Pak. J. Bot., 43(6): 2869-2874, 2011.

ROLE OF NITRATE NUTRITION IN ALLEVIATION OF THE ADVERSE EFFECTS OF DROUGHT STRESS ON MAIZE CULTIVARS: BIOMASS PRODUCTION AND ANTIOXIDATIVE CAPACITY

LIXIN ZHANG[1,2*], KAI WANG[1], XIFENG ZHANG[1], LIXIA LU[3],YUNFEI LI[3], MEI GAO[1,2], CUIYUN WANG[1], JINJIANG HU[1] AND ZONGSUO LIANG[1,2]

[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
[3]College of Forestry, Northwest A & F University, 712100, Yangling, Shaanxi, P.R. China

Abstract

Optimal nitrogen (N) nutrition has been shown to alleviate the negative effects of drought stress (DS) on plants. The mechanisms of beneficial effect of nitrogen form are not conclusive. In this study, the effects of different ratios of nitrate (NO_3^-) to ammonium (NH_4^+) nutrition on the growth and oxidative damage of two maize cultivar i.e. Zhengdan 958 (ZD958) and Jundan 20 (JD20) were investigated under DS and non-DS in nutrient solution. The activities of superoxide dismutase (SOD) and catalase (CAT) increased, while that of peroxidase (POD) remained unchanged in ZD958 with supplies of NO_3^-: NH_4^+ ratios of either 100:0 or 50:50, while in NO_3^-: NH_4^+ ratio of 0:100 in ZD958 and all NO_3^-: NH_4^+ ratios in JD20 all the enzymes showed decreased activities compared to control. Furthermore, DS decreased biomass production, whereas increased the contents of superoxide radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), along with an enhanced accumulation of malondialdehyde (MDA) in the leaves of both cultivars. The above effects were greater in JD20 than those in ZD958. An increased ratio of NO_3^-: NH_4^+ in culture solution increased the activities of SOD, POD and CAT while decreased the production of $O_2^{\bullet-}$ and H_2O_2, thereby diminishing MDA accumulation, and increasing biomass production of drought-stressed plants of both cultivars. The above responses were pronounced in ZD958 than those in JD20. This study demonstrated that increased NO_3^--nutrition played a favored anti-oxidative metabolic role, as compared with NH_4^+-nutrition, in the plants thereby increasing tolerance to DS.

Introduction

Maize (*Zea mays* L.) is an important crop grown all over the world, and its yield is affected by a variety of environmental factors, such as drought stress (DS) (Li, 2007). Two third of total maize planting area in China is frequently subjected to delay in irrigation or DS, hence resulting in significant yield reductions (Lu *et al*., 2010). The DS tolerance in crops is largely dependent on the crop genotype, in particular the cultivar's sensitivity to DS (Zhang *et al.*, 2007a).

The environmental stress induces excessive generation of reactive oxygen species (ROS), such as superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) in plants (Ashraf, 2009; 2010; Bhutta, 2011). Reactive oxygen species can cause lipid peroxidation and even lead to the death of cells (Imlay, 2003). To alleviate the damage from ROS, plants evolve cellular adaptive responses like oxidative stress protectors. Antioxidant defense enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are the systems designed to minimize the concentration of $O_2^{\bullet-}$ and H_2O_2 (Mittler, 2002; Ashraf, 2009; Bhutta, 2011). Malondialdehyde (MDA) content, a measure of lipid peroxidation, is induced by large accumulation of ROS under stress. Therefore, activities of antioxidant enzymes and MDA content are suitable indicators to evaluate the degree of drought tolerance in crop plants (Imlay, 2003). Recent studies have shown that patterns of antioxidant enzymes and $O_2^{\bullet-}$, H_2O_2 and MDA production were associated with the severity of DS, cultivar, and development stage (Imlay, 2003; Zhang *et al.*, 2007b).

One of the factors influencing physiological responses of plants to DS is mineral nutrition (Li, 2007; Liua *et al.*, 2011). Nitrogen (N) has been shown to promote the growth and development of plants by enhancing antioxidative capacity under DS (Zhang *et al*., 2007a). However, impacts of N form on growth and its antioxidant responses in drought-stressed crop plants are rarely studied (Guo *et al*., 2007a; Li, 2007). Some evidence supported that single ammonium (NH_4^+) may be more helpful to increase the drought tolerance of rice (*Oryza sativa* L.) plants than single nitrate (NO_3^-) nutrition (Guo *et al.*, 2007b; Guo *et al*., 2008; Li *et al.*, 2009; Gao *et al*., 2010). As for maize, single NH_4^+ supplied under drying soil culture condition as well as mixed of NO_3^- and NH_4^+ under partial root-zone water stress both promoted plant growth (Mihailovic *et al.,* 1992; Wang *et al.,* 2009). However, the modulated mechanism of increased nitrate nutrition in alleviation of negative effects on maize in integrated root-zone DS are not fully understood. (Mihailovic *et al.*, 1992; Guo *et al*., 2007a; Li *et al*., 2009; Gao *et al*., 2010).

