Pak. J. Bot., 44(6): 1837-1844, 2012.

EFFECTS OF EXOGENOUS NITRIC OXIDE ON GLYCINEBETAINE METABOLISM IN MAIZE (*ZEA MAYS* L.) SEEDLINGS UNDER DROUGHT STRESS

LIXIN ZHANG[1,2*], YONGGUI ZHAO[1], YOUYA ZHAI[1], MEI GAO[1], XIFENG ZHANG[1], KAI WANG[1], WEIGE NAN AND JIANCHAO LIU[1]

[1]*College of Life Sciences, Northwest A&F University, 712100, Yangling, Shaanxi, P.R. China,*
[2]*State Key Laboratory of Soil Erosion and Dryland Farming, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Northwest A&F University, 712100, Yangling, Shaanxi, P.R. China,*
[*]*Corresponding author's e-mail: zhanglixinyangling88@yahoo.com.cn*

Abstract

In this study, we investigated the effects of exogenously applied nitric acid (NO) source i.e., sodium nitroprusside (SNP), NO scavenger i.e., 2-(4-carboxypheny-4,4,5,5-tetramethylimidazoline-1-1oxyl-3-oxide, potassium salt (cPTIO), NO inhibitor i.e., NaN_3, and NOS inhibitor i.e., Nω-nitro-larginine methyl ester hydrochloride (L-NAME) on eglycinbetaine (GB) metabolism in root tips and leaves of maize seedlings under drought stress (DS). The accumulation of NO peaked earlier than that of GB in shoot tips and leaves. The maximum NO content in root tips was attained earlier than that in leaves, while the reverse was observed with respect to GB content. The concentrations of NO, GB and choline in root tips and leaves of maize seedlings under DS were greater in the plants which received exogenous SNP application as compared to those in plants without SNP application. Exogenous SNP application also increased betaine aldehyde dehydrogenase (BADH) activity in leaves. The GB metabolism was negatively influenced by exogenous applications of either PTIO, NaN_3 or L-NAME. The combined application of NaN_3 and L-NAME had the most serious negative effects. These results demonstrate that GB metabolism in drought stressed maize plants was significantly enhanced by exogenous NO application. The accumulation of greater level of NO in maize seedlings contributed to increase GB accumulation by regulating BADH activity and choline content. This study provides a direct evidence of regulation of GB metabolism in maize plants under drought stress by exogenous application of NO.

Introduction

In arid and semiarid regions of the world, drought stress (DS) has been a major limitation to plant growth and crop productivity. Adaptation to water deficit is rather common to all terrestrial plants (Li, 2007; Ashraf, 2010; Anwar *et al.*, 2011). Maize (*Zea mays* L.), an important cereal crop in these areas, is highly sensitive to drought resulting in a significant yield reduction (Ashraf *et al.*, 2007; Zhang *et al.*, 2007; Ali & Ashraf, 2011). Understanding the mechanism of tolerance to these stresses is a prerequisite for developing strategies to improve plant stress tolerance (Ashraf *et al.*, 2007; Ashraf, 2010; Zhang *et al.*, 2011).

Plants sense and adapt to different stresses by altering their physiological metabolism, growth pattern, and mobilizing various defense mechanisms (Arasimowicz & Floryszak-Wieczorek, 2007; Ashraf & Foolad, 2007; Ren *et al.*, 2007; Zhang *et al.*, 2011; Zhang *et al.*, 2012). Therefore, accumulation of osmolytes is a prerequisite for osmotic adjustment of all organisms under DS (Zhang *et al.*, 2009; Ashraf *et al.*, 2011). It is well established that glycinebetaine (GB) accumulates in plants during their adaptation to various types of environmental stresses, including drought (Ashraf & Foolad, 2007; Zhang *et al.*, 2009). Glycinebetaine, a quaternary ammonium compound, is a very effective compatible solute which is found in a wide range of crops (Ashraf & Foolad, 2007; Shahbaz *et al.*, 2011). Glycinebetaine is synthesized from its precursor choline by a two-step oxidation, via the intermediate betaine aldehyde. Choline is synthesized in protoplast and transported to chloroplast and vacuole. The first oxidation step is catalyzed by choline monooxygenase (CMO, EC 1.14.15.7), and the further oxidation to GB is catalyzed by betaine aldehyde dehydrogenase (BADH, EC 1.2.1.81) in chloroplast (Sakamoto & Murata, 2002; Sithtisarn *et al.*, 2009). Glycinebetaine synthesized by mature leaves during stress behaves as an inert end product and upon rewatering is translocated to the new leaves and roots, most probably via the phloem (Ashraf & Foolad, 2007). In maize, GB accumulates in leaves and well responds to water deficit, which was determined by choline content and BADH activity (Ashraf & Foolad, 2007; Rhodes & Hanson, 1993; Zhang *et al.*, 2012).

Nitric oxide (NO) is a free radical involved in numerous and diverse cellular pathways in mammals (Torreilles, 2001; Hancock *et al.*, 2011). In recent years, there has been much research about the presence of NO and its physiological role in the plant kingdom. Thus, evidence has been obtained for the involvement of NO in growth and developmental processes, as well as in defense responses against oxidative stress under various adverse growing conditions (Wojtaszek, 2000; Beligni & Lamattina, 2001; Lamattina *et al.*, 2003; Wang *et al.*, 2004). Recent studies support that NO has a vital antioxidant characteristic, therefore, it is required for activating antioxidant enzyme activities and some osmolytes metabolism under abiotic stresses (Liu & Zhang, 2009; Misra *et al.*, 2011). Plants exposed to drought show an increased accumulation of NO and GB, especially in a drought tolerant maize cultivar (Zhang *et al.*, 2012). Although the signal regulation role of NO has been well documented (Siddiqui *et al.*, 2011), its influence on regulation GB metabolism in maize under drought is not clearly understood (Ruan *et al.*, 2004; Sarath *et al.*, 2007; Liu & Zhang, 2009; Ashraf, 2010; Misra *et al.*, 2011; Zhang *et al.*, 2012). In this study, a drought tolerant maize cultivar Zhengdan 958 was used to investigate the role of NO in induction of GB metabolism in leaves and roots under drought stress.

