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GROWTH, WATER STATUS AND PHOTOSYNTHESIS IN TWO MAIZE (*ZEA MAYS* L.) CULTIVARS AS AFFECTED BY SUPPLIED NITROGEN FORM AND DROUGHT STRESS

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Abstract

Hydroponic experiments were conducted to investigate the effects of nitrogen form on plant growth, water status and photosynthetic characteristics under integrated root-zone drought stress (IR-DS) and non drought conditions (non-DS) with two hybrids of maize cultivars Zhengdan 958 (ZD958) and Jundan 20(JD20). On the 12th day of IR-DS, dry matter (DM) of total plant, shoot and root, relative water content (RWC), chlorophyll (Chl.) content, net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E) of both cultivars in all N forms treatments as well as intercellular CO_2 concentration (C_i) except NH_4-treatment were significantly decreased. However, lower DM production, RWC and P_N as well as drought index (DI) were observed for JD20 than ZD958, thereby the later could be considered as a drought tolerance cultivar comparatively. By comparison with sole ammonium (NH_4^+), sole nitrate (NO_3^-) and the mixture of NH_4^+ and NO_3^- both obviously increased DM of total plant, shoot and root, RWC, P_N and Chl. content while decreased g_s, E and Ci of both cultivars under drought. The effects of NO_3^--supplied were superior than NH_4^++NO_3^--supplied in the above responses. These impacts were more predominant in ZD958 than JD20. Further analysis of variation indicated that the impact of N form treatment on most parameters measured except root DM were, in general, less than water regime while higher than cultivar. It is, therefore, concluded that an increase of ratio of NO_3^- to NH_4^+ in nutrition solution could lead to an enhancement in leaf RWC and photosynthesis of both cultivars subjected to IR-DS, then result in biomass increase, thus alleviate of damage from drought due to their obvious drought-resistance function based on its nutritive role, especially for a drought tolerant cultivar.

Introduction

Drought stress (DS) is considered to be one of the primary causes of crop losses in northern China (Li, 2007). Maize (*Zea mays* L.) is an important food crop grown in these areas, and it is comparatively sensitive to drought, whose production is affected by delay in irrigation or DS to cause yield reduction (Lu *et al.*, 2010). Drought stress generally induces cellular changes of biochemistry and physiology, resulting from a variable decline in relative water content (RWC), which has been reported in various plants imposed to drought (Taiz & Zeiger, 2002; Bai *et al.*, 2006; Masoumi *et al.*, 2010). Numerous studies showed that the decline in RWC lead to a reduction in leaf photosynthetic activity under water deficit (Noreen & Ashraf, 2008; Pinheiro & Chaves, 2010; Sage & Zhu, 2011). The general tolerance of crops to DS is dependent on cultivar, the intensity and duration of stress (Chandrasekar *et al.*, 2000; Zhang *et al.*, 2007a).

Nitrogen (N) nutrition has been reported to be able to promote the growth and development of plants under DS by alleviation of damaging impacts on plants (Li, 2007). Its modulations in drought resistance not only cover osmotic regulation and cell wall elasticity, but photosynthesis and carbohydrate metabolism (Taiz & Zeiger, 2002; Zhang *et al.*, 2009). The above responses are closely dependent on crop species, N form and cultured method (Guo *et al.*, 2007a). Much of researches about the effects of ammonium (NH_4^+) and nitrate (NO_3^-) nutrition on plant growth, water relations, and photosynthesis focus on maize, wheat (*Triticum aestivum* L.), tobacco (*Nicotiana tabacum* L.), beans (*Phaseolus vulgaris* L), and rice (*Oryza sativa* L.) under normal water supply condition (Cramer & Lewis, 1993a,b; Walch-Liu *et al.,* 2000; Guo *et al.*, 2002, 2007 a b,c; Li *et al.*, 2008). Some evidences of an influence of N form on plant response to DS occur in rice. The NH_4^+-fed seedlings recorded higher biomass, photosynthesis rate and water uptake than NO_3^--fed ones (Guo *et al.*, 2002, 2007d, 2008; Li *et al.*, 2009; Gao *et al.*, 2010). In maize, Mihailovic *et al.*, (1992) and Wang *et al.*, (2009) stated 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. Up to now, however, which N form is beneficial for plant growth under integrated root-zone drought stress (IR-DS) maize and its mechanism in relation to water relation and photosynthesis responses is still poorly understood (Lewis *et al.*, 1989; Mihailovic *et al.*, 1992; Chandrasekar *et al.*, 2000; Guo *et al.*, 2002; Guo *et al.*, 2007d; Hamidou *et al.*, 2007; Guo *et al.*, 2008; Mérigout *et al.*, 2008; Li *et al.*, 2009; Gao *et al.*, 2010). The aim of this study was to clarify whether differences existing among NH_4^+, NO_3^- and a mixture of NH_4^+ and NO_3^- supplied two maize cultivars plants influence their response to IR-DS in view of plant growth, water relation and photosynthesis. Additionally, effectiveness of N form was elucidated under both DS and non-DS conditions in the same trial to prove further which N form has more obvious anti-drought function (Guo *et al.*, 2007a; Li, 2007).

