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EFFECTS OF NITROGEN FORMS AND DROUGHT STRESS ON GROWTH, PHOTOSYNTHESIS, AND SOME PHYSICO-CHEMICAL PROPERTIES OF STEM JUICE OF TWO MAIZE (*ZEA MAYS* L.) CULTIVARS AT ELONGATION STAGE

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Abstract

Drought stress (DS) usually causes a serious yield reduction in maize production. Nitrogen (N) has been reported to be able to alleviate DS damage in previous studies; however, which N form is beneficial for plant growth in drought-stress maize and its mechanism is still poorly understood. The objective of this study is to examine the effects of different nitrogen forms on growth, photosynthesis and stem juice characteristics of maize (*Zea mays* L.) under drought, and find out better and convenient measurers for drought resistance ability of the C_4 crop. Pot experiments were carried out using two maize cultivars (Zhengdan 958 and Jundan 20) under integrated root-zone drought stress (IR-DS) by irrigating 10% PEG-6000 solution and non-DS conditions grown for 33 days, and their tested indexes were determined at elongation stage. Dry matter (DM), net photosynthetic rate (Pn), stomatal conductance (*g*s) and oxidation-reduction potential (ORP) in stem juice were decreased while pH, electric conductivity (EC), soluble protein concentration (SPC) and ABA concentration (ABAC) in stem juice increased of both cultivars in all N forms treatments under IR-DS above non-DS. However, the responses of two examined cultivars to DS were different: significantly higher DM production, Pn and drought index (DI) were observed for ZD958 than JD20, therefore the former could be treated as a drought tolerance cultivar comparatively. Better correlations were obtained amongst the above parameters, especially for DM, Pn and pH, EC, ORP, ABAC in stem juice under IR-DS than non-DS. By comparison with sole ammonium (NH_4^+), sole nitrate (NO_3^-) and the mixed supply of NH_4^+ and NO_3^- both obviously improved DM and Pn as well as pH, EC, SPC and ABAC whereas decreased Gs and ORP in both drought-stressed cultivars. The effects of NO_3^--supplied were more evident than NH_4^++NO_3^--supplied in the above responses. These impacts were superior in ZD958 than JD20. Further analysis of variation indicated that the impact of N form treatment on almost all parameters measured were, in general, less than those of water regime. It is, therefore, concluded that pH, EC and ORP in stem juice could be regarded better and convenient indicators for evaluating drought resistance ability of the C_4 crop. The NO_3^- supplied could be more beneficial for enhancing photosynthesis and plant growth by improving stem juice characteristics than NH_4^+ supplied, especially for a drought tolerant cultivar.

Introduction

Maize (Zea mays L.) is a C_4 cereal species which is widely planted in northen China. However, the crop is frequently subjected to delay in irrigation or drought stress (DS) to cause a significant yield reduction due to its considerable sensitivity to drought (Ashraf *et al*., 2007; Jabeen *et al.*, 2008; Lu *et al.*, 2010; Ali *et al*., 2012). The more sensitive to DS a variety is, the more serious yield reduction it will experience (Chandrasekar *et al.*, 2000; Zhang *et al.*, 2007a). Drought stress is one of the main constraints, which may impair growth and hence ultimately the crop productivity. Thus, it is necessary to improve the drought tolerance in plants by selection and breeding for drought tolerance characteristics as well as by application of efficient nitrogen nutrition (Li, 2007; Ashraf, 2010).

To mitigate the deleterious effects of DS on regular metabolism and ensure crops under optimal growth conditions, plants have evolved various strategies to counteract this problem. By necessity, plants possess one mechanism of an increase of pH and ABA concentration in xylem sap which occurs in many crop plants such as maize. This results in reduction of stomatal opening (*g*s), thereby conserving water in plants, and consequently improving leaf photosynthesis and plant growth (Taiz & Zeiger, 2002; Goodger, 2005; Bai, *et al*., 2006; Alvarez *et al*., 2008; Ullah *et al*., 2008; Sharp & Davies, 2009; Ernst *et al*., 2010, Pinheiro & Chaves, 2010). The pH and ABA concentration in xylem sap might be regarded as a good measure for evaluating the drought resistance of crop plants (Borel & Thierry, 2002; Goodger, 2005; Ernst *et al.*, 2010). However, extraction of xylem sap is tedious and requires pressure chamber apparatus, thus, it may not be feasible for most DS responses studies. As an alternative, physic-chemical properties of stem juice were examined in the response of C_4 sugarcane (*Saccharum officinarum*) to DS (Moore, 1995). The patterns of changes in physical and chemical characters of stem juice i.e. pH, electric conductivity (EC), oxidation-reduction potential (ORP), soluble protein and ABA were covered in this study of drought resistance assessment (Borel & Thierry, 2002; Goodger, 2005; Ernst *et al.*, 2010).

