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ORIGINAL ARTICLE

Effect of foliar fertilization application on the growth and mineral nutrient content of maize seedlings under drought and salinity

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Abstract

The objective of the present study was to investigate the interactive effects of foliar fertilization and drought and salinity on the growth of maize. Maize plants were grown in soil under drought or salinity in a greenhouse for 23 days after sowing. At harvest, plant height, shoot biomass, and the lengths, fresh weights and dry weights of the blade in the expanded leaf 3 and expanding leaves 4 and 5 were determined. Mineral elements (Na, K, Ca, Mg, P and N) in individual leaves were analyzed. Although there was a reduction in evapotranspiration, maize growth, such as shoot fresh weight and dry weight, and leaf fresh weight and dry weight under drought and salinity, the application of foliar fertilization did not improve plant growth under short-term drought or salt stress. Drought reduced the uptake of K, Ca, Mg and P, which may be attributed to decreased transpiration. An increase or no change in the nutrient concentration in leaves under saline conditions suggests that an osmotic effect may be responsible for the plant reduction.

Key words: drought, foliar fertilization, leaves, maize seedling, mineral nutrients, salinity.

INTRODUCTION

The increasing frequency of dry periods in many regions of the world and the problems associated with salinity in irrigated areas frequently result in the consecutive occurrence of drought and salinity on cultivated land. Currently, approximately 50% of irrigated land in the world, which has at least twice the productivity of rainfed land and may produce one-third of the world's food, is affected by salinization (Ghassemi *et al*. 1995; Hillel 2000). Although a water deficit or osmotic effect is probably the major physiological mechanism for growth reduction as both stresses lower the soil water potential, drought and salinity may also differentially affect the mineral nutrient relations in plants. In general, drought reduces both nutrient uptake by the roots and transport from the roots to the shoots because of restricted transpiration rates and impaired active transport and membrane permeability (Alam 1999; Viets 1972). A decline in soil moisture also results in a decrease in the diffusion rate of nutrients from the soil matrix to the absorbing root surface (Alam 1999; Pinkerton and Simpson 1986; Viets 1972). Studies in the literature also show that, under drought conditions, an increase in soil fertility enhances plant growth in wheat (Gutierrez-Boem and Thomas 1998) and millet (Bagayoko *et al*. 2000; Brück *et al*. 2000) and increases nutrient uptake in maize (Studer 1993). In contrast to drought, soils contain extreme ratios of Na/Ca, Na/K, Ca/Mg and Cl/NO_3 under saline conditions, which cause reduced plant growth because of specific ion toxicities (e.g. Na and Cl) and ionic imbalance acting on biophysical and/or metabolic components of plant growth (Grattan and Grieve 1999). To date, it is not clear whether water deficit or ionic effects are a major limiting factor to plant growth. Munns (1993) proposed a two-phase model for the inhibition of growth by salinity, that is, the first phase of growth reduction in this model results from the osmotic effect of soil salinity that may cause water deficit, and under extended periods (the second phase) salt begins to accumulate in older leaves and salt injury becomes apparent. To identify a separate effect of the osmotic stress from ionic stress during the first phase, a comparison between NaCl and polyethyleneglycol (PEG) treatments in hydroponics with iso-osmotic conditions

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has been studied in barley (Kawasaki *et al.* 1983; Storey and Wyn Jones 1978), tomato (Perez-Alfocea *et al.* 1993) and maize (Sümer *et al.* 2004). These studies have showed controversial results. To further our understanding of the water deficit or nutrient effect on plants under drought and salinity, therefore, there is a need to study the effect of the supply of mineral nutrients on plant growth and the nutrient status under both stresses.

The supply of nutrients via the roots is restricted under drought and salinized soils because of the negative effect of drought and salinity on nutrient availability. The efficacy of foliar fertilization is higher than that of soil fertilizer application in these situations. The reasons for this are because of the supply of the required nutrient directly to the location of demand in the leaves and its relatively quick absorption (e.g. 0.5–2 h for N and 10–24 h for K), and the independence of root activity and soil water availability (Römheld and El-Fouly 1999). At early growth stages, foliar fertilization could increase P and K supplies at a time when the root system is not well developed (Mallarino *et al.* 2001). Thus, foliar nutrient application under drought and salinity conditions may be able to exclude or include a water deficit or nutrient effect under short-term drought or salt stress.

