Potassium fertilization mitigates the adverse effects of drought on selected *Zea mays* cultivars

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Abstract: In the present study, the role of potassium (K) in mitigating the adverse effects of drought stress (DS) on 2 maize (*Zea mays* L.) cultivars, 'Shaandan 9' (S_9; drought-tolerant) and 'Shaandan 911' (S_{911}; drought-sensitive), was assessed. K application increased dry matter (DM) across all growth stages and grain yield (GY) in both cultivars under DS, but not under control conditions. Drought-tolerant cultivar S_9 was superior to drought-sensitive cultivar S_{911} under DS in terms of DM and GY regardless of K addition. Additionally, K application increased relative water content, nitrate reductase activity, and concentrations of potassium ion, free proline, soluble protein, and endogenous glycine betaine in both cultivars. These positive effects due to K fertilization under DS were greater for S_{911} than for S_9. In contrast, the differences in the above parameters between K-treated plants and plants under control conditions were either nonsignificant or marginal. This study provides direct evidence of the beneficial physiological function of K fertilization in mitigating the adverse effects of DS by increased nitrate assimilation and osmotic regulation, but not due to its nutritive role.

Key words: Relative water content, nitrate reductase activity, osmotic solutes, Poaceae, potassium fertilizer

1. Introduction

Water shortage has become a significant limiting factor for agricultural production in most parts of the world, including China. Due to this problem, sustainable production of food requires new approaches to enhance the use efficiencies of all inputs, including water and nutrients. These approaches are vital not only to achieve enhanced agricultural productivity, but also to protect environmental quality (Zhang et al., 2009a; Saeidnejad et al., 2013). In recent years, the impact of frequent droughts on crop production in most parts of northern China has been tremendous (Ge et al., 2012). Maize (*Zea mays* L.), one of the most important cereal crops in this area, is frequently subjected to drought stress (DS), resulting in marked reductions in economic yields (Zhang et al., 2009a; Lopes et al., 2011; Ge et al., 2012).

DS, in fact, causes a multitude of biochemical and physiological changes as a consequence of osmotic stress. The imposition of osmotic stress causes a significant decline in chemical activity of water and a loss of turgor in plant cells (Taiz and Zeiger, 2002; Zhang et al., 2013; Yıldıztugay et al., 2014). These changes, in turn, may impair nitrate

assimilation by reducing nitrate reductase activity (NRA) (Zhang et al., 2009b). Protein degradation also increases under DS due to enhanced protease hydrolysis activity. The net effect of these changes leads to reduced plant growth and/or plant death as well as reduced relative water content (RWC), the latter being an effective indicator of plant water status (Taiz and Zeiger, 2002; Zhang et al., 2009b).

Plants have evolved various strategies, the most promising one being osmotic regulation, to mitigate the negative effects of DS on nitrate assimilation. Furthermore, osmoregulation provides optimal conditions for plant growth (Ashraf and Foolad, 2007; Chołuj et al., 2008; Ashraf, 2010). Plant cells can mitigate the adverse effects of osmotic stress by reducing the depletion of cellular water and regaining cell turgor, which is mediated by maintaining optimal NRA and accumulation of potassium ions (K^+), as well as concentrations of endogenous glycine betaine (EGB), free proline (FP), soluble proteins (SP), and derivative products from N metabolism (Zhang et al., 2009a, 2009b). Therefore, changes in NRA and concentrations of K^+, EGB, FP, and SP can determine the changes in nitrate

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assimilation and osmotic solute accumulation in response to DS (Chołuj et al., 2008). Different reports have shown that changes in RWC, NRA, and osmotic solutes induced by DS were dependent on cultivars and growth stage as well as the intensity and duration of DS (Shao et al., 2006; Ashraf and Foolad, 2007; Chołuj et al., 2008; Zhang et al., 2009a, 2009b; Ashraf, 2010; Zhang et al., 2013).

Abundant amounts of K are required by most plants. However, its uptake by plants is significantly affected by soil moisture content, which affects rates of root growth and of K^+ diffusion from soil to the root. Hence, K uptake efficiency is rather low in dry-land regions. Thus, it becomes a major limiting factor for attaining optimal crop yield and quality (Ge et al., 2012). Despite acting as an essential macronutrient, K serves as a primary osmoticum to maintain turgor in plants, particularly under stressful environments. Therefore, abundant K^+ accumulation in plant tissues under DS may play a vital role in water uptake from the soil (Cakmak, 2005). In view of several reports, it is now evident that the exogenous application of K can alleviate drought-induced negative effects on plant growth (Andersen et al., 1992; Abdelvahab and Abdalla, 1995; Sudama et al., 1998; Tiwari et al., 1998; Sangakkara et al., 2001; Singh and Kuhad, 2005; Fanaei et al., 2009; Ezzat et al., 2010; Mohammad and Mahmood, 2011).

K application can improve drought tolerance in plants by regulating a variety of processes, such as osmoregulation, charge balance, energy status, and protein synthesis (Maathuis and Sanders, 1996). Reduced water loss of plants grown under adequate K supply is dependent on the osmotic potential of mesophyll cells (Cakmak, 2005). Several studies, often under short durations of DS, have provided evidence of the role of K in mitigating DS by enhancement of NRA and accumulation of K^+, glycine betaine, FP, and SP (Maathuis and Sanders, 1996; Fanaei et al., 2009; Ezzat et al., 2010; Mohamma and Mahmood, 2011).