Materials and Methods

Plant material and trial location: Solution culture experiments were conducted in a controlled growth chamber at the College of Life Sciences of Northwest A & F University, Yangling, P.R. China. Maize (*Zea mays* L.) cultivar Zhengdan 958 was used in this study (Zhang *et al.*, 2011).

Plant growth and experimental design: The seeds were germinated at 28 °C for 72 h in petri dish in dark. The young seedlings were inserted into holes of styrofoam boards placed over plastic containers (inner length: 26 cm; width 18 cm; height 12 cm) containing deionized water initially, which was replaced by one-half-strength and then by full strength nutrient solution 4, and 8 days later, respectively (Hoagland & Arnon, 1950). The growth containers were placed in a growth chamber under the following environmental conditions: average day/night temperature 25/18 °C, relative humidity 60-70%, light intensity 350 μmol/m^2/s and 16 h of photoperiod. The containers were wrapped with black plastic to prevent roots exposure to light. The pH of the nutrient solution was adjusted to 6.30 (±0.05) every day.

When the seedlings were at stage of 3-leaf, DS treatment was initiated by adding 120 g polyethylene glycol (PEG-6000) per kg of nutrition solution to achieve drought (osmotic) stress level of approximately -0.23 MPa (Wang & Li, 2002). No drought stress, i.e., control treatment solution received no PEG.

Experiment 1: Influence of NO donors i.e., sodium nitroprusside (SNP) on GB and NO accumulation in maize under drought for 72 h (Chen *et al.*, 2006). Treatments included factorial combination of : (a) main treatments (2): with or without DS; (b) sub-treatments (2): no NO or with NO, using 0.1 mmol/L SNP as NO donors in nutrient solution for 72h. Each treatment was replicated four times.

Experiment 2: Effects of NO donors i.e., SNP and NO scavenger i.e., cPTIO [2-(4-carboxyphenyl) -4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium salt] on NO content and GB metabolism at 24 h after drought stress treatment (Chen *et al.*, 2006; Tan *et al.*, 2008). Treatments included factorial combination of : (a) main treatments (2): with or without DS; (b) sub-treatments (4): a. without NO donors or scavenger ; b. with NO donors only (0.1 mmol/L SNP); c. with NO scavenger only (400μmol/L cPTO); d. combination of b and c. Total treatments were eight with number of 4 replications.

Experiment 3: Effects of NO synthase (NOS) inhibitor i.e., L-NAME (Nω-nitro-Larginine methyl ester hydrochloride) or/and nitrate reductase (NR) inhibitor i.e., NaN_3 on NO and GB content after 24h of drought treatment (Chen *et al.*, 2006; Tan *et al.*, 2008). Treatments included factorial combination of : (a) main treatments (2): with or without DS; (b) sub-treatments (4): a. without NOS or NR inhibitor; b. with NOS inhibitor L-NAME only (0.025 mmol/L L-NAME); c. with NR inhibitor NaN_3 only (0.1mmol/L NaN_3); d. combination of b and c. Total treatments were eight with number of 4 replications. SNP, cPTIO and L-NAME were from Sigma company (St. Louis, MO, USA), other chemicals were from Shanghai Experiment Reagent Co., Ltd. (Shanghai , China).

Plants were grown in a growth chamber in 3.4 L plastic pots, which were sealed carefully to avoid evaporation, with a sponge wrapped around the interface of the roots and the shoots. All treatment units were replicated four times, with a completely randomized design. The treatment solutions were under continuous aeration.

All experiments were repeated twice. Data presented here are means of four replicates of the two experiments (n=8).

Sampling and recording of data: The maize plants were harvested after a given duration following the treatments as explained above each experiment. Fresh mass of the second or third leaves from top of each plant and root tips were recorded. Some fresh samples were for assay of NO content and BADH activity. The other fresh samples were placed in an oven at 105 °C for 30 min, and then dried to a constant weight at 75°C for measurement of GB and choline content.

NO content was assayed as described by Griess (1879) with some modifications. Fresh plant materials (1.0g) were homogenizied in 1mL 40 mmol/L N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) pH 7.2 buffer, washed with 4 mL buffer, filtered through 2 layers of gauze. The filtered solution was then centrifuged at 4440×g for 10 min at 4°C and supernatants were used for determination of NO concentration according to the indication on the NO assay kit (Beyotime Institute of Biotechnology, Shanghai, China). Aliquots of 1ml were put in test tubes, cooled in ice water for 1 h, before 2 mL Griess Reagent I solution was added. The reaction was maintained 60 min at 37°C, then centrifuged at 710×g for 10 min at 25°C. Aliquots of 2ml supernatant were added in 2 ml Griess Reagent II solution at 25°C. After 10 min, the absorbance was measured at 540 nm with UV-visible spectrophotometer. The NO content was determined based on a standard curve using $NaNO_2$ and expressed as n mol/g DW.

GB content was determined following Grieve & Grattan, (1983) with some modifications. Dried and finely powdered plant material (0.5 g) was shaken with 20 ml of deionized water for 24 h at 25°C. The extracts were diluted 1:1 with 2 N H_2SO_4. Aliquots of 0.5 ml were put in test tubes and cooled in ice water for 1 h, before a cold KI-I2 reagent (200 μl) was added. The tubes were stored at 0-4°C for 16 h and then centrifuged at 12,000×g for 15 min at 4°C. The supernatant was aspirated. The periodite crystals were dissolved in 5 ml of 1, 2-dichloroethane. After 2-2.5 h, the absorbance was read at 365 nm with a UV–visible spectrophotometer. The GB content was determined using a for calibration curve of GB (50–200 g/ml) and expressed as n mol/g DW.