Materials and Methods

Plant material and trial location: Hydroponic experiments were carried out with maize (*Zea mays* L.) cultivars (Zhengdan 958 and Jundan 20) in a climatic growth chamber at college of Life Sciences of Northwest A & F University (Yangling, P.R. China). According to field experiments carried out in the farm belonging to the same university, yield of the Zhengdan 958 cultivar is relatively little affected by drought (Zhang *et al.*, 2007b).

Plant growth and experiment design: After germination on moist filter paper in plastic trays, maize seedlings were sown in the holes of styrofoam boards by using absorbent cotton in deionized water in plastic boxes (inner length 26 cm, width 18cm, and height 12 cm) in the growth chamber. The controlled environmental conditions were kept under optimal relative humidity (RH) of 60-70% and temperature regime of 25°C day and 18°C night with 16hr light period and 350μmolm^{-2}s^{-1} light intensity (Zhang *et al*., 2008). To exclude light from the roots, all containers were individually covered with black plastic. The seedlings were fed with one-half-strength and complete nutrient solution after four and eight days of placement in deionized water respectively (Hoagland and Arnon, 1938). The nutrient solution contained all macronutrients and micronutrients with a mixture of NH_4^+ and NO_3^- (the ratio of NH_4^+ to NO_3^- is 50:50) with a pH range of 6.25-6.35.

Seedlings were supplied with nutrient solutions with different resources of N form at the stage of three-leaf grown in 3.4 L pots, which were sealed carefully to avoid evaporation with sponge wrapped around the interface of the roots and the shoots in the growth chamber. The NH_4^+-N was supplied as $(NH_4)_2SO_4$, NO_3^--N supplied as $Ca(NO_3)_2$, and the mixed nutrition (NH_4^+ +NO_3^--N, the ratio of NH_4^+ to NO_3^- is 50:50) supplied as both $(NH_4)_2SO_4$ and $Ca(NO_3)_2$. In NH_4^+-N-containing nutrient solution, Ca^{2+} was supplied as $CaCl_2$. Three days later, drought stress (DS) was simulated by adding 10% (w/v) polyethylene glycol (PEG, MW 6000) to achieve osmotic potentials (ψs) of -0.15 Mpa (Wang & Li, 2002). Complete nutrient solution without PEG-6000 served as non drought (non-DS). Thus, six treatments were examined: 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). To maintain an identified condition, culture solution in every pot was added with nitrification inhibitor dicyandiamide (DCD). All treatments had four replicates, with a random design. The nutrient solutions were changed every two days to retain desired PEG concentrations, and aerated twelve hours a day. The whole experiment was conducted twice independently exposed to the same growth conditions.

Sample harvest and observation recorded: The plants of two maize cultivars were harvested after twelve days treatments start. For dry matter measurement, the plant samples were divided into toots and shoots, then placed in an oven at 105°C for 30 min, and then dried to a constant weight at 75°C, respectively.

Drought index (DI) was calculated (dry matter basis) following the following expressions (Zhang *et al*., 2007):

$DI=Y_D/Y_N$ Y_D—average dry matter under a drought stress condition
Y_N —average dry matter under an adequate water supply condition

Leaf relative water content (RWC) was evaluated using the following formula (Gao, 2000).

$$RWC (\%) = [(FW-DW) / (TW-DW)] \times 100$$

FW – fresh weight of sample, TW – turgid weight of sample, DW – dry weight of sample.

Chlorophyll (Chl.) content was assayed using new fully expanded leaves extracted with a mixture of acetone: alcohol: distilled water = 4.5:4.5:1.0. The absorbance of extracted solution was determined at 665, 649, and 470 nm for calculation of chl. content (Gao, 2000).