Most past studies have dealt with the effects of K on plant physiological responses to mitigate DS during a single growth stage and a sudden simulated DS, mainly in terms of single factorial effects of a cultivar, water, or fertilizer. Hence, there is a need to investigate the influence of K supplementation on the response of maize cultivars exposed to long-term DS and to elucidate the specific role of K in modulating plant physiological responses to mitigate DS, as well as in the overall improvement of plant vigor by optimal K fertilization, which in turn facilitates enhanced tolerance to DS (Zhu et al., 2005; Fanaei et al., 2009; Ezzat et al., 2010; Mohamma and Mahmood, 2011). Keeping in view the above facts, we hypothesize that greater nitrate assimilation and osmotic regulation in the leaves of maize plants provides a mechanism of DS tolerance in different cultivars with application of K

fertilizer. Thus, the primary objective of this study was to examine the mechanism of amelioration of negative effects of long-term DS by K nutrition in 2 maize cultivars with contrasting responses to DS.

2. Materials and methods

2.1. Plant material and experimental design

This study was conducted at the Northwest A & F University, Yangling (34°20′N, 108°24′E), Shaanxi Province, China. Two maize cultivars, Shaandan 9 (S_9; drought-tolerant) and Shaandan 911 (S_{911}; drought-sensitive) (Zhang et al., 2007), were used in the study. The soil used was a typical manural loessial clay-loam, which was sampled from a depth of 20 cm within Yangling, China. Selected properties of the soil used were determined using the procedures described by Miller and Keeney (1982): pH 8.13; organic matter = 11.8 g kg^{-1} (potassium dichromate volume method); total N = 0.96 g kg^{-1} (micro-Kjeldahl method); available N = 42.3 mg kg^{-1} [2 mol L^{-1} potassium chloride (KCl) extraction, autoanalyzer]; Olsen-P = 23.2 mg kg^{-1} [0.5 mol L^{-1} sodium bicarbonate (NaHCO$_3$), molybdenum blue method]; exchangeable K = 216 mg kg^{-1} [1 mol L^{-1} ammonium acetate (NH$_4$OAc) extraction, flame photometry]; water holding capacity (WHC) = 22.6% (Wilcox method).

The soil was ground well, sieved to 0.5 mm, and then air-dried. The soil was then added to each pot with 12 kg per pot. Each pot had a diameter of 28 cm and a height of 35 cm. All soil-filled pots were placed in a greenhouse. The soil moisture content before planting was 13%. The soil in each pot was supplemented with 0.15 g P kg^{-1} and mulched with wheat straw to prevent water evaporation. In June, 5 seeds of each cultivar were planted in each pot. When the seedlings were at the 3-leaf stage they were thinned to 1 per pot. The treatments included 2 soil water regimes: 1) control, i.e. soil water content maintained at 19 ± 1% of water content (weight basis), which was equivalent to 85 ± 5% of WHC; 2) DS, maintained at 15 ± 1% of water content (weight basis), being equivalent to 65 ± 5% of WHC from seedlings at the 3-leaf stage to full maturity. The K treatments, applied as KCl, were: 1) 0 (K_0; no addition of K fertilizer); 2) 0.42 g K kg^{-1} soil. Full doses of K and P fertilizers, together with one-third doses of N (0.5 g N kg^{-1} soil), were added to the soil before sowing. The remaining N was added in 2 equal split doses at the beginning of the elongation stage and heading stage. The experiment was set up in a completely randomized design with 8 treatments and 17 replications. Soil water content was monitored referring to Zhang et al. (2007).

2.2. Sampling and measurement of different plant parameters

Between 1030 and 1100 hours, the third or fourth fully expanded leaf was sampled from each replicate at the

seedling, elongation, heading, and maturity stages at 25, 38, 65, and 95 days after planting, respectively, then stored in a bucket with dry ice during transportation to the laboratory. The leaf samples were washed with distilled water to remove surface dust. The leaf samples for NRA assay and osmotic solutes measurement were immediately placed in liquid N and stored at –40 °C until their use in experimentation.

The whole shoot was sampled from 4 replicates (pots) at each growth stage for measuring dry matter (DM). Grain yield (GY) was measured at the maturity stage after harvest.

RWC was determined using the following formula (Gao, 2000):

RWC (%) = [(FW – DW) / (TW – DW)] × 100,

where FW is fresh weight of sample, TW is turgid weight of sample, and DW is dry weight of sample.

The measurement of NRA was carried out in vitro (Wray and Filner, 1970). Two portions of 0.25 g from each fresh leaf sample were taken. One was used for DM weight determination and the second portion was homogenized in 5 mL of 0.1 M phosphate buffer (pH 7.5) and 1 mM cysteine and then centrifuged. The reaction mixture containing 0.5 mL of enzyme extract, 0.01 mL of 0.1 M KNO_3, 0.5 mL of phosphate buffer (0.1 M, pH 7.5), 0.1 mL of 1 mM NADH, and 0.1 mL of double-distilled water was subjected to 30 °C for 45 min. The reaction was terminated by adding 1 mL of sulfanilamide (1% in 3 M HCl and 1 mL of 0.02% N-1-naphthylethylenediamine dihydrochloride). The reaction mixture was centrifuged to get rid of proteins and absorbance was read at 520 nm against a blank. The standard curve was constructed using 1 µg mL^{-1} of sodium nitrite ($NaNO_2$). The enzyme activity was worked out in terms of nitrite released per milligram protein per hour (NO_2^- µmol mg^{-1} protein h^{-1}).