BADH activity was assayed as described by Daniell *et al.*, (2001) with some modifications. To obtain crude protein extracts, plant materials were homogenized in 250 μl homogenization buffer containing 50 mM HEPES-

KOH, pH 8.0, 1 mM EDTA, 20 mM sodium metabisulfite, 10 mM sodium borate, 5 mM ascorbic acid, 5 mM dithiothreitol, and 2% (w/v) PVPP. The homogenates were then centrifuged at 12,000×*g* for 15 min at 4°C and the supernatants were used for determination of BADH activity. The BADH activity was determined by measuring absorbance at 340 nm with 0.05 mM betaine aldehyde chloride as a substrate. The activity was calculated using the extinction coefficient of 6220/M cm for NADH. The BADH activity was expressed as µ mol/min/ mg protein.

Choline content was determined following Feng & Ren, (2004) and Richard & Emily, (1945) with some modifications. About 0.5 g dried and finely ground plant material was transferred to a triangle vase (100 ml volume) in 70 ml of deionized water added. After thorough nixing, the vase was bathed in hot water at 80°C for 15 min, shaken in an oscillator for 30 min. The volume was adjusted to 100 ml, and then filtered through a filter paper. Aliquots of 10ml filtered solution were poured into a triangle vase, cooled in ice water to -5°C before 15ml Reinecke salt- methanol solution was added (4 g Reinecke salt was dissolved in 100 ml methanol). The mixed solution was stirred for 30 min and placed in a refrigerator for 12 h. The red water insoluble substance was filtered out, washed in 10 ml portion each of propylalcohol three times, dissolved in acetone, and the volume was adjusted to 25 ml. The choline content was assayed by measuring the absorbance at 520nm with UV–visible spectrophotometer using acetone as a control. Reference standards of choline (9-27 mg ml^{-1}) were used for calibration and estimation of choline concentration, and expressed as n mol/ g DW.

Statistical analysis: Analysis of variance (ANOVA) for different responses parameters were evaluated by SAS software package (Anon., 1996). Least significance difference (LSD) test was used to determine the significant difference among the mean values at the 0.05 level.

Results

Dynamic accumulation of NO and GB in root tips and leaves of maize seedlings during DS period with or without NO donor SNP treatment: Endogenous NO and GB accumulated in root tips and leaves with prolonged period of DS treatment, with or without SNP application. The greatest increase in NO content occurred root tips while that of GB was in leaves. Following the DS initiation, NO content in root tips and leaves attained the peak values after 12 and 24 h with SNP, and 24 and 36 h without SNP, respectively. The respective increases were 7.8- and 7.0-fold, and 6.9- and 6.1-fold of that of the no DS plants. In contrast, GB content in root tips and leaves reached the maximum after 36 and 48 h with SNP, and 48h and 60 h with no SNP in the DS treatment. Their increases were 4.0- and 5.2-fold, 3.2- and 3.7-fold of that of no DS plants (Figs. 1 & 2).

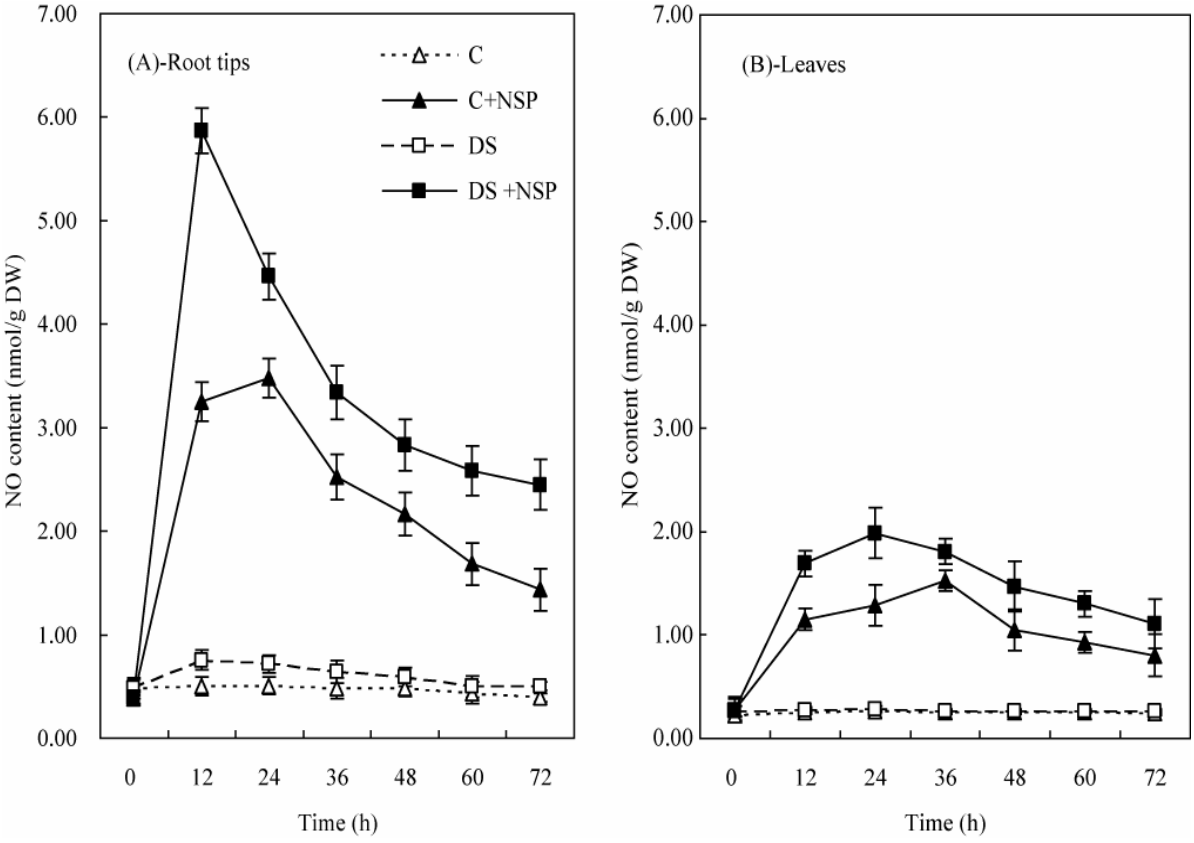

Fig. 1. Dynamic changes of nitric oxide (NO) accumulation in (A) roots tips and (B) leaves of maize seedling during drought stress (DS) period with or without NO donor i.e., sodium nitroprusside (SNP) treatments. Mean±SE (*n*=8). DS and C represent drought stress and non-drought stress (control) respectively.