Like other grasses, vegetative growth of maize is highly sensitive to drought and salinity. Thus, the objectives of the present study were to investigate the effects of drought and salinity on the shoot and leaf growth of maize seedlings to verify whether the direct supply of nutrients via foliar fertilization may play a primary role in increasing maize tolerance to drought and salinity, and to further our understanding of the nutrient effect on maize growth during the initial period of drought and salinity stresses.

MATERIALS AND METHODS

Plant materials and growth conditions

Maize seeds (*Zea mays* L. cv. Rasant) were pregerminated for 1 day after which 10 seeds were sown in 7-L pots filled with loamy soil. One week after sowing, the seedlings were thinned to seven plants per pot. The experiment was carried out in a greenhouse. The air temperature ranged from 37°C (maximum at day) to 10°C (minimum at night). The average daily temperature was approximately 20°C. Relative humidity fluctuated between 30 and 85%, with an average of approximately 60%.

Loamy soil was collected from the soil surface (0–15 cm), air-dried, ground, passed through a 5-mm mesh screen and thoroughly mixed. The soil consisted of 23% clay, 48% silt and 29% sand, and the organic matter content was 1.66%. The pH ($CaCl_2$) was 5.7. The air-dried soil, which had a gravimetric water content of 8%, was filled layer-wise in six layers in 7-L pots. To obtain the final value of 20% soil gravimetric water content, nutrient solution with or without NaCl was added to each layer. Nitrogen was applied as 0.2 g NH_4NO_3 per pot. Both the water content and the amount of nutrient were optimal for plant growth according to our previous tests. For the salinized treatment, the final level of 100 mmol L^{-1} NaCl was obtained by adding NaCl to the nutrient solution and applying it to the top soil layer 10 days after sowing. To reduce evaporation, 400 g of coarse sand (2 mm in diameter) was placed on the soil surface in all treatments. For the control and salinized treatments, the pots were weighed daily and the water loss was replaced by adding tap water during the experiment as necessary. Drought stress was started at day 18 after sowing by replacing only one-quarter of the water losses after this time. During the drought period, the soil matric potentials decreased from –0.02 MPa at day 19 to –0.4 MPa at day 23 after sowing. Salinity treatment at 100 mmol L^{-1} NaCl caused a soil osmotic potential of approximately –0.4 MPa.

The application of foliar fertilization started on the same day as the drought stress began and lasted for 5 days. The optimal level of N, P and K nutrients was chosen based on suggestions by Finck (1992). Four different foliar treatments were applied: (1) tap water was sprayed twice per day (H_2O; osmotic potential –0.02 MPa); (2) NPK solution (7.5 g KNO_3 + 1.5 g KH_2PO_4 + 1 g K_2HPO_4 per liter tap water; osmotic potential –0.40 MPa) was applied twice only on days 1 and 4 during the 5-day period (2×NPK); (3) NPK solution was applied twice per day (NPK); (4) N and K solution (7.5 g KNO_3 per liter tap water; osmotic potential –0.34 MPa) was applied twice per day (NK). A backpack sprayer was used to spray the nutrient solution or water. The foliar fertilization wet the whole leaf surface. All treatments were replicated four times.

Analysis of plant growth

The accumulation of evapotranspiration and evaporation was determined by respectively weighing the pots with and without the plants daily.

Maize plants were harvested at day 23 after sowing and the shoot fresh weight was determined. At the early growth stages, shoot biomass in maize is composed mainly of the leaves. At the final harvest, leaf 3 was fully expanded in all three treatments, whereas leaves 4 and 5 were still expanding. These three youngest leaves were carefully removed from the shoot, which was enclosed in the older leaf sheath. The blade and sheath were separated if present. After the length and fresh

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weight of the leaf blade were measured, the leaf surface was cleaned using a wetted tissue. Plant material was oven-dried at 60°C for 2 days and the dried samples were weighed.

Determination of ion concentrations

The dried samples of leaf blades were ground to pass through a 1-mm diameter sieve. The concentrations of Na, K, Ca, Mg and P were determined with an Inductively Coupled Plasma Emission Spectrometer (ICP model Liberty 200; Varian Australia, Mulgrave Victoria, Australia). Before the analysis, 50 mg of ground dry material was digested by adding 2 mL concentrated HNO_3 (65%) and 1 mL H_2O_2 (30%) for 30 min at 2,600 kPa (80 psi) in a MDS-2100 microwave oven (CEM Corporation, Matthews, NC, USA). After digestion, each sample was brought up to the final volume of 25 mL with deionized water.