In several previous studies, it has been noticed that plant growth and photosynthesis could be improved by increased N application in drought-stressed crop plants (Li, 2007; Taiz & Zeiger, 2002; Zhang *et al.*, 2009a,b). Nitrogen addition might affect nitrate concentration in xylem sap, which plays an important role in affecting proton pump activity resulting in pH change and other physical and chemical traits such as SPC and BAC which affect *g*s (Wilkinson *et al.*, 2007). The change pattern of SPC and ABAC as well as electric conductivity (EC), oxidation-reduction potential (ORP) in stem juice of C_4 crop under drought have not been focused well in past research (Sharp & Davies, 2009; Ernst *et al*., 2010). However, the exhibited responses of N during modulation of drought are closely dependent on different crops with different N forms and cultured method (Guo *et al.*, 2007a). There is some evidence of an influence of N form on plant response to DS, which mainly focuses on rice. These studies demonstrated that under water stress conditions, the growth, photosynthentic rate and water uptake in rice seedlings supplied with NH_4^+ were higher than those with NO_3^- (Guo *et al.*, 2007b; Guo *et al.*, 2008; Li *et al.*, 2009; Gao *et al.*, 2010). In maize, Mihailovic *et al.* (1992) and Wang *et al.* (2009) reported that plant growth of maize was promoted by NH_4^+ using pot culture method under soil drought, and mixed nitrogen source using solution culture method under partial root-zone water stress, respectively. But, up to now, there was little information and unclear conclusion about the effect of N forms on growth, photosynthesis and stem juice characteristics of maize crop cultivars with differential drought tolerance under integrated root-zone DS (IR-DS) (Mihailovic *et al*., 1992; *Guo et al*., 2007a,b; Hamidou *et al.*, 2007; Guo *et al.*, 2008; Mérigout *et al*., 2008; Li *et al*., 2009; Gao *et al.*, 2010). Additionally, effectiveness of N form has not been clarified under both DS and non-DS in the same trial, which could be mainly due to its nutritive role or physiological anti-drought function (Guo *et al.*, 2007a; Li, 2007).

Keeping in view the above facts, we hypothesize that higher constitutive photosynthesis and stem juice characteristics employed by NH_4^+-plant or NO_3^--plant of maize cultivars provide a mechanism of tolerance to different cultivars under DS in terms of application of different N forms. With this aim, we designed pot experiments to clarify the responses of two maize cultivars to N form with respect to plant growth, photosynthesis and stem juice characteristics under IR-DS.

Materials and Methods

Plant material and trial location: Pot experiments were performed in the growth chambers at College of Life Sciences of Northwest A & F University (Yangling, P.R. China). The seed of two maize (*Zea mays* L.) cultivars (Zhengdan 958 and Jundan 20) was supplied for the present experiments by Agronomy College of the same University (Zhang *et al*, 2007b).

Plant growth and experiment design: Seeds were surface-sterilized in 1% (w/v) sodium hypochlorite solution on a magnetic stirrer for 20 min and thoroughly rinsed with sterile deionized water, then transferred to two sheets of sterile filter paper moistened with deionized water after swelling them in deionized water at 28°C for 6 h. In succession, seeds were placed in plastic trays for germination at 28°C for 72 h in the dark, then were sown into holes of styrofoam boards in deionized water in plastic boxes (26×18×12 cm) grown hydroponically in the growth chamber under the conditions with 25/18°C of average day/night temperature, 60-70% relative humidity, and 350μmolm^{-2}s^{-1} light intensity and 16/8h of light/dark regime. Containers were covered with black plastic to exclude light from the roots. Four and eight days after placement of the seedlings in deionized water, the deionized water was replaced by one-half-strength and complete nutrient solution (Hoagland & Arnon, 1938), respectively, which contains to all essential minerals with a mixture of NH_4^+ and NO_3^- (the ratio of NH_4^+ to NO_3^- is 50:50) for plant growth and all solutions were made up of distilled water. The pH of the nutrient solution was adjusted to 6.30 (±0.05).