Measurement of soluble protein concentration (SPC) in fresh leaves was conducted following the method described by Gao (2000). A subsample of 0.5 g of fresh leaves was triturated in 2.5 mL of extraction buffer (30 mmol L^{-1} Tris-HCl, pH 8.7; 1 mmol L^{-1} DTT; 1 mmol L^{-1} vitamin C; 1 mmol L^{-1} EDTA; 5 mmol L^{-1} $MgCl_2$). The mixture was then centrifuged at 5000 × g for 10 min to obtain the supernatant as the protein solution. After treating a subsample of the supernatant with Coomassie Brilliant Blue G-250 solution, the absorbance was read at 595 nm. Bovine serum albumin solution was used as a standard.

Free proline concentration (FPC) in fresh leaf samples was estimated following the procedure of Bates et al. (1973). Leaf samples (0.5 g) were homogenized in 5 mL of 3% sulfosalicylic acid using a mortar and pestle. About 2 mL of extract was placed in a test tube and 2 mL of glacial acetic acid and 2 mL of ninhydrin reagent were added. The mixture was heated in boiling water bath for 1 h. After cooling the mixture in an ice bath, 4.0 mL of toluene was added to it. Absorbance of the colored mixture was read at 520 nm against a blank. Concentration of proline was estimated by referring to a standard curve of proline.

EGB concentration (EGBC) was measured following the method described by Greive and Grattan (1983) with some modifications. Leaf samples (finely ground, 0.5 g) were shaken with 20 mL of deionized water for 48 h at 25 °C. The samples were then filtered and the filtrate was diluted 1:1 with 2 M sulfuric acid. An aliquot (0.5 mL) of this mixture was transferred into a test tube, and then after cooling it for 1 h in an ice bath, cold potassium iodide-iodine reagent (0.2 mL) was added. The samples were centrifuged at 10,000 × g for 15 min at 0 °C, the supernatant was treated with 1,2-dichloroethane (reagent grade), and the absorbance was read at 365 nm. Reference standards of glycine betaine (50–200 µg mL^{-1}) were used for calibration and estimation of glycine betaine concentration in unknown samples.

Concentration of K$^+$ in the leaf samples was determined according to the method described by Bao (2000). Finely ground leaf biomass (0.2–0.3 g) was treated with 18 mol L^{-1} concentrated sulfuric acid and 30 g L^{-1} H_2O_2 at 300 °C for 4–5 h in a digestion tube using the wet ashing method. After wet digestion, the samples were diluted to 100 mL of final volume with deionized water. K$^+$ concentration was determined by flame spectrometry.

The results of the above osmotic nitrides concentrations were expressed in µmol g^{-1} DW based on mean of 4 replicates (n = 4). The relative contribution of a soluble nitride to osmotic adjustment was evaluated by comparing one mole concentration of one solute with another based on DW under DS referring to Zhang et al. (2009b).

2.3. Statistical analysis
The data of each variable were subjected to analysis of variance (ANOVA) separately using the SAS software package (SAS Institute Inc., 1996). Standard errors of the means were calculated. The significance of the treatment effect was assessed using the F-test, and significance of mean separation was worked out by Duncan's multiple range test.

3. Results

3.1. Interactions between K and water regimes in 2 maize cultivars with respect to plant growth, water status, N assimilation, and osmotic solutes
ANOVA showed that the effects of water regime, maize cultivars, and K rates were significant on all measured parameters (Table 1). Two-way interactions among the treatments were also significant for all the parameters. The magnitude of mean squares across the above parameters was, in order: water regime > K rate > cultivar.

Table 1. Mean squares of effects of water regimes (W), potassium rate (K), and cultivar (Cv) on plant growth, water status, nitrate assimilation, and osmotic solute concentration.

Source	df	DM	GY	RWC	KC	NRA	FPC	EGBC	SPC
W	1	701.13***	3289.11***	1540.80***	149,493***	3.256***	5974.93***	37.30***	0.742***
Cv	1	46.20***	8.30***	13.71***	15,527***	0.004***	8.81***	0.17***	0.006***
K	1	32.43***	27.77***	42.93***	77,584***	0.866***	537.13***	0.68***	0.029***
W×Cv	1	226.93***	138.53***	96.00***	526***	0.072***	22.00***	0.29***	0.013***
W×K	1	19.44***	24.16***	27.39***	16,232***	0.516***	660.87***	0.73***	0.025***
Cv×K	1	2.19***	3.96***	0.13	75**	0.003***	0.57	0.02***	0.0002**
W×Cv×K	1	6.00**	3.01**	0.05	23	0.004***	0.71	0.04***	0.0006***
Error	21	0.15	0.20	0.16	8	0.0001	0.24	0.001	0.00002

*: P = 0.05, **: P = 0.01, ***: P = 0.001.
DM, Dry matter; EGBC, endogenous glycine betaine concentration; FPC, free proline concentration; GY, grain yield; KC, K$^+$ concentration; NRA, nitrate reductase activity; RWC, relative water content; SPC, soluble protein concentration.

3.2. Plant growth and plant water status

DS caused a marked reduction in DM weight and GY as well as RWC in both cultivars, though this was more marked in S_{911} than in S_9 (Figure 1; Table 2). The adverse effects of DS on the earlier mentioned parameters were significantly alleviated by exogenous K application. K application led to a significant increase in DM production, GY, and RWC in both cultivars under DS; such a K-induced positive effect was not observed in plant growth and water relations under control conditions. Applied K increased DM of S_{911} by 10.9%–18.5% and that of S_9 by 4.1%–6.6%, and GR of S_{911} by 11.2% and that of S_9 by 8.5% when exposed to DS. RWC also increased by 6.5% and 7.8% for S_9 and S_{911}, respectively (Figure 1; Table 2). The investigated parameters were higher in S_9 as compared with S_{911} with addition of K fertilizer or no K application under DS.