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 two leaves of one plant were determined and repeated three replicates. The determination covered net photosynthetic rate (P_N, μmol CO_2 m^{-2} s^{-1}), transpiration rate (E, H_2O_2 mmol m^{-2} s^{-1}), intercellular CO_2 concentration (C_i, μmol mol^{-1}) and stomatal conductance (g_s mol 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 whole measurements were conducted under the conditions with leaf temperature range of 25.5±2°C, and the RH of roughly 45% in the leaf chamber.

Statistical analysis: All statistical analyses were performed with SAS software package (Anon., 1996). The analysis of variance (ANOVA) was followed by the least significant difference (LSD) to determine the significance of the means at the 0.05 level. All data were analyzed from every treatment, with eight replicates.

Results

Plant growth: Negative effects of integrated root-zone drought stress (IR-DS) on maize growth were observed in all N form treatments (Fig. 1). Compared with non-DS, dry matter (DM) production of total plant, shoot and root of Jundan 20 (JD20) was decreased by 29-46%, 31-54%, and 17-18% respectively under IR-DS. Their corresponding values were 20.1-40.1% (total plant), 24-47% (shoot) and 9%/non-sifnificant (root) in Zhengdan 958 (ZD958). Drought index (DI) of ZD958 was 0.599-0.799 while that of JD20 was 0.544-0.711. The above responses to IR-DS differed among the nitrogen forms. As a result, ZD958 maintained greater DM production and DI than JD20 under IR-DS with the same N form except NH_4^+- treated plants (Fig. 1).

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

Plant water status: Measurements of relative water content (RWC) in leaf tissues are commonly used to evaluate the water status of plants (Taiz & Zeiger, 2002; Bai *et al*., 2006; Table 1). Leaf RWC decreased by 9 and 13% in JD20 and 4 and 9% in ZD958 exposed to NO_3^- and NH_4^++NO_3^- supplied treatments respectively against IR-DS above non-DS (Fig. 2). Consequently, ZD958 showed higher values of RWC except NH_4^+ treated plants under IR-DS. However, in response to non-DS, leaf RWC had no significant difference between both cultivars.