Thus, the objective of this study was to uncover the anti-oxidative mechanism of two maize cultivars to increased nitrate concentrations in solution culture imposed to root-zone DS.

Materials and Methods

Plant material and trial location: Solution culture experiments were performed in a growth chamber at the College of Life Sciences, Northwest A & F University, Yangling, P.R. China, using maize (*Zea mays* L.) cultivars Zhengdan 958 and Jundan 20. Cultivar Zhengdan 958 has a relatively greater drought resistance than Jundan 20 under the field experiments (Zhang *et al*., 2007b).

*Corresponding author E-mail: zhanglixinyangling88@yahoo.com.cn

Plant growth and experiment design: Seeds were immersed in 1% (w/v) Sodium hypochlorite solution for 30 min, soaked for 6 h in deionized water at 28°C, then transferred to sterile filter paper moistened with deionized water. After these treatments, seed germination was initiated in plastic trays at 28°C for 72 h in the dark, then placed into holes of styrofoam boards in deionized water in plastic boxes (26×18×12 cm) grown in nutrition solution in a growth chamber under the environmental condition of day/night temperature of 25/18°C, 60-70% relative humidity, 350 μmol $m^{-2}s^{-1}$ light intensity, and 16/8h of light/dark regime. Containers were covered with black plastic to exclude light exposure to the roots. The seedlings were grown in deionized water for the first 4 d, followed by 4 d of growth in half-strength nutrient solution (Hoagland & Arnon, 1938), and subsequently in full strength nutrient solution. The ratio of NH_4^+ to NO_3^- was 50:50. When seedlings attained three-leaf stage, drought stress (DS) treatment was imposed by adding 10% (w/v) polyethylene glycol (PEG-6000) dissolved in complete nutrient solution to achieve osmotic potentials(ψs) of -0.15 MPa (Guo *et al.,* 2007a). Complete nutrient solution without PEG-6000 served as non-DS (control). The sub-treatments were different ratios of NO_3^- to NH_4^+, i.e., 100:0 (N), 50:50 (NA), and 0:100 (A). In NH_4^+-containing nutrient solution, Ca^{2+} was supplied as $CaCl_2$. The pH of the nutrient solution adjusted daily to 6.30 ±0.05 by adding HCl or NaOH every day.

Plants were grown in 3.4 L pots in the growth chamber, which were sealed carefully to avoid evaporation, with a sponge wrapped around the interface of the roots and the shoots. Nitrification inhibitor dicyandiamide (DCD) was added to every pot to keep an identified condition. All treatments had four replicates. Desired PEG concentrations were maintained by irrigating sufficiently with new solution every 2 days. The solution was aerated for 12 h a day.

The experiment was carried out twice under the same environmental conditions. Data presented are the means of four replicates of the two experiments (n=8).

Sample harvest and observations recorded: Maize plants were harvested 12 d after the start of N treatments. Shoot samples were placed in an oven at 105°C for 15 min, and then dried to a constant weight at 75°C.

Drought index (DI) was calculated based on dry matter using the following relationship (Zhang *et al*., 2007a):

DI=YDS/YCK
YDS—average dry matter under drought stress condition
YCK —average dry matter under no drought stress.

All assays for measurement of antioxidant parameters were conducted using completely developed third or fourth leaf from the top of the plant. The leaves were cleaned in distilled water, surface moisture wiped, cut into small pieces, and 1.0g was mixed and homogenized in ice-cold 4 ml 50 mmol.L^{-1} phosphate buffer (pH 7.8) containing 1% PVP (V/V) and a little quartz sand with pre-chilled pestle and mortar. The homogenate was transferred to centrifuge tubes and centrifuged at 4°C for 20 min at 10,000 $_g$. The supernatant was used to measure antioxidant enzyme activities.

Superoxide dismutase (SOD) activity was estimated by recording the decrease in absorbance (560 nm) of superoxide-nitroblue tetrazolium complex by enzyme. One unit of enzyme activity was taken as the quantity of enzyme which reduced the absorbance reading of samples to 50% as compared to that without the enzyme (Dhindsa et al., 1981). Peroxidase (POD) activity was determined specifically with guaiacol at 470 nm and one unit of enzyme activity was taken as the rate of guaiacol which was oxidized in three minutes (Puter, 1974). Catalase (CAT) was assayed by measuring the residual H_2O_2 by tris-HCl reagent. Absorbance was recorded immediately at 240 nm every one minute in four minutes and one unit of enzyme determined the amount necessary to decompose 1 μmol of H_2O_2 per min at 25 °C (Dhindsa et al., 1981). The activities of all antioxidant enzymes were expressed as U mg^{-1}protein. Protein concentration of the crude extract was measured by the method of Gao (2000).