3.3. Nitrate assimilation

The reduction in NRA of plants across different growth stages under DS as compared to that of the control plants was greater for the drought-sensitive cultivar S_{911} than that of the drought-tolerant S_9 (Figure 2). Across different growth stages, the NRA of plants under DS was significantly lower than that of plants under control conditions (Figure 3). NRA increased from seedling to elongation stage, and then decreased towards heading and maturity stages (Figure 3). K application greatly increased NRA in both cultivars under DS compared to control treatments. K application increased NRA by 86% and 61% compared to plants with no K application under DS for S_{911} and S_9, respectively. In control plants, NRA also increased with K application, but the extent of such an increase was much lower than that in stressed plants (Figure 1).

3.4. Accumulation of osmotic solutes

The present study showed that K$^+$ concentration, SPC, EGBC, and FPC were greater in plants of both cultivars under DS than in the controls (Figures 1 and 2).

Furthermore, the magnitude of increase in concentrations of K$^+$ and osmotic nitrides with K application was much greater in plants under DS than in those under control conditions. The concentrations of the above osmotic solutes were greater in S_9 than in S_{911} across both K treatments. In the control treatment, no significant difference was evident between the 2 cultivars with respect to these osmotic solutes, except K$^+$ concentration (Figure 2). Concentrations of osmotic nitrides increased from the seedling stage to the elongation/heading stage, followed by a decrease during the maturity stage, while K$^+$ concentration continuously decreased through the entire growing period (Figure 3). The concentrations of K$^+$ and osmotic nitrides in plants under DS increased with K application in both cultivars. With K application, the magnitude of increase in K$^+$ concentration was much greater in plants grown under DS than that of the plants under control conditions (Figure 1). Across all treatments and both cultivars, K$^+$ concentration and FPC were much greater than those of EGBC or SPC (Figures 1–3).

3.5. Correlations among parameters of plant growth, water status, N assimilation, and osmotic solutes

The correlation coefficients among different plant growth and physiological characteristics under DS and controlled growing conditions are presented in Table 3. In general, correlation coefficients among DM, GY, RWC, NRA, and osmolyte concentrations were greater for the plants under DS than for plants under control conditions (Table 3). Correlations between RWC versus either K$^+$ concentration, NRA, FPC, EGBC, or SPC were significant for plants under DS, but not for those under control treatments.

4. Discussion

Different crop cultivars maintain differential productivity, water status, N assimilation, and osmotic solute accumulation under DS, which can be altered by K
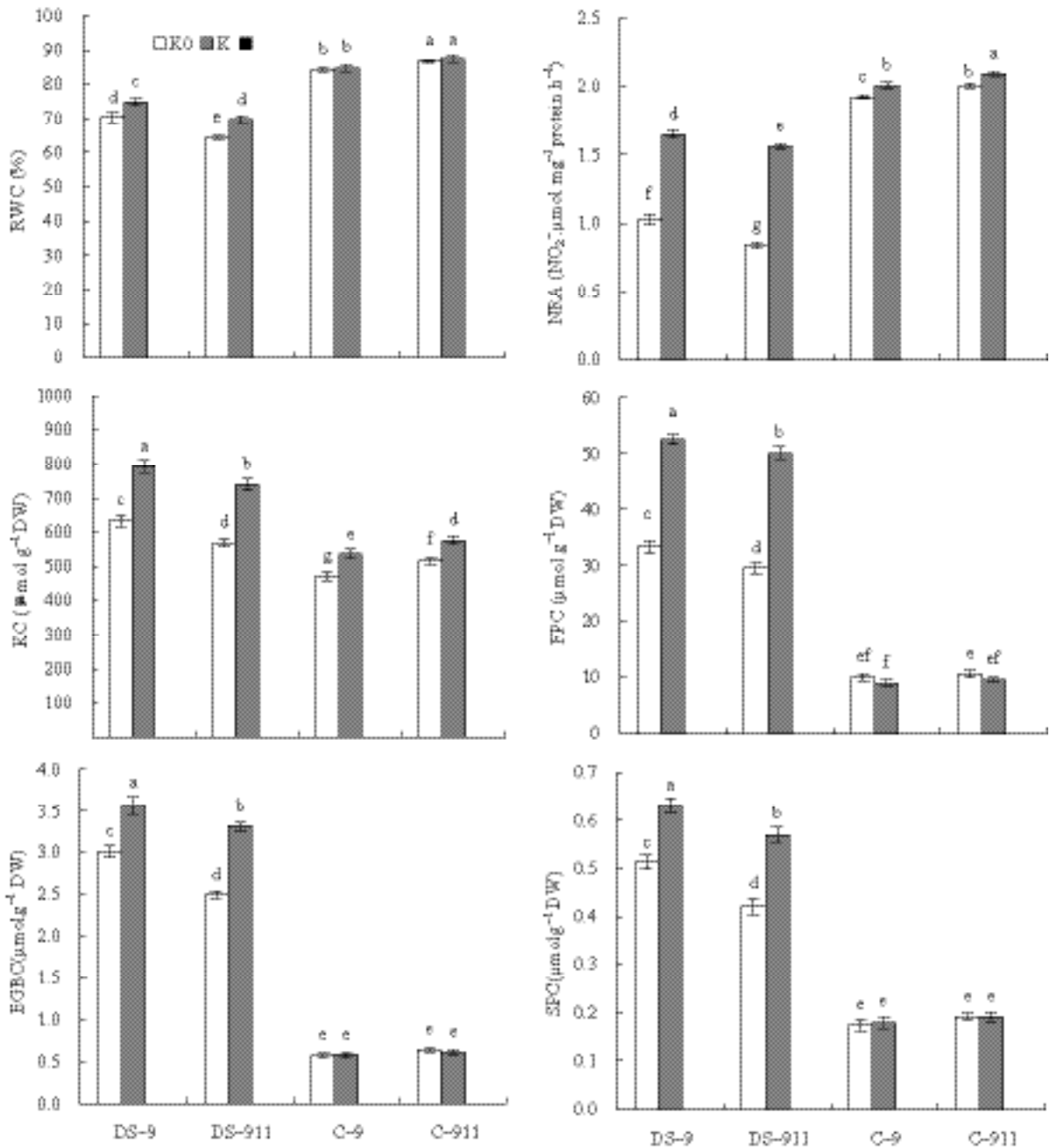
Figure 1. Effects of potassium application on relative water content (RWC), nitrate reductase activity (NRA), K+ concentration (KC), free proline concentration (FPC), endogenous glycine betaine concentration (EGBC), and soluble protein concentration (SPC) in 2 maize cultivars under drought stress (DS) or well-watered treatment (C). Cultivars Shaandan 9 and Shaandan 911 are represented by 9 and 911, respectively. Each value is the mean across 4 growth stages. Different letters at the top of histograms within each response variable indicate significant difference (P < 0.05).