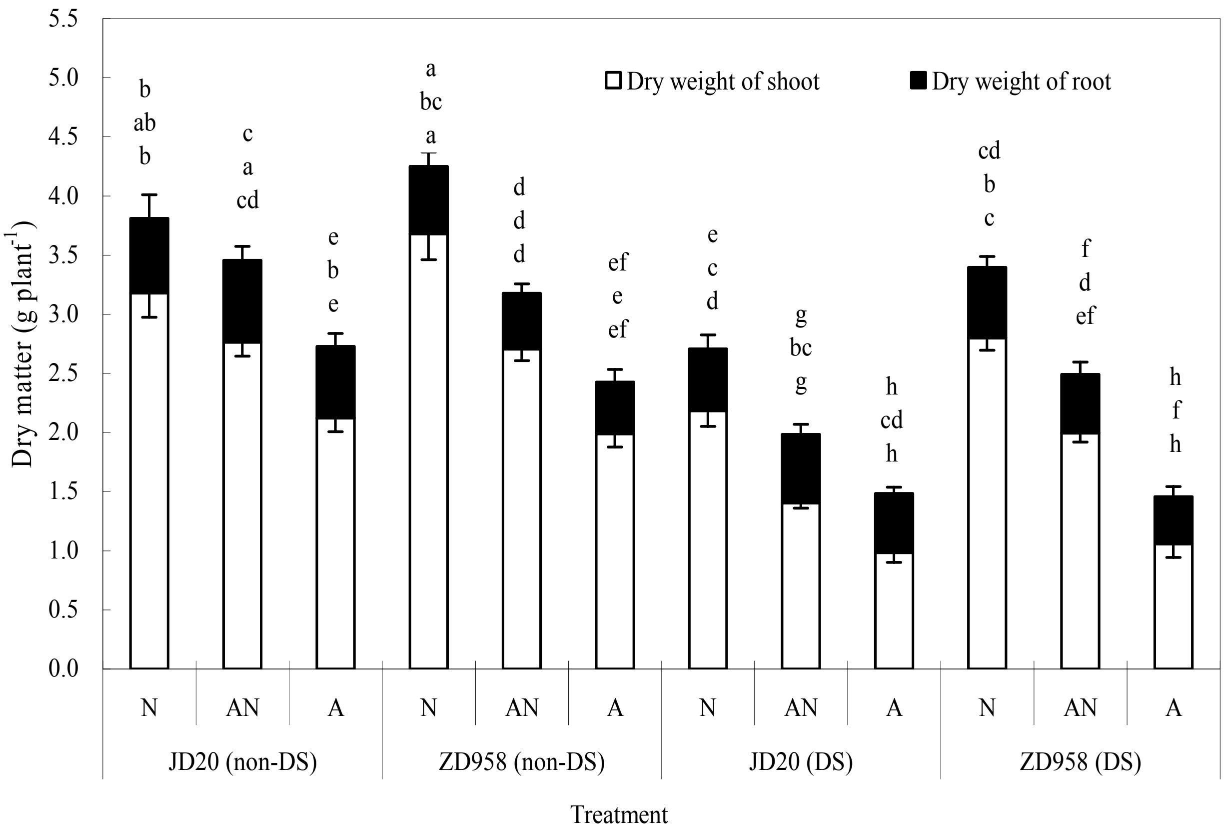

Fig. 1. Responses of dry matter in Jundan20 (JD20) and Zhengdan958 (ZD958) at seedling stage (12 days after treatment start) to nitrate (N, NO_3^-), ammonium (A, NH_4^+), and the mixed supply (AN, NH_4^++NO_3^-, the ratio of NH_4^+ to NO_3^- is 50:50) under drought stress (DS) and non drought stress (non-DS). Different letters from the top down on each column show significant differences among means for dry matter of total plant, root and shoot respectively at $p<0.05$. Means ± SD (n = 8).