Three seedlings at the stage of three-leaf were grown in plastic pots (3.8 L) in the growth chamber. Each pot was filled to 20 cm with quartz sand and supplied with distilled water. The sand was leached with 1 N HCl, flushed with distilled water, and sterilized in an oven at 180°C for 30 h. Two days after replacement, seedlings were thinned to one of a uniform size per pot. Treatments were then initiated, and all pots immersed in nutrient solutions. Drought stress (DS) treatment was served by irrigating 10% (w/v) polyethylene glycol (PEG-6000) dissolved in complete nutrient solution to achieve osmotic potentials (ψs) of -0.15 MPa determined by a vapor pressure Wescor 5500 osmometer for 33 days (Zhang *et al*., 2009b). Complete nutrient solution without PEG-6000 served as non-DS (control, CK). For each water treatment, the seedlings were irrigated as different N form solution i.e. NH_4^+-N supplied as $(NH_4)_2SO_4$, NO_3^--N supplied as KNO_3 and Ca $(NO_3)_2$, and the mixed nutrition (NH_4^++NO_3^--N, the ratio of NH_4^+ to NO_3^- is 50:50) supplied as NH_4NO_3. In NH_4^+-N-containing nutrient solution, Ca^{2+} was supplied as $CaCl_2$. Thus, the six treatments were: NH_4^+ (CK-A), NO_3^- (CK-N), NH_4^+ and NO_3^- (CK-AN), NH_4^++PEG (DS-A), NO_3^-+PEG (DS-N), and NH_4^+ and NO_3 + PEG (DS-AN). The pH of the nutrient solutions used was controlled at 6.30 ±0.05 by adding HCl or NaOH. A nitrification inhibitor dicyandiamide (DCD) was added to the irrigation solution for every pot to keep an identified condition. All treatments had four replicates, with a random design. Desired PEG concentrations were maintained by irrigating sufficiently with fresh solution every two days.

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

Sample harvest and observation recorded in dry matter and photosynthesis: Aboveground dry matter at elongation stage (33 days after sowing) was measured. Drought index (DI) was estimated based on procedure described by Zhang *et al*. (2007). Photosynthesis parameters were measured using the second completely developed leaf from the top of sample plant before harvest from 9:00 to 11:00 h, respectively using LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA). The 2 leaves of one plant were determined and repeated three times. The determination covered net photosynthetic rate (Pn, μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (*g*s, mol H_2O m^{-2} s^{-1}) under the conditions of photosynthetically active radiation (*PAR*) of 1200 μmol m^{-2} s^{-1} and ambient CO_2 concentration of 360 μmol mol^{-1}. The leaf temperature was 25.5±2°C, and the relative humidity in the leaf chamber was roughly 45% throughout the measurements (Gao, 2000).

Stem juice characteristics measurement: All samplings were taken from the middle part of stem for each plant. An electric crusher was used to extract the stem juice. The extracted juice was then filtered through six layers of cheesecloth, and the stem juice parameters described below were measured.

The pH, electric conductivity (EC, μS cm^{-1}) and oxidation-reduction potential (ORP, mv) were measured using pH meter and DDA-11 electric conductivity meter, respectively. Soluble protein concentration (mg ml^{-1}) of the crude extract was measured by colorimetric method of Coomassie Brilliant Blue (Gao, 2000). The ABA concentration (μmol ml^{-1}) was assayed by the method of enzyme-linked mmunosorbent assay (ELISA) as described by Weiler (1982).

Statistical analysis: All data were subjected to analysis of variance (ANOVA) using the SAS software package (SAS Institute Inc., Cary, NC, USA, 1996). Appropriate standard errors of the means (SE) were calculated for presentation with table and line diagrams. The significance of the treatment effect was determined using *F*-test, and to determine the significance of the means at the 0.05 level the LSD (t) range test was employed.