application. The significant interactions in this study showed that choice of cultivar, optimal water, and K management are important to overcome the adverse effects of DS in terms of plant growth, water status, N assimilation, and osmotic solute accumulation. These

results are in agreement with those of Zhang and Li (2005) and Zhang et al. (2007).

DS is generally characterized by a disorder in growth and a reduction in yield, which is dependent on the cultivar's drought tolerance and nutrient supplies

Table 2. Dry weights and grain yield of 2 maize cultivars at different growth stages influenced by drought stress (DS) and potassium (K) application.

Treatment			Dry matter (g plant^{-1}) at different growth stages				Grain yield
Water regime	Cultivar	K rate	Seedling	Elongation	Heading	Maturity	(g plant^{-1})
DS	S_9	K_0	4.81 b	47.86 b	72.80 b	85.06 b	44.01 b
		K	5.13 a	50.72 a	76.65 a	88.56 a	47.74 a
	S_{911}	K_0	4.06 b	36.54 b	65.46 b	74.45 b	40.24 b
		K	4.81 a	42.67 a	72.64 a	83.13 a	44.76 a
Mean of S_9			4.97 A	49.29 A	74.73 A	86.81 A	45.88 A
Mean of S_{911}			4.43 B	39.61 B	69.05 B	78.79 B	42.50 B
Control	S_9	K_0	11.92 a	68.93 a	102.26 a	107.04 a	49.47 a
		K	12.24 a	68.89 a	100.66 a	108.54 a	51.60 a
	S_{911}	K_0	12.47 a	70.45 a	106.28 a	115.04 a	60.00 a
		K	12.39 a	70.96 a	107.06 a	114.84 a	58.92 a
Mean of S_9			12.08 A	68.91 A	101.46 B	107.79 B	50.53 B
Mean of S_{911}			12.43 A	70.71 A	106.67 A	114.94 A	59.46 A

K_0 = no K, K = 0.42 g K kg^{-1} soil; Control = well-watered plants, DS = drought stress, S_9 = Shaandan 9, S_{911} = Shaandan 911. Means with different letters within a column are significantly different at P < 0.05. Lowercase letters: effects of K rates by each cultivar and water regime treatment. Uppercase letters: cultivar comparison means across both K rates by each water regime treatment.

(Cakmak, 2005). On the one hand, the drought-tolerant maize cultivar S_9 had a greater DM production and GY regardless of K application (Table 2) and N application (Zhang et al., 2007). On the other hand, numerous studies have shown that the application of K fertilizer mitigates the adverse effects of DS on growth of different crops such as barley (*Hordeum vulgare* L.) (Andersen et al., 1992), faba beans (*Vicia faba* L.) (Abdelvahab and Abdalla, 1995), sugar cane (*Saccharum officinarum* L.) (Sudama et al., 1998), and rice (*Oryza sativa* L.) (Tiwari et al., 1998). K fertilization increased DM production and GY of both cultivars under DS to levels above those of plants with no K supply. However, K fertilization had no significant influence on the above parameters of both cultivars under nonstressed conditions (Maathuis and Sanders, 1996; Tabatabaii Ebrahimi and Yarnia, 2011; Table 2). The results demonstrate the beneficial effects of K in terms of mitigating the negative effects of DS on maize plants by its role in maintaining optimal water relations under DS conditions, which in turn support various physiological processes to enhance plant tolerance to DS; none of this is due to the nutritive role of K (Cakmak, 2005; Table 2).

RWC is an appropriate index of plant water status in terms of the physiological consequences of cellular water deficit. DS generally induces a decrease in RWC (Cakmak, 2005). Fanaei et al. (2009) reported a significant increase in RWC of 2 oilseed species with K fertilization across a range

of DS. Similar responses to K were also reported in mung bean (Nandwal et al., 1998), maize (Premachandra et al., 1991), and wheat (Pier and Berkowitz, 1987) under DS. In both maize cultivars, the RWC of the plants under DS was greater with K fertilization than in the plants without K supply (Figure 1). However, this trend was not observed in nonstressed control plants. Application of K contributes to improved cell turgor through osmotic adjustment (Maathuis and Sanders, 1996). Plants absorb K from the soil to maintain normal growth and development under DS (Elumalai et al., 2002).