Malonaldehyde (MDA) was extracted with 10% trichloroacetic acid and determined at 450, 532 and 600 nm with 0.6% thiobarbituric acid as described by Gao (2000).

Superoxide radical ($O_2^{\bullet-}$) production was assayed according to the method of Wang & Lou (1990). One ml of enzyme extract as described above for SOD was mixed with 1 ml of 1 mM hydroxylammonium chlroride, and then incubated for 30 min at 30°C. One ml of incubated solution was then added to 1 ml of 17 mM 3-aminobenzenesulfonic acid and 1 ml of 7 mM 1-naphthylamine, and then further incubated for 20 min at 30°C. The absorbance of the solution was monitored at 530 nm. The $O_2^{\bullet-}$ production was expressed as nmol /g DW min.

Hydrogen peroxide (H_2O_2) content was determined following the procedure described by Mukherjec & Choudhuri (1983). Fresh leaf tissue (1.0 g each sample) was ground in cold acetone (10 ml) and centrifuged at 3000 *g* for 10 min. One ml of the supernatant was mixed with 0.1 ml titanium reagent and 0.2 ml of 17 M ammonia solution and then centrifuged at 3000 *g* for 10 min. The precipitate was washed five times with acetone by resuspension, drained, and dissolved in 3 ml of 1 M H_2SO_4. The absorbance of the solution was measured at 410 nm against blanks, which had been prepared similarly but without plant tissue. The H_2O_2 production was expressed as μmol /g DW.

Statistical analysis: All data were subjected to analysis of variance (ANOVA) using SAS software (Anon., 1996). The significance of the treatment effect was determined using *F*-test, and mean separation was analyzed by LSD test.

Results

Plant growth: Two week-old plants of two maize cultivars subjected to PEG-induced root-zone drought stress (IR-DS) showed a significant decrease in growth (Fig. 1). Compared with non-DS, shoot biomass (SB) of Jundan 20 (JD20) decreased by 31-54% under IR-DS. Their corresponding values were 24-46% in Zhengdan 958 (ZD958). Drought index (DI) of ZD958 was 0.53-0.76 while that of JD20 0.46-0.69. The above responses to IR-DS differed among the nitrogen forms. As a result, ZD958 maintained greater SB production and DI than JD20 under IR-DS except ratio of NO_3^- (0) to NH_4^+ (100) (Fig. 1).

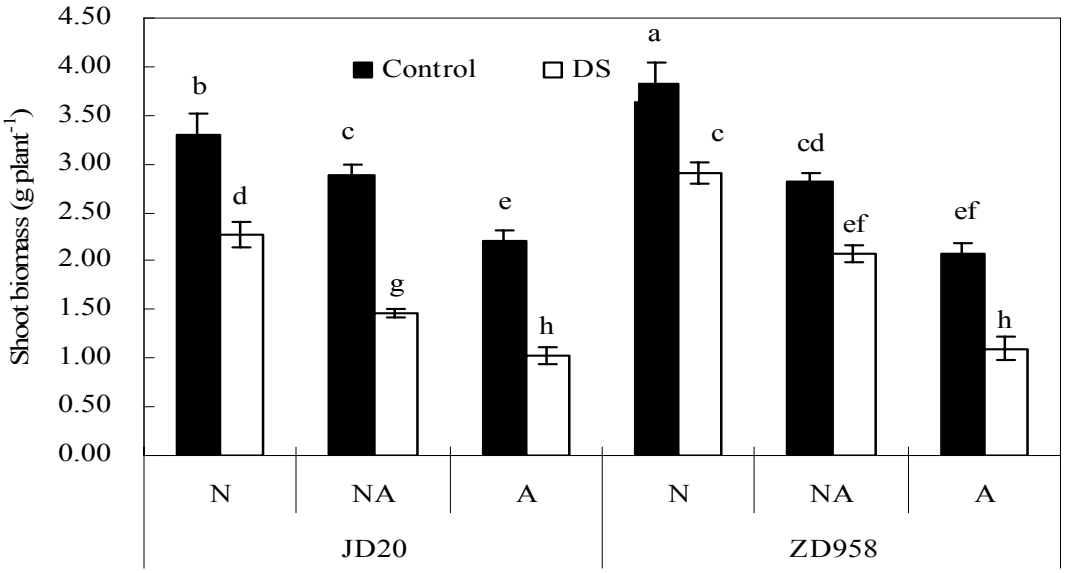

Fig. 1. Effects of increased nitrate nutrition and drought stress interaction on shoot biomass of maize plants at seedling stage (12 days after treatment)

Each values is the mean ± S.E. of eight replicates each treatment (n=8). JD20 and ZD958 represent Jundan 20 and Zhengdan 958 respectively. N, NA and A represent ratios of NO_3^-(100) to NH_4^+(0), NO_3^-(0) to NH_4^+(100), NO_3^-(50) to NH_4^+(50). DS and Control represent with drought stress and non-DS respectively.