Results

Plant growth and photosynthesis: Negative effects of integrated root-zone drought stress (IR-DS) on maize growth and photosynthesis were observed in all N form treatments. In the present work, plant growth and photosynthesis were measured in terms of dry matter (DM) and net photosynthesis rate (Pn) (Fig. 1). Compared with non-DS, DM of Jundan 20 (JD20) and Zhengdan 958 (ZD958) decreased by 31-56% and 18-46% under IR-DS, respectively. The corresponding values decreased of Pn were by 38-50% and 34-43%. Additionally, drought index (DI) of ZD958 was 0.53-0.77 while that of JD20 0.44-0.72. The above responses to IR-DS differed among the nitrogen forms. As a result, ZD958 maintained greater DM production, Pn and DI than those of JD20 under IR-DS with the same N form (Fig. 1).

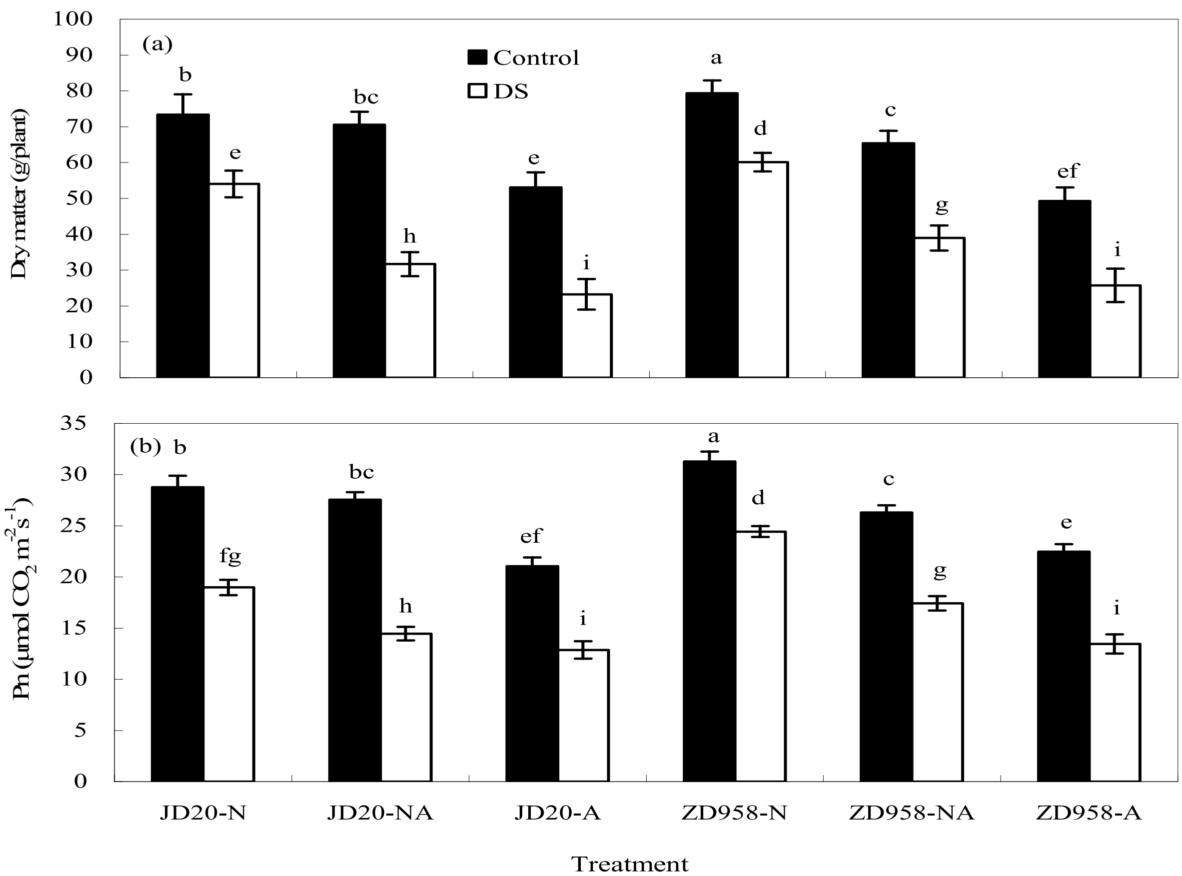

Fig. 1. Effects of nitrogen form and drought stress interaction on dry matter (a) and net photosynthetic rate (Pn) (b) of maize plants at elongation stage (33 days after treatment)

Each values is the mean±S.E. of eight replicates each treatment (*n*=8). JD20-N, JD20-NA, JD20-A , and ZD958-N, ZD958-NA, ZD958-A represent Jundan 20 and Zhengdan 958 supplied by nitrate (NO_3^-), ammonium (NH_4^+), the mixed supply (NH_4^++NO_3^-, the ratio of NH_4^+ to NO_3^- is 50:50) respectively. DS and Control represent drought stress and non- drought stress respectively. At the top of each column, different letters indicate significant differences for dry matter and Pn respectively among treatments. Mean values with the same letter within variables are not significantly different at the 0.05 level.