Nitrate reductase plays a key role in nitrate assimilation. Several crop species adapt to DS conditions by enhancing their NRA. Thus, NRA could be used as an index for assessing plant response to DS (Caravaca et al., 2003). Previously, it has been shown that changes in NRA under DS were dependent on the inherent capability of a given crop/cultivar to respond to DS and K supplies and growth stage (Zhang et al., 2011). DS was found to induce a greater reduction in NRA of the drought-sensitive cultivar S_{911} than of the drought-tolerant S_9 at different growth stages (Figure 2). Guan et al. (1996) reported that NRA in corn leaves increased gradually from the elongation stage, reached maximum at blossoming, and then decreased considerably at the maturity stage. In contrast, NRA showed an increasing trend from seedling to elongation stage, and then a decreasing trend to the end of growth
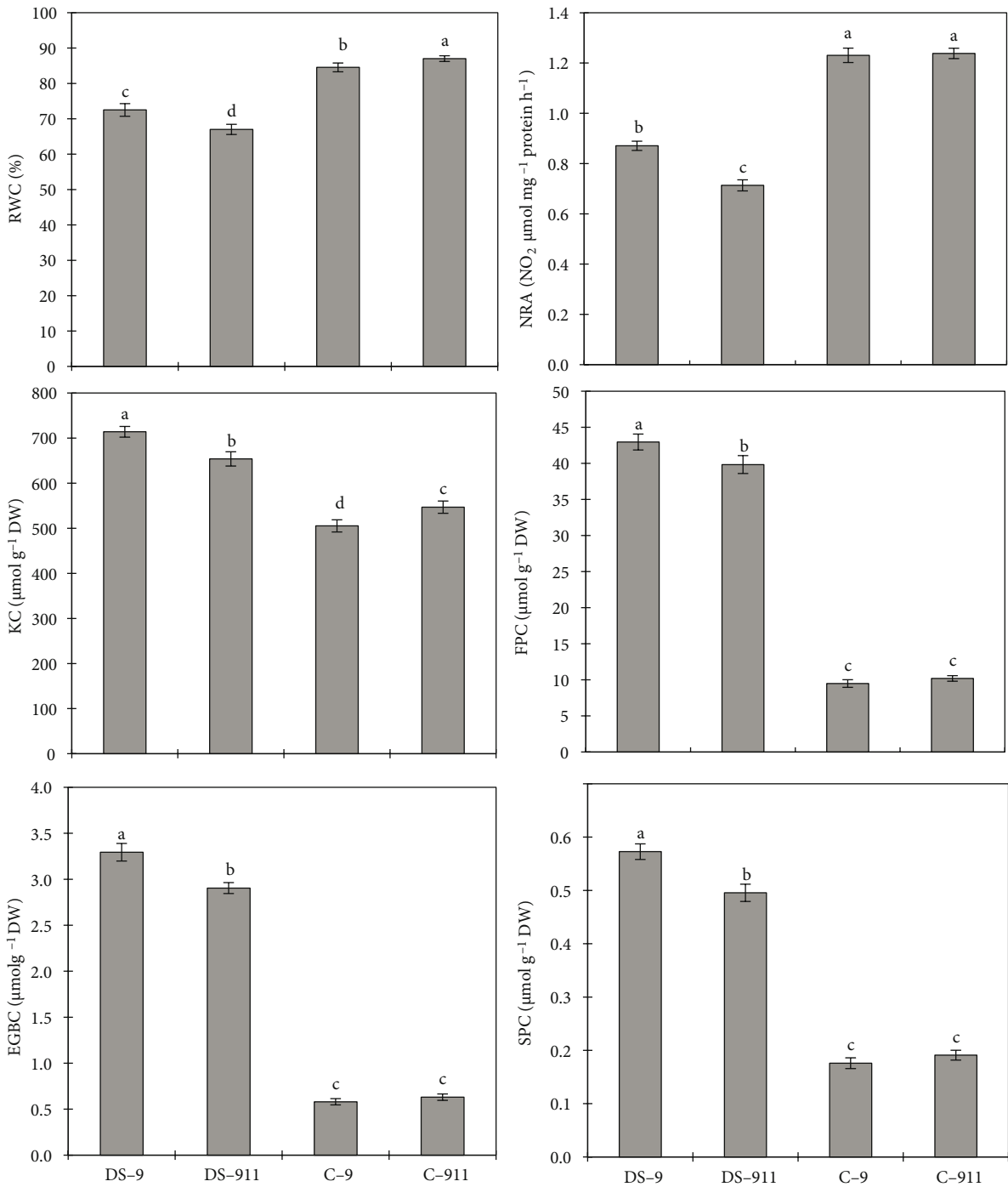
Figure 2. Effects of maize cultivar on relative water content (RWC), nitrate reductase activity (NRA), K⁺ concentration (KC), free proline concentration (FPC), endogenous glycine betaine concentration (EGBC), and soluble protein concentration (SPC) under drought stress (DS) or well-watered treatment (C). Cultivars Shaandan 9 and Shaandan 911 are represented by 9 and 911, respectively. Each value is the mean across 4 growth stages and 2 K treatments. Different letters at the top of histograms within each response variable indicate significant difference (P < 0.05).