At the top of each column, different letters indicate significant differences for shoot biomass among treatments. Mean values with the same letter within variables are not significantly different at the 0.05 level.

By comparison with ratio of NO_3^-(0) to NH_4^+(50) in the growth medium, ratios of NO_3^-(50) to NH_4^+(50) and NO_3^-(100) to NH_4^+(0) both more obviously increased SB of ZD958 than JD20 under drought. The above effects of ratio of NO_3^-(100) to NH_4^+(0) were superior to those of NH_4^+(50) and NO_3^-(100) with the same cultivar. The similar responses due to the above two ratios were also found under non-DS. However, the greater increments of SB in both cultivars occurred under IR-DS than that in non-DS with ratio of NO_3^-(0) to NH_4^+(100) (Fig. 1).

Activities of key antioxidant enzymes: The activities of superoxide dismutase (SOD) and catalase (CAT) increased in ZD958 with both supplies of NO_3^-: NH_4^+ ratios of either 100:0 0 or 50:50, while peroxidase (POD) remained constant from control to IR-DS. But with the ratio of NO_3^-: NH_4^+ of 0:100, all the enzymes in ZD958 showed decreased activities. With respect for JD20, all the enzymes exposed to all treatments of ratios of NO_3^-: NH_4^+ showed the reduced activity against IR-DS above non-DS (Table 1). Consequently, ZD958 documented higher values of the activities of these key antioxidant enzymes than those in cv. JD20 except ratio of NO_3^-(0) to NH_4^+(100) under IR-DS.

Table 1. Effects of increased nitrate nutrition and drought stress (DS) interaction on activities of SOD, CAT, POD (U mg^{-1} protein) and MDA content (μmol g^{-1} DM) of maize cultivars Jundan 20 (JD20) and Zhengdan 958 (ZD958) exposed to DS or no DS (Control) for 12 days.

Water regime	Cultivar	NO_3^- NH_4^+ ratio	SOD activity	POD activity	CAT activity t	MDA content
Control	JD20	N(100:0)	47.34 ± 2.06 b	29.12 ± 1.42 a	24.70 ± 1.22 b	7.33 ± 3.13 f
		NA(50:50)	46.19 ± 1.56 b	25.86 ± 2.92 ab	23.56 ± 1.76 bc	8.01 ± 2.67 f
		A(0:100)	41.37 ± 1.88 c	24.76 ± 2.48 bc	20.16 ± 0.74 c	11.03 ± 2.85 e
	ZD958	N(100:0)	46.00 ± 1.78 b	25.56 ± 1.54 b	23.08 ± 0.98 bc	6.96 ± 2.48 f
		NA(50:50)	43.64 ± 2.25 bc	24.12 ± 1.88 bc	21.68 ± 0.82 c	7.10 ± 1.20 f
		A(0:100)	39.19 ± 2.90 cd	22.76 ± 1.56 c	16.68 ± 0.72 d	12.01 ± 1.01 e
DS	JD20	N(100:0)	38.72 ± 1.98 d	17.64 ± 1.26 d	15.48 ± 1.09 d	22.36 ± 2.67 c
		NA(50:50)	31.69 ± 2.15 e	12.08 ± 0.72 e	12.34 ± 0.19 e	28.17 ± 2.94 b
		A(0:100)	24.58 ± 0.99 f	10.30 ± 0.56 f	10.26 ± 0.22 f	39.92 ± 2.48 a
	ZD958	N(100:0)	55.30 ± 2.28 a	26.32 ± 1.24 ab	29.26 ± 0.62 a	18.68 ± 2.21 d
		NA(50:50)	48.02 ± 1.95 b	24.05 ± 0.48 bc	24.58 ± 0.56 b	24.04 ± 2.02 c
		A(0:100)	31.20 ± 1.79 e	11.66 ± 0.50 f	10.44 ± 0.34 f	37.66 ± 3.40 a

Means in each column followed by different letters indicate significant difference at p<0.05.

The supplies of ratios of NO_3^- (50) to NH_4^+ (50) and NO_3^- (100) to NH_4^+ (0) induced a marked rise in all enzymes activities of both cultivars as compared with ratio of NO_3^- (0) to NH_4^+ (100) under IR-DS. Greater increments of these enzymes activities were recorded in ZD958 than JD20 when submitted to the above two increased ratios of NO_3^- to NH_4^+. In contrast, the increases became less under non-DS than those in IR-DS above ratio of NO_3^- (0) to NH_4^+ (100) (Table 1).