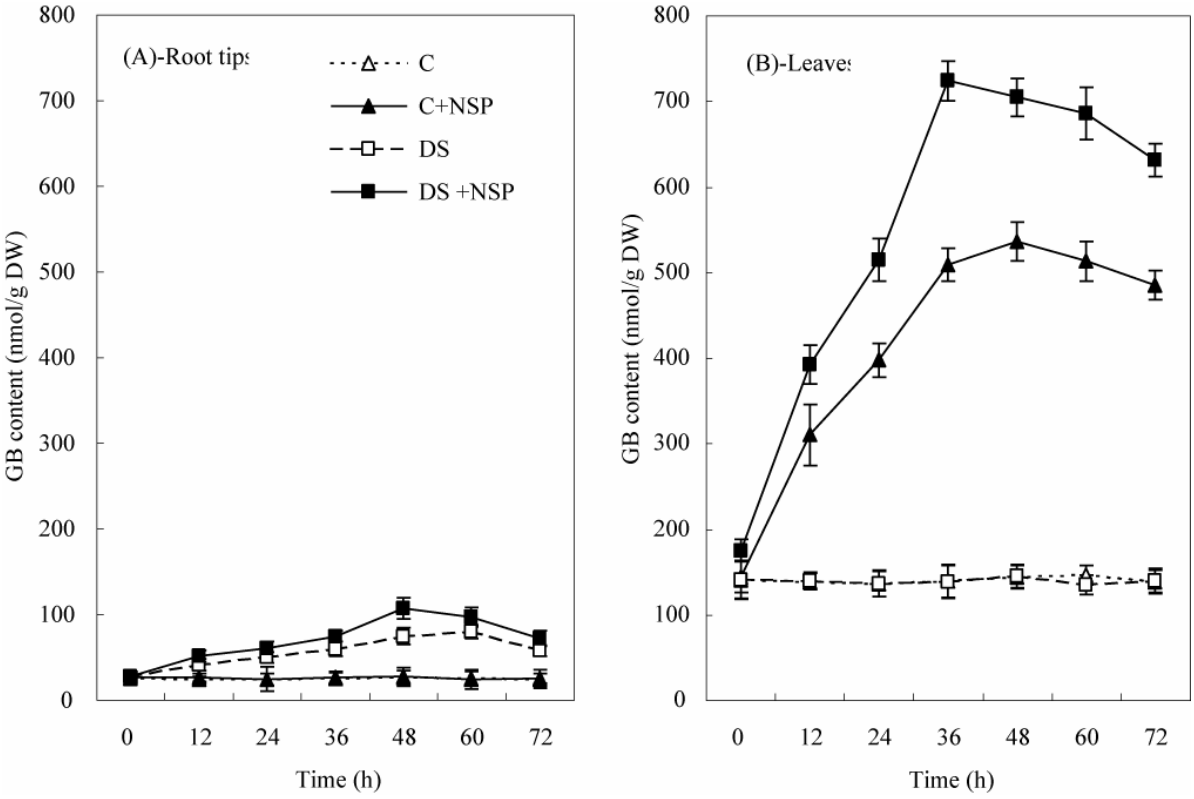

Fig. 2. Dynamic changes of glycinebetaine (GB) accumulation in (A) roots tips and (B) leaves of maize seedling during drought stress (DS) period with or without NO donor i.e., sodium nitroprusside (SNP) treatments. Mean±SE (*n*=8). DS and C represent drought stress and non-drought stress (control) respectively.

The dynamic accumulations of NO and GB in root tips and leaves were enhanced by SNP application under DS. The SNP application increased NO and GB in root tips by 31-81% and 20-31%, respectively, compared to the respective values of the plants without SNP application. The corresponding increases in leaves were 18-55% and 27-42%. The SNP application showed no significant effects on GB content in roots tips and leaves of plants which were not under DS. The NO content in both roots tips and leaves attained the peak earlier than in time required for peak values of GB content. The peak NO values attained much earlier in root tips as compared to that for leaves (Figs. 1 & 2).

Effects of NO donor i.e., SNP and NO scavenger i.e., cPTIO on NO content and GB metabolism parameters in roots tip and leaves of maize seedlings under DS: NO content in root tips was greater than that in leaves, while converse was true for GB content. The BADH activity was detectable in leaves only. Exogenous SNP treatment increased NO, GB and choline contents in root tips and leaves as well as BADH activity in leaves. The cPTIO application on plants under DS decreased all response parameters as compared to those without cPTIO application. The responses to combination of SNP and cPTIO applications were very similar to those with application of only cPTIO and weaker than those with SNP application of the plants under DS. As for no DS, SNP or cPTIO treatment induced less increase/decrease only in NO content in root tips of DS plants (Fig. 3).

Effects of different NO synthesis inhibitors i.e., NaN_3 and L-NAME on NO content and GB metabolism parameters in roots tips and leaves of maize seedling during DS: NOS inhibitor L-NAME or NR inhibitor NaN_3 treatments induced a decrease in NO, GB and choline contents in both root tips and leaves as well as BADH activity in leaves only. The combination of both inhibitors had greater negative effects as compared that with application of single inhibitor. Application of NaN_3 and/or LNAME decreased NO content in root tips only in the plants under no DS. However, the decrease rate was lower than those in DS plants (Fig. 4).