Comparison with sole ammonium (NH_4^+), the sole nitrate (NO_3^-) and the mixed supply of nitrate and ammonium (NH_4^++NO_3^-) both obviously increased DM and Pn under drought. The increased effects of NO_3^--supplied were superior to those of NH_4^++NO_3^--supplied to the same cultivar. These responses were more predominant in ZD958 than JD20 when NO_3^- and NH_4^++NO_3^- supplied than those when only NH_4^+ supplied. The similar responses due to N form were also found under non-DS. However, the greater increments of DM and Pn in both cultivars due to NO_3^- and NH_4^++NO_3^- treatments occurred under IR-DS than in non-DS with NH_4^+ treatment (Fig. 1).

Stem juice characteristics and stomatal conductance (*g*s): The pH, electric conductivity (EC), oxidation-reduction potential (ORP), soluble protein concentration (SPC) and ABA concentration (ABAC) might be better parameters of evaluation of stem juice characteristics of a C_4 crop at elongation stage. The IR-DS treatment induced a greater increase in pH, EC, SPC and ABAC and decrease in ORP and *g*s in ZD958 than those in JD20. Such higher pH, EC, SPC and ABAC, and lower ORP and *g*s were recorded in drought-stressed ZD958 than JD20 except at NH_4^+-treatment. In non-DS condition, however, there were no significant differences between cultivars for all N form treatments (Table 1).

Table 1. Effects of nitrogen form and drought stress interaction on pH, EC (electric conductivity, µS cm^{-1}), ORP (oxidation-reduction potential, mv), soluble protein concentration (SPC, mg ml^{-1}) and ABA concentration (ABAC, µmol ml^{-1}) in stem juice and gs (mol H_2O m^{-2} s^{-1}) of maize plants at elongation stage (33 days after treatment).

Treatment		pH	EC	ORP	SPC	ABAC	*g*s
Drought stress (DS)							
JD20	DS-N	6.63 ± 0.09 b	542 ± 25 b	63 ± 2 de	2.68 ± 0.12 b	44.18 ± 2.71 b	0.154 ± 0.027 e
	DS-AN	5.96 ± 0.06 d	479 ± 16 d	70 ± 5 c	1.86 ± 0.06 d	35.01 ± 1.19 d	0.177 ± 0.012 d
	DS-A	5.74 ± 0.06 e	451 ± 12 e	76 ± 2 b	1.48 ± 0.05 e	28.91 ± 1.41 e	0.201 ± 0.010 c
ZD958	DS-N	6.89 ± 0.09 a	581 ± 18 a	58 ± 3 e	2.88 ± 0.09 a	49.64 ± 1.88 a	0.135 ± 0.019 g
	DS-AN	6.38 ± 0.12 c	521 ± 12 c	66 ± 2 d	2.35 ± 0.03 c	39.11 ± 2.63 c	0.156 ± 0.006 e
	DS-A	5.78 ± 0.08 e	468 ± 17 e	73 ± 4 c	1.61 ± 0.13 e	27.27 ± 1.63 e	0.205 ± 0.010 c
Non-drought stress (non-DS,CK)							
JD20	CK-N	5.69 ± 0.12 e	256 ± 12 f	79 ± 2 b	0.89 ± 0.09 f	18.18 ± 1.70 g	0.295 ± 0.007 a
	CK-AN	5.48 ± 0.09 ef	234 ± 19 g	85 ± 4 b	1.05 ± 0.01 ef	18.78 ± 2.90 fg	0.276 ± 0.012 ab
	CK-A	5.41 ± 0.04 ef	236 ± 11 g	88 ± 3 ab	1.09 ± 0.14 ef	20.48 ± 0.28 f	0.268 ± 0.009 b
ZD958	CK-N	5.83 ± 0.09 de	262 ± 12 f	80 ± 5 b	0.99 ± 0.09 f	17.88 ± 1.71 g	0.308 ± 0.017 a
	CK-AN	5.42 ± 0.04 ef	233 ± 9 g	86 ± 4 ab	1.03 ± 0.05 ef	17.64 ± 0.96 g	0.278 ± 0.010 ab
	CK-A	5.53 ± 0.13 ef	235 ± 13g	81 ± 3 b	1.13 ± 0.12 e	20.77 ± 2.32 f	0.260 ± 0.014 b

