March 2017)