Discussion

A widely occurring adaptation in plants to counteract abiotic stress is an accumulation of stress signal molecules such as NO and compatible organic solutes such as GB (Taiz & Zeiger, 2006; Anwar *et al.*, 2011; Ashraf *et al.*, 2011; Siddiqui *et al.*, 2011). Zhang *et al.*, (2012) reported an obvious increase in NO and GB accumulation in leaves under DS maize plants. The roles of these two stress-related substances have attracted the attention most of the stress physiologists (Arasimowicz & Floryszak-Wieczorek, 2007; Ashraf & Foolad, 2007; Ren *et al*., 2007; Liu & Zhang, 2009; Misra *et al*., 2011; Zhang *et al*., 2012). On one hand, a series of studies have been conducted about exogenous application of stress signal molecules such as NO and hydrogen peroxide, ethylene etc., has tremendous potential to improve stress

tolerance (Zhu, 2002; Taiz & Zeiger, 2006). Among these substances, NO, a common signal molecule in plants, has been documented to influence plant growth and development as well as their physiological responses under DS (Wojtaszek, 2000; Beligni & Lamattina, 2001; Lamattina *et al.*, 2003; Himmelbach *et al.*, 2003; Tan *et al.*, 2008). For example, the role of NO in closing stomata of DS plants has been widely reported (Ruggiero *et al.*, 2004; Bright *et al.*, 2006; Neill *et al.*, 2008). This effect is suggested to be vital for fast growth resumption and recovery of water content of the plants (Durner & Kessig, 1999). NO not only possesses a vital antioxidant characteristic, but a signal function in some osmolytes metabolism such as proline under abiotic stress (Liu & Zhang, 2009; Misra *et al.*, 2011). On the other hand, plant growth inhibition and relative adaptive physiological processes such as osmotic regulation caused by environmental stress might be regulated by the application of osmolytes (Achard *et al.*, 2006; Ashraf & Foolad, 2007; Ashraf *et al.*, 2011; Hancock *et al.*, 2011). As a major organic osmolyte, accumulation of GB has been reported in a variety of plant species in response to different stresses (Ashraf & Foolad, 2007; Ashraf *et al.*, 2011). The accumulation of GB has been found in many organisms, including higher plants. Since accumulation of GB in plant tissues serves as an index of the internal water status of plants in crop plants under drought stress suggests that GB may play a protective role in preventing cell damage from stress-induced dehydration (Rhodes & Hanson, 1993; Ashraf & Foolad, 2007). There are many reports demonstrating positive effects of exogenous GB on water relations, plant growth and final crop yield under water deficit conditions (Rhodes & Hanson, 1993; Ashraf & Foolad, 2007; Zhang *et al.*, 2011).

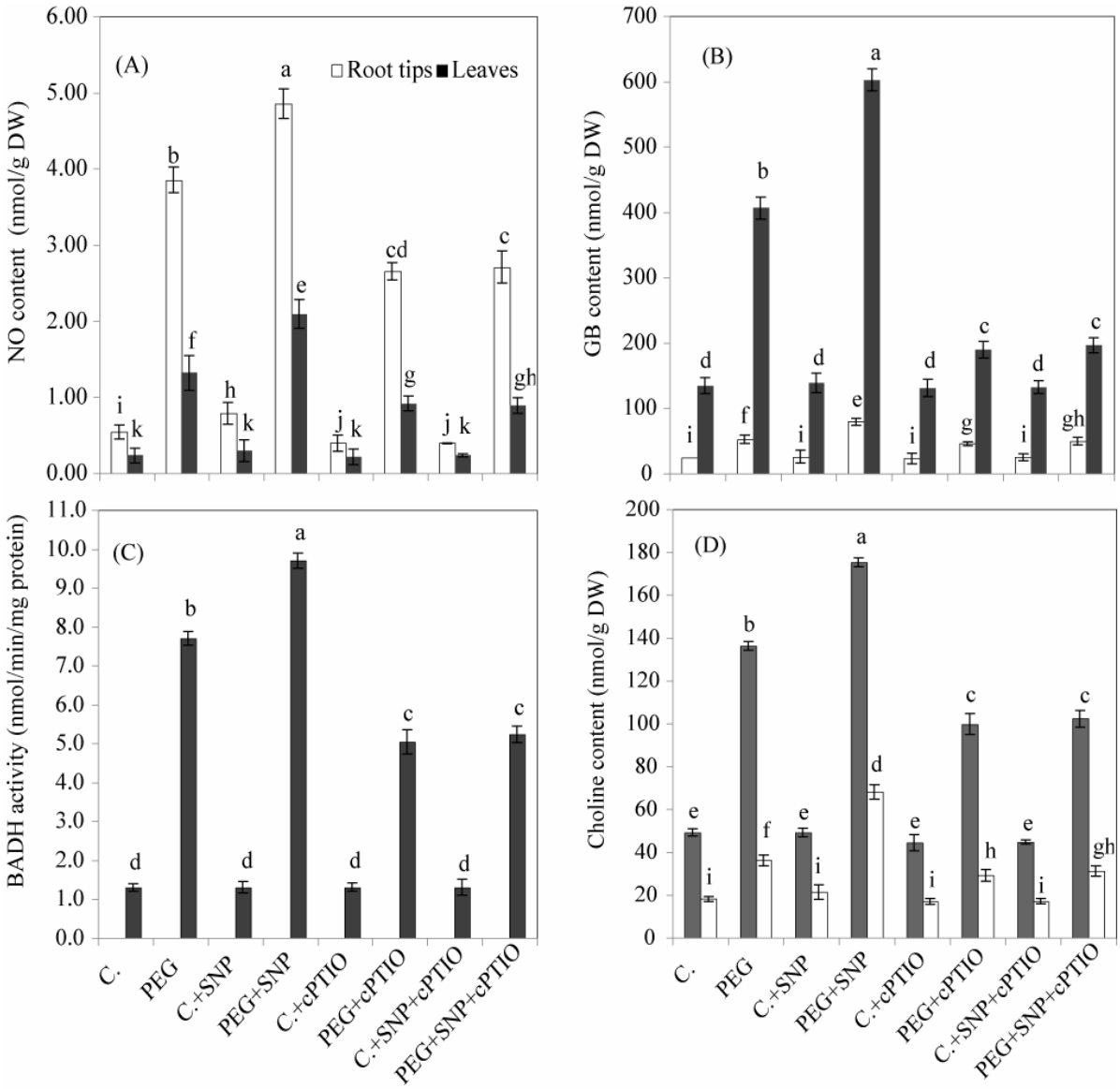

Fig. 3. Effects of NO scavenger i.e., 2-(4-carboxypheny-4,4,5,5-tetramethylimidazoline-1-1oxyl-3-oxide, potassium salt (cPTIO) on NO and GB contents in roots tips and leaves of maize seedling during DS. Mean±SE (n=8). DS and C represent drought stress and non-drought stress (control) respectively. Different letters at the top of histograms indicate significant differences (p<0.05).