Accumulation of superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA): Compared with non-DS, accumulation of $O_2^{\bullet-}$, H_2O_2 and MDA in leaves greatly increased in JD20 than those in ZD958, which held lower production of reactive oxygen species (ROS) and weaker lipid peroxidation except ratios of NO_3^- (0) to NH_4^+ (100).

The above positive responses due to IR-DS on accumulation of $O_2^{\bullet-}$, H_2O_2 and MDA were all decreased by increased ratios of NO_3 to NH_4^+. Ratios of NO_3^- (50) to NH_4^+ (50) supplied plants had clearly lower accumulation of $O_2^{\bullet-}$, H_2O_2 and MDA as compared to ratios of NO_3^- (0) to NH_4^+ (100), while higher than ratios of NO_3^- (100) to NH_4^+ (0) in both cultivars. The decreased effects with increase in ratio of NO_3^- to NH_4^+ were more obvious in ZD958 than those in JD20. However, a less rise in these parameters in ratios of NO_3^- (100) to NH_4^+ (0) and NO_3^- (50) to NH_4^+ (50) occurred under non-DS than under IR-DS above ratio of NO_3^- (0) to NH_4^+ (100) (Fig. 2; Table 1).

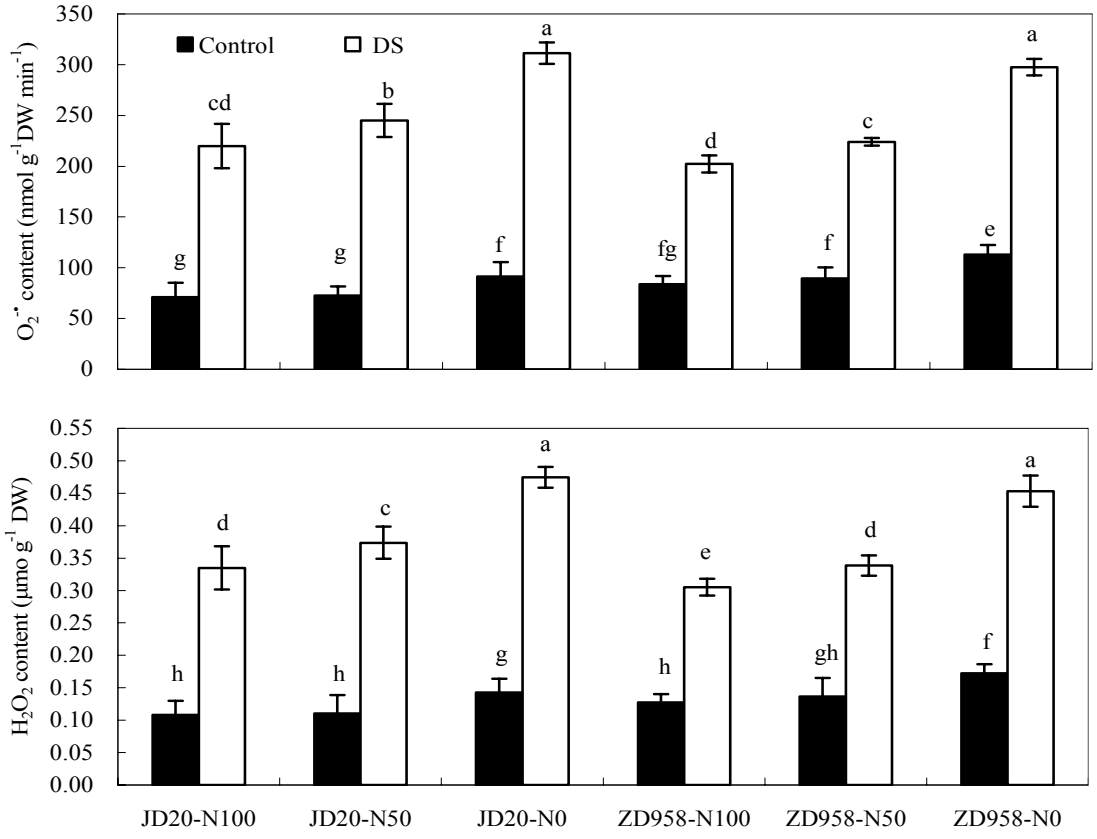

Fig. 2. Effects of increased nitrate nutrition and drought stress interaction on contents of $O_2^{\bullet-}$ and H_2O_2 in leaves of maize plants at seedling stage (12 days after treatment)

Each values is the mean ± S.E. of eight replicates each treatment (n=8). JD20 and ZD958 represent Jundan 20 and Zhengdan 958 respectively. N, NA and A represent ratios of NO_3^-(100) to NH_4^+(0) , NO_3^-(0) to NH_4^+ (100), NO_3^-(50) to NH_4^+(50). DS and Control represent with drought stress and non-DS respectively.