(Figure 3). Applied K exhibited a significant influence on NRA, as it increased in both cultivars under DS compared with NRA in control plants (Figure 1). These findings

support the hypothesis that K is required for maintaining the activation of enzymes and protein synthesis in plants, because the whole structure of proteins and protein
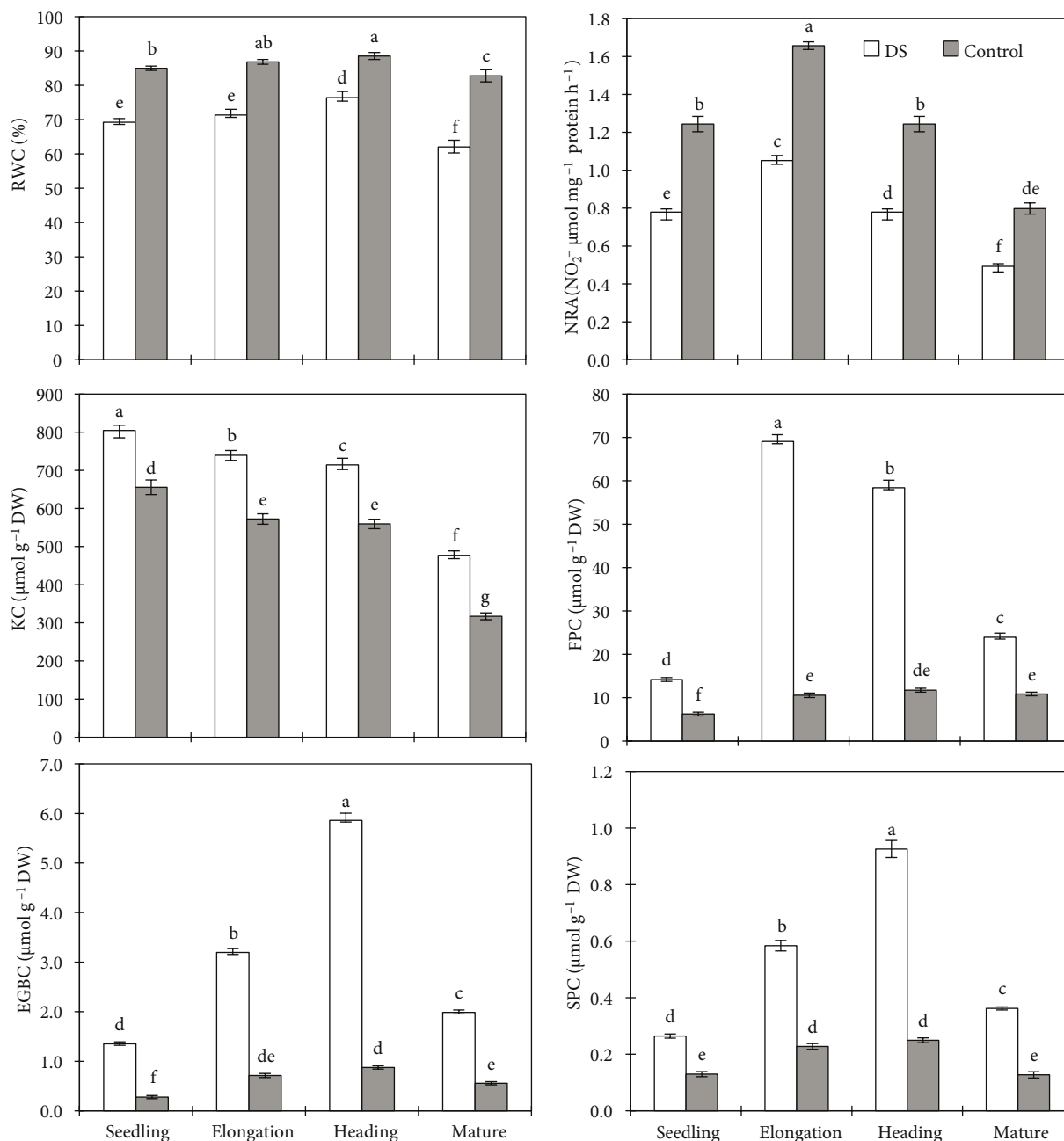
Figure 3. Effects of drought stress (DS) on relative water content (RWC), nitrate reductase activity (NRA), K^+ concentration (KC), free proline concentration (FPC), endogenous glycine betaine concentration (EGBC), and soluble protein concentration (SPC) in maize at different growth stages. Control represents well-watered treatment. Each value is the mean across 2 cultivars and 2 K treatments. Different letters at the top of histograms across all growth stages indicate significant difference ($P < 0.05$).

activity needs high concentrations of K^+ in the cytosol for optimum plant functions (Taiz and Zeiger, 2002; Cherel, 2004).

Exposure to DS may induce osmotic stress, resulting in detrimental effects on plant growth and development. Therefore, maintenance of osmotic homeostasis is vital for plant adaptation to DS. Osmoregulation in plants is achieved

by the absorption, transportation, and compartmentation of water and solutes, including K, which is an important ion present in large amounts in cells for maintaining membrane potential and turgor and for the regulation of osmotic pressure (Taiz and Zeiger, 2002; Cherel, 2004; Ashraf and Foolad, 2007; Chołuj et al., 2008; Ashraf, 2010; Yıldıztugay et al., 2014). An increased accumulation of K

Table 3. Correlation coefficients among different parameters of 2 maize cultivars as influenced by DS (above diagonal) and control (below diagonal) growing conditions.