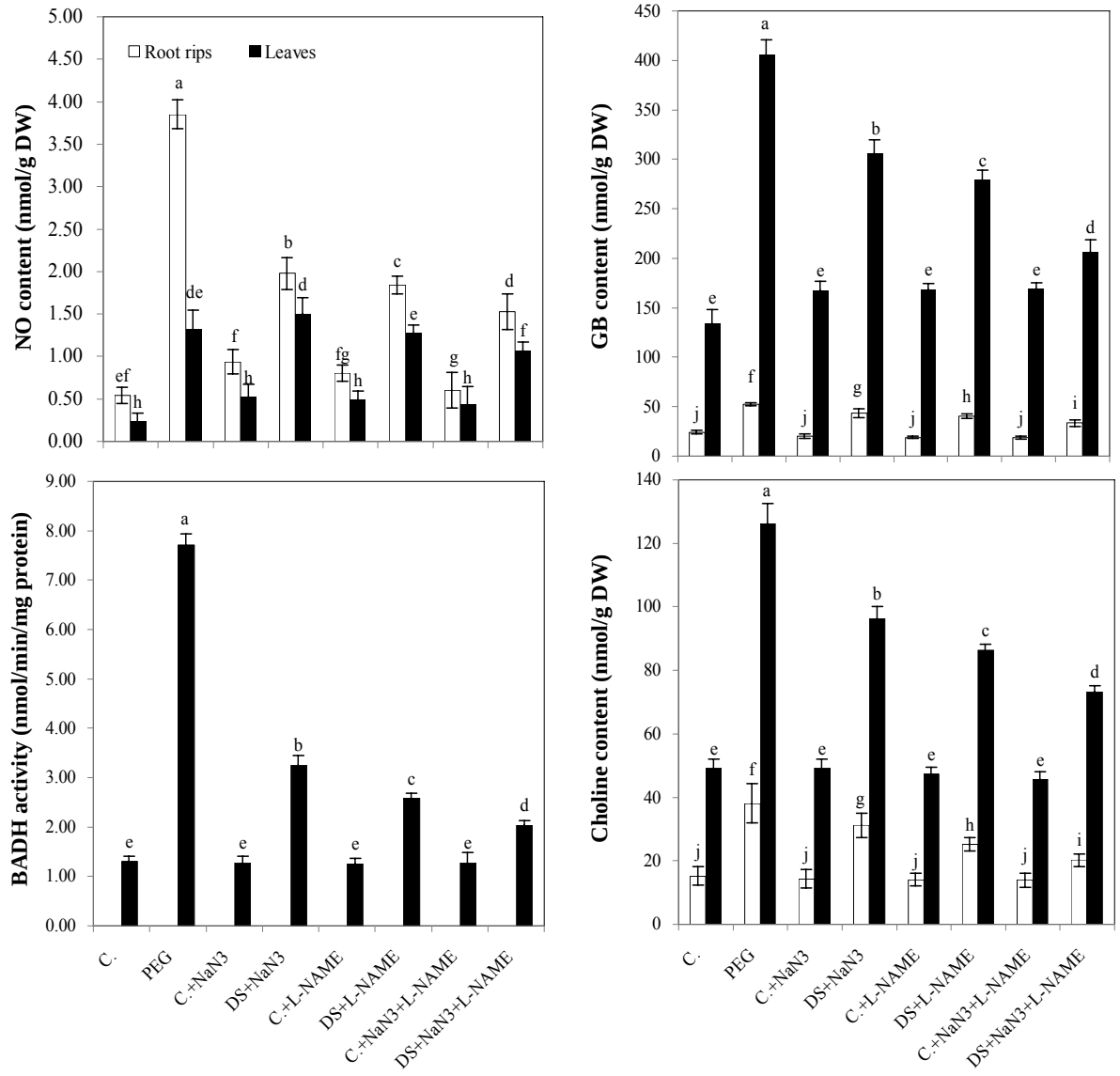

Fig. 4. Effects of different NO synthesis inhibitors i.e.,, NaN_3 and Nω-nitro-larginine methyl ester hydrochloride (L-NAME) on NO and GB contents in roots tips and leaves of maize seedling during DS. Mean±SE (n=8). DS and C represent drought stress and non-drought stress (control) respectively. Different letters at the top of histograms indicate significant differences (p<0.05).

Up to now, there are no conclusive experimental data to clarify the relationship of NO and GB accumulation under DS plants. The confirmed promoting role of NO on GB accumulation in DS maize is not well understood (Siddiqui *et al.*, 2011; Ashraf *et al.*, 2011; Zhang *et al.*, 2012). The results of this study clearly showed greater increase in NO and GB contents in root tips and leaves in DS maize plants with application of NO donor i.e., SNP as compared with no application of SNP. The application of SNP provoked the earlier occurrence of maximum GB accumulation by NO burst as compared to those in plants receiving no SNP application under DS (Figs. 1 & 2). These findings suggest that root tips are the primary site of NO production, GB synthesis occurs in leaves and translocates to roots. The SNP addition enhanced these physiological processes, especially for accumulation of NO in roots and that of GB in leaves.