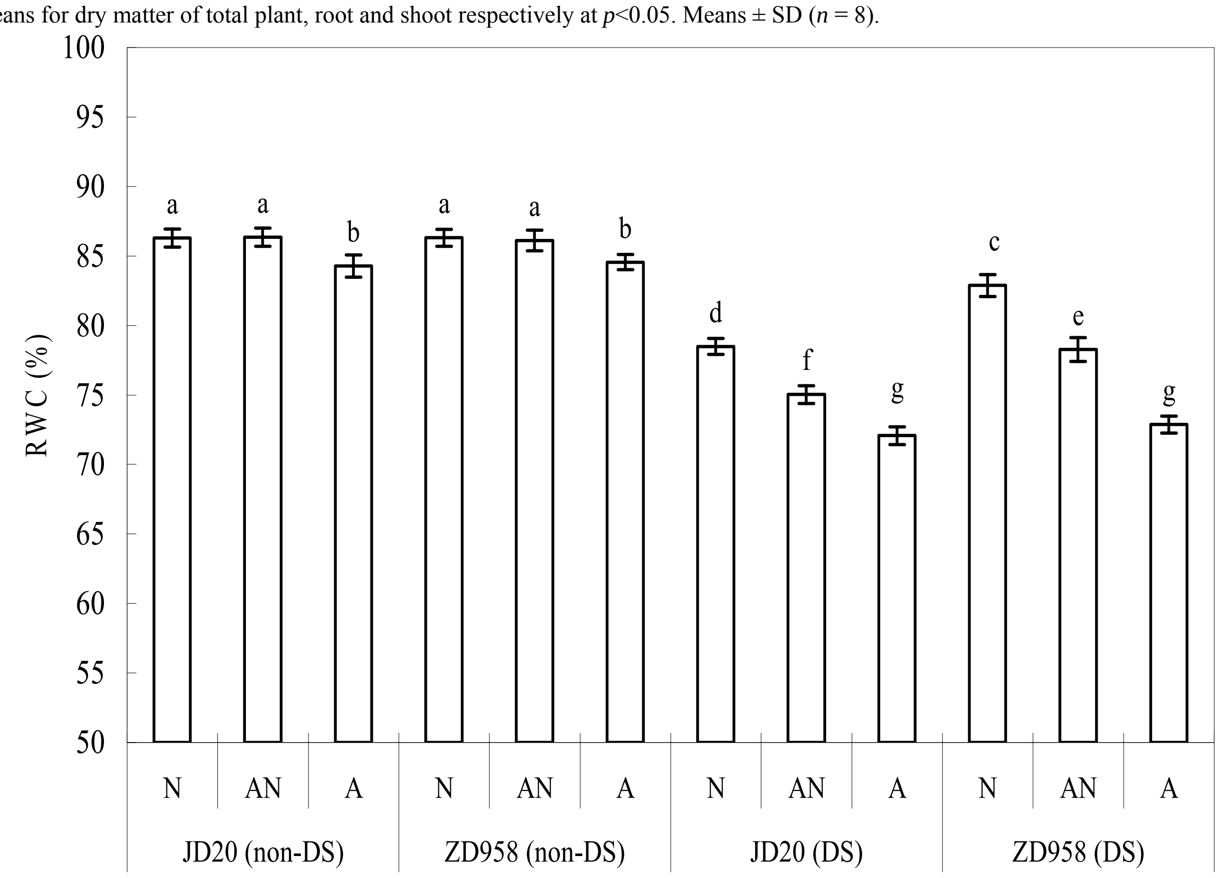

Fig. 2. Responses of leaf relative water content (RWC) in Jundan20 (JD20) and Zhengdan958 (ZD958) at seedling stage (12 days after treatment) to nitrate (N, NO_3^-), ammonium (A, NH_4^+), and the mixed supply (AN, NH_4^++NO_3^-, the ratio of NH_4^+ to NO_3^- is 50:50)

under drought stress (DS) and non- drought stress (non-DS DS). Different letters at the top of each column each column show significant differences among means at p<0.05. Means ± SD (n = 8).

Table 1. Responses of net photosynthetic rate (P_N) , transpiration rate (E), stomatal conductance (gs), intercellular CO_2 concentration (Ci) and chlorophyll (Chl) content in Jundan20 (JD20) and Zhengdan958 (ZD958) at seedling stage (12 days after treatment start) to nitrate (N, NO_3^-), ammonium (A, NH_4^+), and the mixed supply (AN, NH_4^++NO_3^-, the ratio of NH_4^+ to NO_3^- is 50:50) under drought stress (DS) and non- drought stress (non-DS).

Treatment		P_N [µmol(CO_2) m^{-2} s^{-1}]	E [mmol(H_2O)m^{-2} s^{-1}]	g_s [mol(H_2O) m^{-2} s^{-1}]	Ci [µmol (CO_2)mol^{-1}]	Chl. content (mg g^{-1}DW)
Drought stress (DS) with different nitrogen form supply						
JD20	N	17.79 ± 0.53 f	4.24 ± 0.09 h	0.254 ± 0.007 e	180 ± 9 e	2.57 ± 0.08 f
	AN	13.77 ± 0.42 h	4.82 ± 0.16 f	0.277 ± 0.012 d	191 ± 10 de	2.16 ± 0.07 h
	A	11.46 ± 0.65 i	5.02 ± 0.14 e	0.309 ± 0.010 c	205 ± 15 d	2.01 ± 0.09 i
ZD958	N	19.99 ± 0.72 e	4.03 ± 0.11 i	0.235 ± 0.019 f	162 ± 8 f	2.88 ± 0.10 e
	AN	15.51 ± 1.03 g	4.51 ± 0.08 g	0.256 ± 0.006 e	184 ± 7 e	2.42 ± 0.11 g
	A	11.99 ± 0.42 i	5.08 ± 0.12 e	0.305 ± 0.010 c	209 ± 11 d	2.05 ± 0.13 i
Non-drought stress (non-DS) with different nitrogen form supply						
JD20	N	25.61±0.63 b	6.43±0.12 b	0.395 ±0.007 a	245±13 b	3.79 ± 0.07 b
	AN	23.43±0.83 c	5.87±0.17 c	0.376±0.012 ab	217±11 cd	3.26 ± 0.12 c
	A	21.76±0.93 d	5.31±0.15 de	0.368±0.009 b	193±16 d	3.02 ± 0.08 d
ZD958	N	27.85±1.03 a	7.26±0.17 a	0.408±0.017a	264±10 a	4.08 ± 0.12 a
	AN	24.54±0.55 bc	6.37±0.12 b	0.378±0.010 ab	229±12 c	3.48 ± 0.09 bc
	A	22.14±0.69 cd	5.41±0.16 d	0.360±0.014 b	197±18 d	3.05 ± 0.11 d

Different letters in each column show significant differences among means at p<0.05. Means ± SD (n = 8).

The addition of NO_3^--N and NH_4^++ NO_3^--N could increased markedly leaf RWC of both cultivars as compared with NH_4^+-N addition under IR-DS. Greater increase percentage of RWC were calculated in ZD958 (14 and 7%) than JD20 (9 and 5%) when submitted to NO_3^--N and NH_4^++ NO_3^--N nutrition respectively. However, the non-significant impact of N form on RWC was found under non-DS condition (Fig. 2).