Plants were supplied with sole nitrate (NO_3^-), the mixed supply (NH_4^++NO_3^-, the ratio of NH_4^+ to NO_3^- is 50:50) , sole ammonium (NH_4^+) under either drought stress (DS) simulated by adding PEG (6000) (NO_3^-+PEG as DS-N, NH_4^++NO_3^-+PEG as DS-AN, NH_4^++PEG as DS-A) or non-drought stress (non-DS,CK) (NO_3^-+control as CK-N, NH_4^++NO_3^-+ control as CK-AN, NH_4^++ control as CK-A). Data are means of eight replications ± SE (*n*=8). Significant differences (p<5%) between treatments of maize are indicated by different letters in each column

Addition of NO_3^--nutrition and the mixed nutrition (NH_4^++NO_3^-) significantly increased pH, EC, SPC and ABAC, while it decreased ORP and *g*s in both cultivars in NH_4^+-nutrition under IR-DS. The above effects were predominant in NO_3^--supplied plants than those in NH_4^+ - and NO_3^--supplied plants. The ZD958 plants recorded greater responses than those of JD20. In contrast, no impacts of different N forms applying on pH, EC and ORP were found in the non-DS treatment. Additionally, sole NH_4^+-supplied plants recorded higher SPC and ABAC, whereas lower *g*s than those in sole NO_3^--supplied ones for both cultivars.

Correlations among all parameters measured: Correlation coefficients among all traits evaluated were greater under IR-DS than those under non-DS. A significant correlation of pH, EC, ORP, and ABA concentrations in stem juice, and DM/Pn were evident for plants under IR-DS but not under non-DS treatment (Table 2).

Table 2. Correlation coefficients of dry matter (DM, g plant^{-1}), net photosynthetic rate (Pn, µmol CO_2 m^{-2} s^{-1}), pH, EC (electric conductivity, µS cm^{-1}), ORP (oxidation-reduction potential, mv), soluble protein concentration (SPC, mg ml^{-1}) and ABA concentration (ABAC, µmol ml^{-1}) in stem juice and *g*s (mol H_2O m^{-2} s^{-1}) of both cultivars under drought stress (above diagonal) and control conditions (below diagonal).

Character	DM	Pn	pH	EC	ORP	SPC	ABAC	*g*s
DM		0.985***	0.985***	0.979***	-0.887***	0.978***	0.983***	-0.788***
Pn	0.892***		0.996***	0.989***	-0.821**8	0.989***	0.978***	-0.792***
pH	0.571	0.404		0.993***	-0.896***	0.940***	0.946***	-0.814***
EC	0.471	0.419	0.961***		-0.904***	0.925***	0.934***	-0.790***
ORP	-0.102	-0.303	-0.312	-0.193		-0.856***	-0.871***	0.847***
SPS	-0.522*	-0.487*	-0.146	-0.101	0.660**		0.998***	-0.742***
ABAC	-0.388	-0.338	0.118	0.193	0.461	0.896***		-0.752***
*g*s	0.759***	0.922***	0.841***	0.679**	-0.057	-0.261	-0.272	

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

Interaction of maize cultivars, water supply and N form for all parameters: Analysis of variation indicated the presence of a considerable amount of variability for maize cultivar, water supply and N form treatment for DM, Pn, *g*s and stem juice characteristics under IR-DS and non-DS. The magnitudes of *F* values for all parameters measured for N form (NF) treatment were, in general, lower than those for water regime (W), while higher than those for cultivar (Cv). Moreover, *F* values due to interaction of W ×Cv, W×NF and Cv×NF as well as Cv×W×NF were also significant for most of the parameters except Cv×NF for *g*s and Cv×W×NF for EC (Table 3).