As previous reports, it is well known that GB accumulates in many plants subjected to DS (Ashraf & Foolad, 2007; Ashraf *et al.*, 2011). Glycinebetaine is synthesized in chloroplast from its precursor choline by a two-step oxidation via betaine aldehyde catalyzed by choline monooxygenase (CMO), a ferredoxindependent soluble Rieske-type protein, betaine aldehyde dehydrogenase (BADH), and soluble NAD^+ dependent enzyme (Rhodes & Hanson, 1993). Choline is synthesized in protoplast and transported to chloroplast and vacuole (Sakamoto & Murata, 2002; Sithtisarn *et al.*, 2009). The chloroplast in mature leaves synthesizes GB and translocated to other organs such as new leaves and roots during stress behaves as an inert end product and upon rewatering, most probably via the phloem (Ashraf & Foolad, 2007). However, there are not published reports

of influence of NO on GB metabolism in DS maize plants (Ashraf & Foolad, 2007; Ashraf *et al*., 2011; Misra *et al*., 2011; Siddiqui *et al*., 2011; Zhang *et al*., 2012). This study has shown that application of SNP to DS maize plants increased BADH activity and choline content, in turn, enhanced accumulation of GB (Fig. 3). In contrast application of NO-scavenger i.e., cPTIO and/or NO inhibitor i.e., NaN_3 and NOS inhibitor i.e., L-NAME had negative effects on NO and GB accumulation. The above negative effects were more predominant in combination of NaN_3 and L-NAME treatments. The positive impacts of SNP were more prominent in root tips for NO and in leaves for GB (Figs. 3-4). It is, therefore, concluded that endogenous NO is involved in the regulation of GB metabolism by increasing BADH activity and choline content in enhancing osmotic ability under drought, especially in leaves. These results imply that NO rmediates the synthesis and accumulation of GB in maize under DS. Such increases in both BADH activity and choline content were correlated with enhanced accumulation of GB in the maize plants in response to DS with application of NO.

Conclusion

In conclusion, changes in the contents of NO, GB and choline as well as BADH activity are good indicators of plants to DS. The results showing significant positive effects of exogenously applied NO and negative effects of NO-scavenger cPTIO, and/or NO and/or NOS inhibitor on the above parameters supported that NO mediates GB synthesis and accumulation in drought-stressed maize plants. It is, therefore, suggested that application of NO can be a practical strategy to improve plant tolerance to DS by modulation of GB accumulation in maize plants.

Acknowledgements

This work was supported by the China Postdoctoral Science Foundation and Chinese Universities Scientific Fund, Project No. QN2009069, and by the Foundation of State Key Laboratory of Soil Erosion and Dryland Farming, Project No. 10501-J-2.

References

Achard, P., H. Cheng, L. De Grauwe, J. Decat, H. Schoutteten, T. Moritzn, D. Van Der Straeten, J.R. Peng and N.P. Harberd. 2006. Integration of plant responses to environmentally activated phytohormonal signals. *Science,* 311: 91-94.

Ali, Q. and M. Ashraf. 2011. Induction of drought tolerance in maize (*Zea mays* L.) due to exogenous application of trehalose: growth, photosynthesis, water relations and oxidative defense mechanism. *J. Agron. Crop Sci.* 197: 258-271.

Anonymous. 1996. *Getting started with PROC ANOVA. SAS.* Institute Inc., Cary, NC.

Anwar, J., G.M. Subhani, M. Hussain, J. Ahmad, M. Hussain and M. Munir. 2011. Drought tolerance indices and their correlation with yield in exotic wheat genotypes. *Pak. J. Bot.*, 43(3): 1527-1530.

Arasimowicz, M. and J. Floryszak-Wieczorek. 2007. Nitric oxide as a bioactive signalling molecule in plant stress responses. *Plant Sci.*, 172: 876-887.

Ashraf, M. 2010. Inducing drought tolerance in plants: some recent advances. *Biotech. Adv.*, 28: 169-183.

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

Ashraf, M., N.A. Akram, F. Al-Qurainy and M.R. Foolad. 2011. Drought tolerance: Roles of organic osmolytes, growth regulators and mineral nutrients. *Adv. Agron.* 111: 249-296.

Ashraf, M., S. Nawazish and H.R. Athar. 2007. Are chlorophyll fluorescence and photosynthetic capacity potential physiological determinants of drought tolerance in maize (*Zea mays* L.). *Pak. J. Bot.*, 39(4): 1123-1131.

Beligni, M.V. and L. Lamattina. 2000. Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. *Planta*, 210: 215-221.

Bright, J., R. Desikan, J.T. Hancock, I.S. Weir and S.J. Neill. 2006. ABA induced NO generation and stomatal closure in *Arabidopsis* are dependent on H_2O_2 synthesis. *Plant J.*, 45: 113-122.

Chen, K., J. LI, J. Tang, F.G. Zhao and X. Liu. 2006. Involvement of nitric oxide in regulation of salt stress-induced ABA accumulation in maize seedling. *J. Plant Physiol. Mol. Biol.*, 32: 577-582.

Daniell, H., B. Muthukumar and S.B. Lee. 2001. Marker free transgenic plants: the chloroplast genome without the use of antibiotic selection. *Curr. Genet.*, 39: 109-116.

Durner, J. and D.F. Kessig. 1999. Nitric oxide as a signal in plants. *Curr. Opin. Plant Biol.*, 2: 369-374.

Feng, Z.X. and A.N. Ren. 2004. Mensuration of the content of choline in diospyros leaves. *Tianjin J. Traditional Chin. Med.* , 21: 248-249.

Griess, P. 1879. Bemerkungen zu der abhandlung der H.H. Weselsky und Benedikt "Ueber einige azoverbindungen." *Chem. Ber*., 12: 426-428.

Grieve, C.M. and S.R. Grattan. 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant Soil*, 70: 303-307.

Hancock, J.T., S.J. Neill and I.D. Wilson. 2011. Nitric oxide and ABA in the control of plant function. *Plant Sci.*, 181: 555-559.

Himmelbach, A., Y. Yang and E. Grill. 2003. Relay and control of abscisic acid signaling. *Curr. Opin. Plant Biol.*, 6: 470-479.

Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soils. *Col Agr. Exp Sta.Cir.* , 347: 1-32.