Chlorophyll content and net photosynthetic rate (P_N): Compared with non-DS, the Chlorophyll (Chl.) content and net photosynthetic rate (P_N) of new fully expanded leaves greaterly decreased by 40 and 38% in NO_3^- solution, 48 and 50% in the mixed solution in JD20, respectively, than by 35 and 34% in NO_3^- solution, 44 and 43% in the mixed solution in ZD958, respectively. The negative effects of IR-DS on Chl. content and P_N of both cultivars were greatest due to NH_4^+ treatments above non-DS and non-significant difference occurred between both cultivars in NH_4^+- treated plants under IR-DS.

By comparison with NH_4^+-supplied plants, NO_3^- and NH_4^++NO_3^--supplied plants both increased Chl. content and P_N under drought. The above responses were more predominant in ZD958 than JD20. The NO_3^--treated plants recorded more positive effects than NH_4^++NO_3^--treated ones above NH_4^+-supplied ones in both cultivars. As for non-DS, the similar effects of N form on Chl. content and P_N occurred in both cultivars. However, the increase impacts due to NO_3^- and NH_4^++NO_3^-- treatments became less under non-DS than IR-DS above NH_4^+ treatment (Table 1).

Gas exchange parameters: Drought stress greaterly decreased stomatal conductance (g_s), transpiration rate (E) and intercellular CO_2 concentration (Ci) in ZD958 by 56, 51 and 39% in NO_3^--nutrition and 44, 33 and 22% in mixture nutrition than in JD20 by 47, 39 and 31% in NO_3^--nutrition and 36, 20 and 14% in mixture nutrition respectively, whereas the lowest decrease responses on g_s and E due to IR-DS were obtained with NH_4^+-supplied treatments as compared with non-DS and no significant differences were observed between both cultivars with NH_4^+ contained nutrition under IR-DS. But for Ci, there existed no significant effects of whether IR-DS or maize cultivar when submitted to NH_4^+-nutrition.

Under IR-DS, NH_4^++NO_3^-- supplied plants had a clearly lower g_s and E in both cultivars as compared to sole NH_4^+, while higher to sole NO_3^- in both cultivars. The decreased effects due to NO_3^- and NH_4^++NO_3^- supplied were more obvious in ZD958 than JD20 above NH_4^+- supplied. With respect for Ci, significant differences occurred among the both two sole N forms and their mixture in ZD958 as well as between NH_4^+ and NO_3^- treatments under drought. However, a rise responses of these parameters due to NO_3^- and NH_4^++NO_3^- treatments occurred under non-DS above NH_4^+ treatment. These effects were more predominant in ZD958 than JD20 and NO_3^--treated plants recorded greater responses than NH_4^++NO_3^-- supplied plants with the same cultivar (Table 1).

Interaction of maize cultivar, water regime and N form for all parameters: Analysis of variation showed the presence of a considerable amount of maize cultivar, water regime and N treatment variability for DM of shoot, root and total plant, RWC, Chl. content, P_N, g_s, E and Ci under IR-DS and non-DS. The F values for most of parameters measured except root DM followed in order of water regime (W) > N form (NF) > cultivar (Cv). Additionally, F values of interaction of W ×Cv, W×NF and Cv×NF as well as Cv×W×NF were also significant for most traits measured except g_s and Ci due to Cv×NF (Table 2).

Table 2. *F*-values of nitrogen form with two maize cultivar in drought stress (DS) and non drought stress (non-DS) environments for dry matter (DM), leaf relative water content (RWC), net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and chlorophyll (Chl) content.

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
Shoot DM	3598.00***	251.10***	2417.42***	94.15***	3.61*	102.28***	24.61***
Root DM	153.62***	477.69***	180.58***	169.52***	5.79**	140.20***	6.42**
Total DM	7735.91***	206.03***	5311.08***	326.67***	10.92***	311.89***	54.42***
RWC	76836.10***	1877.68***	8057.70***	1833.71***	3194.37***	215.75***	316.96***
P_N	15147.40***	358.26***	2229.09***	2.48*	82.20***	42.25***	2.31*
E	21543.50***	253.05***	270.86***	960.71***	4650.69***	53.44***	221.32***
g_s	2951.10***	17.38**	30.29***	28.76***	246.06***	1.87	3.23*
C_i	1043.61***	4.47*	38.72***	71.47***	623.56***	0.84	23.41***
Chl. content	24278.50***	536.62***	4042.11***	43.35***	20.87***	84.25***	23.01***