Table 3. *F*-values of nitrogen form with two maize cultivar in drought stress (DS) and non-DS environments for dry matter (DM, g plant^{-1}), net photosynthetic rate (Pn, µmol CO_2 m^{-2} s^{-1}), pH, EC (electric conductivity, µS cm^{-1}), ORP (oxidation-reduction potential, mv), soluble protein concentration (SPC, mg ml^{-1}) and ABA concentration (ABAC, µmol ml^{-1}) in stem juice and *g*s (mol H_2O m^{-2} s^{-1})

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
DM	2789.57***	4.10*	1180.71***	71.53***	43.37*	6.13*	3.87*
Pn	6699.06***	116.28***	863.77***	13.54**	7.64**	50.44***	8.24**
pH	2300.99***	140.92***	809.78***	31.05***	208.30***	10.27**	37.39***
EC	15050.00***	52.85***	260.84***	65.94***	143.67***	7.27**	1.62
ORP	1276.36***	50.19***	170.94***	4.15*	41.06***	5.03*	11.60***
SPC	4866.31***	80.96***	393.99***	69.93*	650.62***	9.63**	15.47***
ABA	3404.04***	19.86***	210.19***	16.25***	425.77***	5.35*	23.23***
*g*s	2084.26***	4.32*	8.26**	8.89***	137.74***	0.68	12.68***

**P*=0.05, ***P*=0.01, ****P*=0.001

Discussion

Effects of drought stress (DS) on stem juice characteristics, photosynthesis and plant growth: Much evidence has proved that stomatal closure during soil water deficit may be attributable to the involvement of ABA in plant protection against drought stress. In fact, ABA is produced in roots in contact with drying soil, and it is transported to the shoot in the xylem vessels via the transpirational stream, and then it accumulates in leaves in the vicinity of the stomata (Sharp & Davies, 2009). Abscisic acid (ABA) is reported to bind to receptors on the external surface of the stomatal guard cell plasma membrane and induces a reduction in guard cell turgor such that stomatal pores undergo closure, which resulting a reduction of stomatal conductance (*g*s). Thus,it causes an improvement of net photosynthetic rate (Pn) and plant growth (Schwartz *et al*., 1994; Sharp & Davies, 2009; Ernst *et al*., 2010). The composition of xylem sap from un-watered crop plants has shown that in addition to the expected increase in ABA concentration, there were also changes in the cation, anion and amino acid concentration of the sap, and in its pH and buffering capacity. The only factors which, when taken into account, improved the correlation between xylem ABA concentration and stomatal conductance were sap pH, and nitrate and calcium concentrations (Gollan *et al*., 1992; Schurr *et al.*, 1992; Sharp & Davies, 2009; Ernst *et al.*, 2010). The above studies showed considerable changes in xylem sap characteristics under DS. However, there is little information in the literature on stem juice responses of plants, in particular, for the C_4 plants, when subjected to DS (Glassop *et al*., 2007; Vu *et al*., 2009). In maize, the stem is made up of internodes at various stages of development (Moore, 1995; Lingle, 1999), and metabolic activities in the stem storage tissues are not similar from one internode to another, as sucrose concentration starts to increase in the internodes as elongation ceases (Rae *et al.*, 2005; Glassop *et al.*, 2007). The total soluble solids in maize stem juice extract consists of high percentage of water-soluble components such as soluble protein, and it also provides a good estimate of the sugar content for stem juice (Moore, 1995). In our studies, drought index (DI) of ZD958 was greater than that of JD20, which could be attributed as a drought tolerant cultivar. Lu *et al.*, (2010) confirmed these results in their field experiments. The present studies have further demonstrated the correlations among DM production, Pn, *g*s and physical and chemical characters of stem juice i.e. pH, electric conductivity (EC), oxidation-reduction potential (ORP), soluble protein concentration (SPC) and ABA concentration (ABAC) under DS. The DS treatment induced increases in pH, EC, and concentrations of soluble protein and ABA while decreases in ORP for both cultivars. Then, *g*s was correspondingly decreased, which could be beneficial for photosynthesis and plant growth. These responses were more evident for a drought-tolerant cultivar (ZD968) than sensitive one (JD20) (Table 1). Moreover, a significant correlation among pH, EC, ORP, ABAC in stem juice, and DM/Pn for plants under DS, but not under non-DS treatment has depicted some better and convenient indicators i.e. pH, EC and ORP for drought resistance assessment (Table 2). In addition, the variation of the environment factors (water and N from) over cultivar could provide a scope for optimal N form dose matching water supply in terms of water use efficiency and N use efficiency under DS (Guo *et al*., 2007b; Zhang *et al*., 2007; Guo *et al.*, 2008; Table 3).