Lamattina, L., C. Garcı´a-Mata, M. Graziano and G. Pagnussat. 2003. Nitric oxide: The versatility of an extensive signal molecule. *Annu. Rev. Plant Biol.*, 54: 109-136.

Li, S.X. 2007. *Dry land Agriculture in China*. Science Press, Beijing, China.

Liu, Y.G. and J.H. Zhang. 2009. Rapid accumulation of NO regulates ABA catabolism and seed dormancy during imbibition in *Arabidopsis*. *Plant Signal. Behav.*, 4: 905-907.

Misra, A.N., M. Misra and R. Singh. 2011. Nitric oxide ameliorates stress responses in plants. *Plant Soil Environ.*, 57: 95-100.

Neill, S.J., R. Barros, J. Bright, R. Desikan, J. Hancock and J. Harrison. 2008. Nitric oxide, stomatal closure and abiotic stress. *J. Exp. Bot.*, 59: 165-176.

Ren, H., G. Zhihui, C. Lin, W. Kaifa, L. Jing, F. Yijuan, J.D. William, J. Wensuo and Z. Jianhua. 2007. Dynamic analysis of ABA accumulation in relation to the rate of ABA catabolism in maize tissues under water deficit. *J. Exp. Bot.*, 58: 211-219.

Rhodes, D. and A.D. Hanson. 1993. Quaternary ammonium and tertiary sulfonium compounds in higher-plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., 44: 357-384.

Richard, J.W. and R.M. 1945. Emily Spectrophotometric determination of small amounts of choline. *J. Biol. Chem.*, 159: 395-397

Ruan, H.H., W.B. Shen and L.L. Xu. 2004. Nitric oxide involved in the abscisic acid induced proline accumulation in wheat seedling leaves under salt stress. *Acta Bot. Sin.*, 46: 1307-1315.

Ruggiero, B., H. Koiwa, Y. Manabe, T.M. Quist, G. Inan, F. Saccardo, R.J. Joly, P.M. Hasegawa, R.A. Bressan and A. Maggio. 2004. Uncoupling the effects of abscisic acid on plant growth and water relations. Analysis of sto1/nced3, an abscisic acid-deficient but salt stress tolerant mutant in Arabidopsis. *Plant Physiol.*, 136: 3134-3147.

Sakamoto, A. and N. Murata. 2002. The role of glycinebetaine in the protection of plants from stress: clues from transgenic plants. *Plant Cell Environ.*, 25: 163-171.

Sarath, G., G. Hou, L.M. Baird and R.B. Mitchell. 2007. Reactive oxygen species, ABA and nitric oxide interactions on the germination of warmseason C4-grasses. *Planta* , 226: 697-708.

Shahbaz, M., Y. Masood, S. Perveen and M. Ashraf. 2011. Is foliar-applied glycinebetaine effective in mitigating the adverse effects of drought stress on wheat (*Triticum aestivum* L.)? *J. Appl. Bot. Food Qual.*, 84: 192-199.

Siddiqui, M.H., M.H. Al-Whaibi and M.O. Basalah. 2011. Role of nitric oxide in tolerance of plants to abiotic stress. *Protoplasma*, 248: 447-455.

Sithtisarn, S., P. Harinasut, S. Pornbunlualap and S. Cha-um. 2009. Accumulation of glycinebetaine and betaine aldehyde dehydrogenase activity in *Eucalyptus camaldulensis* clone T5 under *in vitro* salt stress. *Kasetsart J. (Nat. Sci.)*, 43: 146-152.

Taiz, L. and E. Zeiger. 2006. *Plant Physiology*, 4th edn. Sinauer Associates Inc. Publishers, Sunderland, MA, USA.

Tan, J.F., H.J. Zhao, J.P. Hong, Y.L. Han, H. Li and W.C. Zhao. 2008. Effects of exogenous nitric oxide on photosynthesis, antioxidant capacity and proline accumulation in wheat seedlings subjected to osmotic stress. *World J. Agri. Sci.*, 4: 307-313.

Torreilles, J. 2001. Nitric oxide: one of the more conserved and widespread signaling molecules. *Front. Biosci*, 6: 1161-1172.

Wang, J. and D.Q. Li. 2002. Effects of water stress on AsA-GSH cycle and H_2O_2 content in maize root. *Chin. J. Eco-Agri.*, 2: 94-96.

Wang, X.Y., W.B. Shen and L.L. Xu. 2004. Exogenous nitric oxide alleviates osmotic stress-induced membrane lipid peroxidation in wheat seedling leaves. *J. Plant Physiol. Mol. Biol.*, 30: 195-200.

Wojtaszek, P. 2000. Nitric oxide in plants: to NO or not to NO. *Phytochemistry*., 54: 1-4.

Zhang, L.X., M. Gao, S.Q. Li, S.X. Li and Z.S. Liang. 2011. Modulation of plant growth, water status and antioxidantive system of two maize (*Zea mays* L.) cultivars induced by exogenous glycinebetaine under long term mild drought stress. *Pak. J. Bot.*, 43(3): 1587-1594.

Zhang, L.X., S.X. Li and Z.S. Liang. 2009. Differential plant growth and osmotic effects of two maize (*Zea mays* L.) cultivars to exogenous glycinebetaine application under drought stress. *Plant Growth Regul.*, 58: 297-305.

Zhang, L.X., S.X. Li, H. Zhang and Z.S. Liang. 2007. Nitrogen rates and water stress effects on production, lipid peroxidation and antioxidative enzyme activities in two maize (*Zea mays* L.) genotypes. *J. Agron. Crop Sci*., 193: 387-397.

Zhang, L.X., X.F. Zhang, K. Wang, Y.G. Zhao, Y.Y. Zhai, M. Gao, Z.F An, J.C. Liu and J.J. Hu. 2012. Foliar urea application affects nitric oxide burst and glycinebetine metabolism in two maize cultivates under drought. *Pak. J. Bot.*., 44(1): 81-86.

Zhu, J. K. 2002. Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Biol.*, 53: 247-273.

(Received for publication 6 February 2012)