At the top of each column, different letters indicate significant differences for contents of $O_2^{\bullet-}$ and H_2O_2 among treatments. Mean values with the same letter within variables are not significantly different at the 0.05 level.

Interaction of maize cultivar (Cv), water regime (W) and N form (NF) for all parameters: Analysis of variation showed that *F* values for all parameters in NF treatment were less than W treatment while greater than Cv treatment. Moreover, *F* values due to interactions of W ×Cv, W×NF and Cv×NF as well as Cv×W×NF were also significant for most parameters except contents of $O_2^{\bullet-}$ and H_2O_2 due to Cv×NF and Cv×W×NF as well as CAT activity and MDA content due to Cv×W×NF (Table 2).

Correlations: Correlations coefficients for IR-DS amongst all the traits were higher than in non-DS. The significant coefficients of SB and contents of H_2O_2 and MDA as well as activities of POD and CAT were even disappeared under non-DS (Table 3).

Table 2. Analysis of variance for shoot biomass (SB, g^{-1} plant); contents of $O_2^{\bullet-}$ (nmol g^{-1} DW min^{-1}) and H_2O_2 (µmol g^{-1} DW); activities of SOD, CAT, POD (U mg^{-1} protein); and MDA content (µmol g^{-1}DM) of two maize cultivars subject to drought stress (DS) or non-DS for 12 days at different N nutrition (i.e. NO_3^- : NH_4^+ ratios of 100:0, 50:50, and 0:100).

Source of variation	Water regime (W)	Cultivar (Cv)	N form (NF)	W×Cv	W×NF	Cv×NF	Cv×W×NF
d.f.	1	1	2	1	2	2	2
SB	3695.18***	257.76***	2480.45***	95.56***	3.59*	104.11***	24.91***
$O_2^{\bullet-}$ content	12566.30***	4.02*	603.53***	141.10***	196.25***	1.95	0.61
H_2O_2 content	13201.70***	4.60*	643.20***	147.27***	217.87***	2.40	1.87
SOD activity	9795.68***	6.57*	957.82***	110.91***	20.92***	30.60***	5.31*
POD activity	3571.65***	4.38*	253.49***	146.04***	36.44***	5.81**	4.43*
CAT activity	4943.88***	18.10***	475.65***	131.27*	6.31*	13.23***	3.01
MDA content	6723.78***	51.59***	792.45***	45.79***	283.89**	5.47*	0.32

*, **, *** Significance at 5%, 1% and 0.1% level of significance, respectively

Table 3. Correlation coefficients of shoot biomass (SB, g $plant^{-1}$); contents of $O_2^{\bullet-}$ (nmol g^{-1} DW min^{-1}) and H_2O_2 (µmol g^{-1} DW); activities of SOD, CAT, POD (U mg^{-1} protein); and MDA content (µmol g^{-1} DM) of two maize cultivars under drought stress (DS) (above diagonal) and non-DS (below diagonal).

Character	SB	$O_2^{\bullet-}$ content	H_2O_2 content	SOD activity	POD activity	CAT activity	MDA content
SB		-0.885***	-0.873***	0.947***	0.985***	0.976***	-0.880***
$O_2^{\bullet-}$ content	-0.603*		0.995***	-0.814***	-0.835***	-0.913***	-0.993***
H_2O_2 content	-0.441	0.987***		-0.791***	-0.825***	-0.900***	-0.996***
SOD activity	0.560*	-0.669**	-0.553*		0.935***	0.952***	-0.785***
POD activity	0.489	-0.545*	-0.404	0.819***		0.967***	-0.830***
CAT activity	0.401	-0.842***	-0.738***	0.937***	0.820***		-0.898***
MDA content	-0.463	0.675**	0.764***	-0.293	-0.056	-0.387	

*, **, *** significance at 5%, 1% and 0.1 % level of significance, respectively

Discussion

Plant response to DS evaluated based on plant growth and drought index (DI) is cultivar dependant (Zhang *et al.*, 2007a). Numerous studies have shown that maize plants are sensitive to DS, as evident from reduced leaf expansion and cell division when subjected to DS (Mihailovic *et al.*, 1992; Zhang *et al.*, 2007a; Wang *et al.*, 2009; Lu *et al.*, 2010). Drought Index (DI) of ZD958 (0.53-0.76) was greater than that of JD20 (0.46-0.69), which indicates that the former is more drought tolerant than the latter. The shoot biomass (SB) of DS plants was greater for ZD958 than that of JD20 at increased NO_3^- concentrations in the nutrient solution i.e. NO_3^-: NH_4^+ ratio of 50:50 or 100:0 (Fig. 1). Similar response was also reported for these cultivars in a field experiment (Lu *et al.*, 2010).