Effects of nitrogen form on stem juice characteristics, photosynthesis and plant growth under drought: Nitrogen is fundamental to the growth and productivity of plants, which is essential for photosynthesis process (Li, 2007). On one hand, it usually plays nutritive role in normal growth environment. On the other hand, its moderate supply can modulate the drought resistance of crops by improving water relations and photosynthesis in drought stressed plant (Saneoka *et al*., 2004; Guo *et al*., 2007a,b; Zhang *et al*., 2007a). Several reports show that N addition may affect nitrate concentration in xylem/apoplastic sap of many crop plants, thereby resulting in pH and ABA increase, such as reducing *g*s under DS (Hoffmann & Kosegarten, 1995; Sharp & Davies, 2009). However, the differences in stem juice characteristics, photosynthesis and plant growth between stressed and control plants during the drought depend on the nitrogen form (Guo *et al.*, 2007a, b).

Previous studies focus on the responses of rice (*Oryza sativa* L.) , whereas rarely in maize to N form under drought (Li, 2007; Guo *et al.*, 2007b; Guo *et al*., 2008; Li *et al.*, 2009; Gao *et al.*, 2010). Under water stress, NH_4^+ nutrition resulted in a higher Pn and biomass of rice seedlings than NO_3^- nutrition (Guo *et al*., 2007b, 2008). The effect of N form on photosynthesis is also associated with *g*s. Plants supplied with ammonium had a higher assimilation rate and *g*s than those supplied with nitrate (Guo *et al.*, 2007a). Mihailovic *et al.*, (1992) pointed out that the NH_4^+-fed plants of each hybrid maintained higher turgor pressure during the drought by better osmotic adaptation in a pot experiment (the quantities of N in available form in the soil before starting were as follows: 0mg/100g of NH_4^+-N, 20 mg/100 g of NO_3^--N and 130 mg/100g of organic N). Wang *et al.*, (2009) stated that growth of maize plants was promoted by a mixed nitrogen source. In our studies, the authors systematically clarified the responses of two maize cultivars with respect to stem juice characteristics, photosynthesis and plant growth subjected to integrated root DS by comparison to non-DS (Fig. 1; Table 1). By comparison with single NH_4^+, single NO_3^- more significantly decreased *g*s and ORP while increased pH, EC, APC and ABAC as well Pn and DM in both cultivars under DS than the mixed supply of NO_3^- and NH_4^+ under drought. These impacts were more evident in ZD958 than those in JD20 (Fig. 1 & Table 1). The results of the above experiment showed that the effect of N form was associated to form and rate of N in the culture medium, type of the medium as well as DS type. Additionally, the low increments in DM and Pn of both cultivars due to NO_3^- treatment occurred under non-DS than in IR-DS with NH_4^+ treatment. The NO_3^- treatment induced non significant impact on stem juice parameters except SPC for both cultivars and ABAC and *g*s for ZD958 under non-DS unlike IR-DS as compared with NH_4^+ treatment

(Fig. 1 & Table 1). These results clearly demonstrated that NO_3^- could obviously raise DM production of maize crop as compared with NH_4^+ under drought but importantly its anti-drought role acts as a signal to initiate coordinated changes in stem juice characteristics accompanying with stomatal opening and carbon fixation during photosynthesis to promote plant growth under DS (Crawford, 1995; Scheible *et al.*, 1997; Fig. 1; Table 1).

Conclusion

In conclusion, the pH, EC and ORP of stem juice proved to be useful for assessing drought resistance of maize plants. An increase of ratio of NO_3^- to NH_4^+ under DS led to a decreased *g*s and ORP while an increase in pH, EC, APC and ABAC and photosynthesis in both cultivars. All these factors resulted in biomass increase, alleviating drought-induced adverse effects on maize plants. The increased magnitude in DM production and Pn of maize plants due to NO_3^- supply were higher under IR-DS than under non-DS with NH_4^+ supply. These conclusions have proved that NO_3^- has obvious drought-resistance function acting as a signal based on its nutritive role under DS and there exists an overlapping effect between NO_3^- supply and the drought-resistance of cultivar as compared with NH_4^+ supply. Furthermore, field experiments are needed to be conducted to clarify which N from is beneficial for plant growth of maize under DS depending on the N status in soil.

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