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

Discussion

Effects of drought stress and maize cultivar on water status, photosynthesis and plant growth: Drought stress (DS) act as a considerable adverse role in productivity of a crop in view of crucial changes in water homeostasis. Relative water content (RWC) in leaf is usually treated as an important and efficient measure of plant water status (Taiz & Zeiger, 2002; Bai *et al.*, 2006). Chlorophyll (Chl.) content is an indicator of a plant's ability to drought tolerance (Taiz & Zeiger, 2002). Drought stress can create loss of homeostasis in water status in plants to lead to a decrease of RWC, Chl. degradation and photosynthesis disorder (Bai *et al.*, 2006; Pinheiro & Chaves, 2010; Sage & Zhu, 2011). Stomatal conductance (g_s) and transpiration rate (E) have main influences on exchange of CO_2 and water vapour affecting the CO_2 fixation rate in leaf mesophyll tissue as well as net photosynthetic rate (P_N) and intercellular CO_2 concentration (C_i). The above parameters, thereby, can be good indicators for evaluating photosynthesis responses characters of crop plant under DS (Pinheiro & Chaves, 2010; Sage & Zhu, 2011). Plants respond to stresses such as drought by decreasing leaf expansion and closing stomatal pores. In this way, they preserve the water, nutrients and carbohydrates required for survival, which is beneficial for maintain optimal water status, photosynthesis and plant growth (Guo *et al.*, 2007d; Noreen & Ashraf, 2008; Pinheiro & Chaves, 2010).

Numerous evidences indicate that different crop cultivars recorded different biological and physiological responses to DS (Chandrasekar *et al.*, 2000; Hamidou *et al.*, 2007; Zhang *et al.*, 2007). Much studies pointed out maize is a crop being sensitive to DS: water deficit diminishes leaf expansion and cell division rates of leaves and ultimately lead to a decrease in leaf RWC (Mihailovic *et al.*, 1992; Bai *et al.*, 2007; Zhang *et al.*, 2007; Wang *et al.*, 2009; Lu *et al.*, 2010). In addition, drought index (DI), a measure of plant responses to DS, varied with different maize cultivars due to their different drought resistance (Zhang *et al.*, 2007). Drought index (DI) of ZD958 was greater than that of JD20 in this study, which could be considered as a drought-tolerance cultivar comparatively. Lu *et al.*, (2010) supported this result in their filed experiments. The present studies have further elucidated that integrated root-zone DS (IR-DS) stimulated more serious reduction in RWC, Chl. content, P_N, g_s and E in all three N form treatments and Ci except NH_4^+-nutrition supplied in a drought sensitive maize cultivar Jundan 20 (JD20) than a drought tolerance cultivar Zhengdan 958 (ZD958). Such, the drought tolerance cultivar ZD958 recorded obviously higher DM under IR-DS regardless of N form (Zhang *et al.*, 2007; Fig. 1; Fig. 2; Table 1).

Effects of nitrogen form on water status, photosynthesis and plant growth under drought: Nitrogen is essential for photosynthesis process, which affecting plant growth and productivity (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 crop by improving water relation and photosynthesis in drought-stressed plant (Saneoka *et al.*, 2004; Guo *et al.*, 2007a, d; Zhang *et al.*, 2007). However, the differences in water status and photosynthesis parameters as well as growth between stressed and control treated plants are closely associated with nitrogen form supplied (Guo *et al.*, 2007a, d). Previous studies focus on the responses of rice to N form under drought (Guo *et al.*, 2002; Guo *et al.*, 2007d; Guo *et al.*, 2008; Li *et al.*, 2009; Gao *et al.*, 2010). The NH_4^+-fed plant recorded a greater Chl. content, P_N, g_s and biomass of rice seedlings as compared with NO_3^--fed one under water stress. Furthermore, no significant difference occurred between average Chl. contents of both cultivars in the same N form, but obvious significant differences between NH_4^+ and NO_3^- treated plants within the same cultivar (Guo *et al.*, 2007d; Guo *et al.*, 2008). No negative impact on transpiration rate of rice plants due to drought was observed in NH_4^+ nutrition (Gao *et al.*, 2010).