Nitrogen is the most important nutrient for plant growth and productivity (Li, 2007; Liua *et al.*, 2011). Additionally, N modulates the drought resistance mechanism of plants, thus, contributing to plant growth and development under drought (Guo *et al.*, 2007a; Zhang *et al.*, 2007a). These responses were associated with N form and severity of water stress (Li, 2007). Previous studies focus on the responses of rice to N form under water stress. The NH_4^+ nutrition resulted in a greater biomass of rice seedlings than that of the plants that received NO_3^- nutrition in solution culture experiments (Guo *et al*., 2002; Guo *et al.*, 2007b; Guo *et al.*, 2008; Li *et al.*, 2009; Gao *et al.*, 2010). Mihailovic *et al.* (1992) concluded that in the NH_4^+-form of N, maize plants maintained higher turgor pressure during the drought by better osmotic adaptation in a pot experiment with the quantities of N in available form in the soil before subjecting to N 150mg/100g soil (0mg/100g of NH_4^+-N, 20 mg/100 g of NO_3^--N and 130 mg/100 g of organic N). Wang *et al.* (2009) stated that maize plant growth was promoted by mixed N source, but water use efficiency of the plants subjected to partial root-zone water stress improved with NH_4^+-N nutrition. Indeed, SB weights of both cultivars were greater with NO_3^- : NH_4^+ ratio of 100:0 and 50:50 than that of 0:100 across DS as well as non-DS treatments (Fig. 1). These results demonstrate the beneficial role of NO_3^- form of N on maize plant growth as compared with that of NH_4^+ form of N. This is not simply the N nutritional role, instead it appears that NO_3^- nutrition under DS holds anti-drought ability to improve water relations and promote plant growth under DS (Crawford, 1995; Scheible *et al*., 1997; Table 1).

In higher plants, drought damage is characterized by production of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) and antioxidant defense enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), resulting in lipid peroxidation, and even leading to the death of cells (Imlay, 2003; Ashraf, 2009; 2010). It is necessary to improve the drought tolerance in plants by selection and breeding for tolerance characteristics as well as application of efficient N nutrition to improve growth and enhance yield (Li, 2007; Zhang *et al.*, 2007b).

Different crop cultivars maintain different antioxidant ability under DS, which are significantly affected by different nitrogen forms (Li, 2007; Guo *et al.*, 2007a). Our studies have elucidated that DS stimulated greater

production of $O_2^{\bullet-}$ and H_2O_2 and more serious reduction in activities of SOD, POD and CAT in a drought sensitive JD20 than those in tolerant ZD958 cultivar across all NO_3^-:NH_4^+ treatments. Antioxidant ability of ZD958 was stronger than that of the JD20 under DS (Zhang *et al*., 2007a; Fig. 1; Fig. 2; Table 1).

The mechanism of the effects of different N forms application on antioxidant responses in drought-stressed crop plants are rarely investigated (Mihailovic *et al*., 1992; Guo *et al*., 2007a; Guo *et al*., 2007b; Guo *et al*., 2008; Li *et al*., 2009; Gao *et al*., 2010). In the present study, the negative effects of DS decreased with increasing ratio of NO_3^- to NH_4^+ across both cultivars. Plants subjected to NO_3^- to NH_4^+ ratio of 50:50 or 100:0 showed greater enzyme activities and decreased H_2O_2 and $O_2^{\bullet-}$ contents in both cultivars as compared with those of the plants receiving only NH_4^+ form N. The above changes were greater with NO_3^-: NH_4^+ ratio of 100:0 as compared to 50:50. Furthermore, NO_3^- nutrition-induced alleviation of DS effects were greater for ZD958 than those for JD20.

Analysis of variation (ANOVA) indicated the shoot biomass (SB) of maize cultivars was significantly influenced by the DS, N forms, and antioxidative capacity parameters (Table 2). The impact of nitrogen form below DS while over cultivar treatment showed that nitrogen form should be matched to water regime which is also associated with a selected cultivar. Furthermore, correlations among the biomass production, and antioxidant metabolism were greater for the plants subjected to DS than those of the plants under non-DS. For the plants under non-DS, the correlation between the biomass and H_2O_2 and MDA contents, and the activities of POD and CAT were non-significant (Table 3). These results suggest that the activities of SOD, POD and CAT and contents of H_2O_2, $O_2^{\bullet-}$ and MDA are the most important traits for plants' ability to survive under DS (Zhang *et al*., 2007a; Fig 2; Table 1). Increased NO_3^- nutrition enhanced antioxidative capacity and improved plant growth by enhancing antioxidant enzymes activities thereby reducing ROS (H_2O_2 and $O_2^{\bullet-}$) in two drought-stressed maize cultivars.