Character	DM	GY	RWC	KC	NRA	FPC	EGBC	SPC
DM		0.925***	0.977***	0.810***	0.701**	0.677**	0.904***	0.898***
GY	0.874***		0.956***	0.874***	0.781**	0.786**	0.917***	0.925***
RWC	0.461	0.440		0.869***	0.771**	0.756**	0.930***	0.938***
KC	0.608*	0.616*	0.493		0.982***	0.980***	0.976***	0.981***
NRA	0.641*	0.741**	0.337	0.687**		0.996***	0.936***	0.938***
FPC	0.649*	0.648*	0.503	0.444	0.603*		0.924***	0.928***
EGBC	0.373	0.489	0.429	0.253	0.593*	0.743**		0.994***
SPC	0.624*	0.636*	0.513	0.374	0.827***	0.476	0.924***	

*, **, ***: Significant at 5%, 1%, and 0.1 %, respectively.
DM, Dry matter; DS, drought stress; EGBC, endogenous glycine betaine concentration; FPC, free proline concentration; GY, grain yield; KC, K^+ concentration; NRA, nitrate reductase activity; RWC, relative water content; SPC, soluble protein concentration; S_9, Shaandan 9; S_{911}, Shaandan 911.

was observed under DS in tropical grasses, soybean, and maize for osmotic adjustment to help retain water (Taiz and Zeiger, 2002; Cakmak, 2005). For this reason, tissue K^+ concentration of plants under DS is much greater than the recommended optimal K concentration for plants under controlled growing conditions (Cakmak, 2005). Ma et al. (2004) reported that *Brassica napus* accounted for about 25% of drought-induced changes in osmotic adjustment from K^+ accumulation in the expanding leaves in 2 genotypes.

The accumulation of soluble osmotic nitrides is an expected response of plants under DS, as derivative products from N metabolism are proposed to counteract the dehydration caused by DS. Therefore, the levels of K^+ and soluble osmotic nitrides in plant tissues are an indication of responses to DS. These compounds contribute to osmoregulation and hence osmotolerance to maintain desirable RWC and mitigate the adverse effects of DS (Taiz and Zeiger, 2002; Ashraf and Foolad, 2007; Chołuj et al., 2008; Ashraf, 2010; Zhang et al., 2013; Yıldıztugay et al., 2014). Drought may induce synthesis and accumulation of organic osmolytes such as SP, EGB, and FP as well as K^+, which vary between and within plant species (Zhang et al., 2009a, 2009b; Figures 1 and 2). The above responses were greater for both cultivars with K application under DS than for those under control conditions. They were also greater for drought-sensitive S_{911} than for the tolerant cultivar S_9. Contents of the osmotic solutes were greater in the drought-tolerant cultivar (S_9) than in the sensitive cultivar S_{911} under DS, irrespective of K treatments. However, the 2 cultivars showed similar contents of these osmotic solutes, except for K^+ in control plants (Figure 2). This is clear evidence of the role of K^+ and osmotic nitrides

in mitigating the effects of DS, particularly in the case of a DS-tolerant cultivar (Figures 1 and 2).

Accumulation of osmotic nitrides in maize plants varied with different growth stages, which followed an increasing trend from seedling to elongation/heading stage and then a decreasing trend towards the maturity stage. Inorganic osmolytes such as K^+ decreased across the whole period (Figure 3). This trend was also observed in some previous studies (Zhang et al., 2002, 2009). DS-induced accumulation of certain osmotic solutes, mostly in the cytoplasm, can protect cell membranes, proteins, and metabolic machinery and consequently preserve subcellular structures from damage under cell dehydration caused by DS (Taiz and Zeiger, 2002).

Adequate supply of K to crop plants may cause osmotic adjustment, in which leaf water potential of the cells is reduced to maintain turgor pressure. This facilitates DS toleration in plants by improving plant growth and increasing yield (Cakmak, 2005). The addition of K application could strengthen the accumulation of K^+ and osmotic nitrides in plants, especially for a drought-sensitive cultivar under DS (Figure 1). Additionally, the comparison of all osmolytes measured in this study indicated the relative contribution of K^+ and FP to osmotic adjustment in all treatments and both cultivars had reasonably high amounts of EGB and SP (Zhang et al., 2009; Figures 1–3).

Correlations between different sets of parameters show that those among DM, GY, and most of the parameters measured under DS were stronger than the correlations under well-watered treatments. In particular, significant positive correlations were found between RWC and K^+ concentration, NRA, FPC, EGBC, and SPC under DS; the same correlations were nonsignificant under control

conditions. Thus, it appears that K⁺ and these organic osmotic solutes play an important role in improving plant growth, water status, and N assimilation in plants tissues under DS (Zhang et al., 2007; Mohammad and Mahmood, 2011).

The mechanism of K-induced DS tolerance was found to be due to an increase in RWC, NRA, K⁺ concentration, FPC, EGBC, and SPC in maize cultivars. The beneficial effects of K were greater on the drought-sensitive cultivar (S_{911}) than on the drought-tolerant cultivar (S_9). Thus, accumulation of K and osmotic nitrides appeared to improve plant water status and N assimilation under DS, which increased plant growth and yield.

In conclusion, this study demonstrated that the negative impacts of DS on plant growth could be significantly mitigated by adequate K supply, which, in turn, can improve plant water status, N assimilation, and osmotic adjustment, though not due to its nutritive role. The positive responses to the application of K under DS were greater in the drought-sensitive S_{911} than those in the drought-tolerant S_9. Leaf water status of the plants, N assimilation, and osmotic adjustment parameters under DS were greater in the drought-tolerant cultivar (S_9) than in the drought-sensitive one (S_{911}).

Acknowledgments

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