Studies on responses of maize crop to N form have been conducted mostly under well-watered condition whereas rarely under DS. Li, (2007) summarized the effect of nitrogen form on the physiological characteristics of maize under normal growth condition. Nitrogen form had the remarkable effect on the every tache of photosynthesis including the content of cholorophyll, photosynthetic rate, activity of Rubisco and the photorespiration. Most researches showed that the amount of Chl. was higher under the mixed nitrogen nutrition under well-watered condition (Li, 2007; Guo *et al.*, 2007a). Under nitrate nutrition, the photorespiration of leaf was obviously higher. The N form could change the pathway of respiration (Guo *et al.*, 2007a; Li, 2007). Li *et al.*, (2008) stated that g_s and E were all higher than either the ammonium treatment or the ammonium together with nitrate mixed treatment. Such, nitrate is most suitable for corn seedlings under normal growth condition (Guo *et al.*, 2007a; Li, 2007). The above results are in accordance with the conclusion under non-DS in this study (Guo *et al.*, 2007a; Li, 2007; Fig. 1; Fig. 2; Table 1). But, up to now, there was little information and unclear conclusion about the effect of N form on the growth of maize crop under DS (Mihailovic *et al.*, 1992; Chandrasekar *et al.*, 2000; Guo *et al.*, 2002; Guo *et al.*, 2007d; Hamidou *et al.*, 2007; Guo *et al.*, 2008; Mérigout *et al.*, 2008; Li *et al.*, 2009; Gao *et al.*, 2010).

In an earlier pot study on drought-stressed maize plants, Mihailovic *et al.*, (1992) pointed out that the NH_4^+-fed plants of each cultivar maintain higher chl. contents under two water regimes, especially greater turgor pressure during the drought by better osmotic-regulation (Mihailovic *et al.*, 1992). This result was closely related to quantities of N in available form in the tested soil i.e. 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) (Mihailovic *et al.*, 1992). Wang *et al.*, (2009) stated that plant growth of maize was promoted by mixed nitrogen source, but water use efficiency improved by ammonium supply under partial root-zone water stress conditions. In our studies, the authors systematically elucidated the responses of both maize cultivars in view of water relation, photosynthesis and plant growth under IR-DS by comparison to non-DS using solution culture method (Figs. 1 & 2; Table 1). We observed that maize seedlings under simulated IR-DS were affected by different nitrogen forms applying. By comparison with single NH_4^+, single NO_3^- more significantly increased DM, RWC, P_N and Chl. Content while decreased g_s and E, then resulted in a reduction of Ci of both cultivars than the mixed supply of NO_3^- and NH_4^+ under drought. These impacts were more evident in ZD958 than JD20 (Fig. 1; Fig. 2; Table 1). Our results are mostly opposite to the above reports conducted by Mihailovic *et al.*, (1992) in a pot experiment and Wang *et al.*, (2009) in a solution culture experiment. The above conclusions appear to show that the effect of N form is associated to form and rate of N in culture medium, culture method as well as DS type. Additionally, the less increments in P_N, Chl. content and DM production, and non-significant impact on RWC of both cultivars due to NO_3^- treatment occurred under non-DS than IR-DS above NH_4^+ treatment (Fig. 1; Fig. 2; Table 1). The result has clearly demonstrated that NO_3^- could obviously raise DM production of maize crops as compared with NH_4^+ under drought due to not its nutritive function, but importantly its anti-drought role to initiate coordinated changes in stomatal opening and carbon fixation during photosynthesis and N metabolism to improve water relation and promote plant growth under DS (Crawford, 1995; Scheible *et al.*, 1997; Table 1).

Interaction of maize cultivar, water supply and N form for all parameters: Cultivar and resource of N form both affected differently on DM, RWC and photosynthesis features under two water regimes (Guo *et al.*, 2007d; Zhang *et al.*, 2007; Gao *et al.*, 2010). Further variation analysis about interaction amongst cultivar, water regime and N form for all measured parameters in the present study suggested that the choice of environmental factors and cultivar was appropriate. The evidence about variation of environments (water regime and N from) over cultivar indicates optimal N form should be chosen to match to water status in growth medium to exert ultimately the inherent water use efficiency and N use efficiency of a crop cultivar under drought (Guo *et al.*, 2007d; Zhang *et al.*, 2007; Guo *et al.*, 2008; Table 2).

Conclusions

In summary, an increase of ratio of NO_3^- to NH_4^+ under IR-DS could lead to an enhancement in leaf RWC and photosynthesis of both cultivars, result in biomass increase, therefore alleviate damage from drought. These impacts were more predominant in a drought tolerant cultivar (ZD958) than sensitive one (JD20). The increased magnitude in DM production and P_N of maize plants due to sole NO_3^- supplied are obviously higher under IR-DS than non-DS above sole NH_4^+ supplied. These conclusions have proved that NO_3^- has obvious drought-resistance function based on its nutritive role under drought compared with NH_4^+ supplying for maize crop. Furthermore, the field experiments are very needed to clarify which N from is beneficial for plant growth of maize under IR-DS depending on the N status in soil.

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