Acknowledgements

This study was made possible by generous support by the China Postdoctoral Science Foundation, Chinese Universities Scientific Fund (QN2009069), and Foundation of State Key Laboratory of Soil Erosion and Dryland Farming (10501-J-2).

References

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

Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnol. Adv.*,27: 84-93.

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

Bhutta, W.M. 2011. Antioxidant activity of enzymatic system of two different wheat (*Triticum aestivum* L.) cultivars growing under salt stress. *Plan, Soil Environ.*, 57: 101-107.

Crawford, N.M. 1995. Nitrate: nutrition and signal for plant growth. *Plant Cell,* 7: 859-868.

Dhindsa, R.S., P. Plumb-Dhindsa and T.A. Throne. 1981. Leaf senescence: correlation with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.,* 32:93-101.

Gao, J.F. 2000. *Experiment Technique of Plant Physiology*. Xi'an World Books Press Company, Xi'an,China.

Gao, Y.X., Y. Li, H.J. Li, Q.R. Shen and S.W. Guo. 2010. Ammonium nutrition increased water absorption of rice seedlings (*Oryza sativa* L.) under water stress. *Plant Soil,* 331:193-201.

Guo, S., Y. Zhou, Q. Shen and F. Zhang, 2007a. Effect of ammonium and nitrate nutrition on some physiological processes in higher plants-growth, photosynthesis, photorespiration, and water relations. *Plant Biol.,* 9:21-29.

Guo, S., G.Y. Chen, Y. Zhou and Q. Shen. 2007b. Ammonium nutrition increases photosynthesis rate under water stress at early development stage of rice (*Oryza sativa* L.). *Plant Soil,* 296:115-124.

Guo, S., Y. Zhou, Y. Li , Y. Gao and Q. Shen. 2008. Effects of different nitrogen form and water stress on water use efficiency of rice plants. *Ann. Appl. Biol.,* 153:127-134.

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.

Imlay, J.A. 2003. Pathways of oxidative damage. *Ann. Rev. Microbiol.,* 57 :395-418.

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

Li, Y., Y.X. Gao, L. Ding, Q.R. Shen and S.W. Guo. 2009. Ammonium enhances the tolerance of rice seedlings (*Oryza sativa* L.) to drought condition. *Agr. Water Manage.,* 96 :1746-1750.

Liua, W.K., Q.C. Yanga, L.F. Dub, R.F. Chenga and W.L. Zhoua, 2011. Nutrient supplementation increased growth and nitrate concentration of lettuce cultivated hydroponically with biogas slurry. *Acta Agr. Scand. B – S. P.,* 61:391-394.

Lu, G.H., D.L. Ren, X.Q. Wang, J.K. Wu and M.S. Zhao. 2010. Evaluation on drought tolerance of maize hybrids in China. *J. Maize Sci.,* 3 :20-24.

Mukherjec, S.P. and M.A. Choudhuri. 1983. Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in vigna seedlings. *Physiol. Plantarum,* 58: 166-170.

Mihailovic, N., G. Jelic, R. Filipovic, M. Djurdjevic and Z. Dzeletovic. 1992. Effect of nitrogen form on maize response to drought stress. *Plan. Soil,* 144:191-197.

Putter, J. 1974. Peroxidases. In: *Methods of Enzymatic Analysis*. (Ed): H.U. Bregmeyer. Academic Press, New York. pp. 685-690.

Scheible, W.R, A. Gonzalez-Fontes, M. Lauerer, B. Muller-Rober, M. Caboche and M. Stitt. 1997. Nitrate acts as a signal to induce organic acid metabolism and repress starch. *Plant Cell,* 5:783-798.

Wang, A.G. and G.H. Lou. 1990. Quantitative relation between the reaction of hydroxylamine and superoxide anion radicals in plants. *Plant Physiol. Comm.,* 6:55-57.

Wang, H.H., L.Z. Shu, X.J. Zhou, P.F. Zhu and F.D. Liu. 2009. Regulation and the mechanisms of nitrogen form on water utilization of maize seedlings under fixed partial root-zone water stress. *Chin. Agric. Sci. Bull.,* 18:155-160.

Zhang, L.X., S.X. Li, H. Zhang and Z.S. Liang. 2007a. 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, Y.K., L.X. Wang, J.H. Yang, D.J. Liang, X.L. Wang and L.Y. Xi. 2007b. China maize potential yield developing technique advanced. *Chin. Agri. Sci. Bull.*, 7: 267-269.

(Received for publication 4 February 2011)