The total nitrogen content was determined with an Isotope Radio Mass Spectrometry (IRMS) combined with a preparation unit (ANCA SL 20-20; Europe Scientific, Crewe, UK). Two milligram samples were weighed with a super microbalance (Sartorius AG, Götingen, Germany).

Statistical analysis

A randomized complete block design was used. All treatments were replicated four times. Data were analyzed by ANOVA using JMP 4.02 (SAS 2000) to test the significance of the main effects. Means separation on data was conducted using least significant difference (LSD) multiple range tests. Terms were considered significant at $P < 0.05$.

RESULTS

Cumulative evapotranspiration and plant growth

Figure 1 shows that cumulative evapotranspiration was significantly affected by drought at day 1 or 2 after the treatment began (i.e. at day 19 or 20 after sowing) and by salinity (salinized at the beginning) at day 16 after sowing, regardless of the foliar fertilization treatments ($P < 0.05$). At day 23 after sowing, evapotranspiration of the plants with an average of foliar fertilization treatments under drought stress was reduced by approximately 31%, which was similar to the values of the salinized plants. However, there was no significant difference in evapotranspiration for the control treatment among foliar application. Under drought conditions, higher evapotranspiration for the tap water application was observed, whereas under saline conditions, evapotranspiration with tap water was lower than the other foliar applications, but not significantly lower.

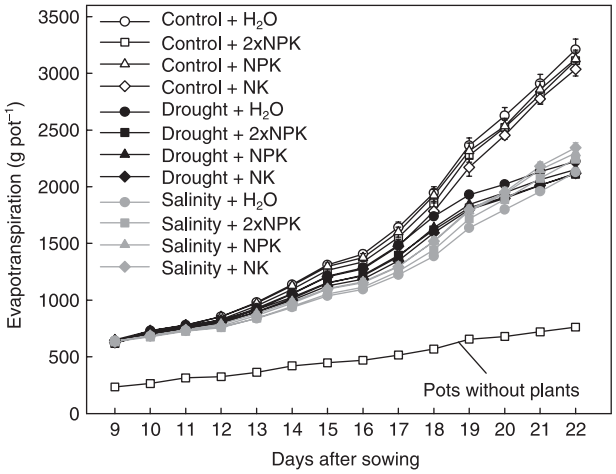

Figure 1 Accumulative evapotranspiration (g pot^{-1}) with time under control, drought and saline conditions applied with four different foliar treatments: tap water (H_2O); NPK solution applied only twice (2×NPK); NPK solution daily (NPK); N and K solution (NK). Error bars represent standard errors and fit within the plot symbol if not visible ($n = 4$).

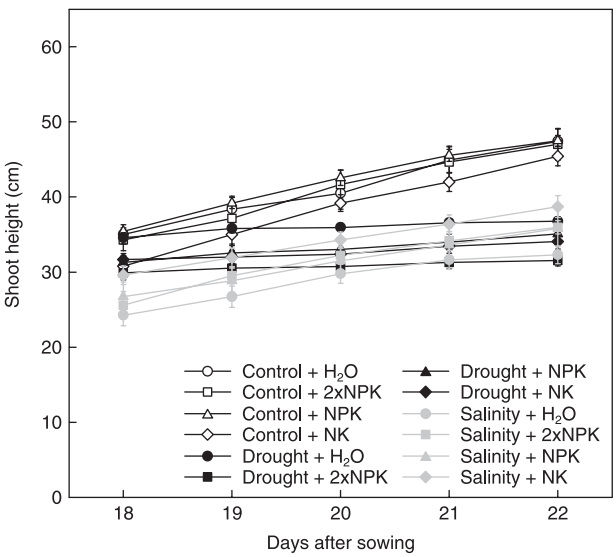

Figure 2 Plant height of maize seedlings with time under control, drought and saline conditions applied with four different foliar treatments: tap water (H_2O); NPK solution applied only twice (2×NPK); NPK solution daily (NPK); N and K solution (NK). Error bars represent standard errors and fit within the plot symbol if not visible ($n = 4$).

The graph of plant height over time (Fig. 2) illustrates that at day 2 after imposing drought, plant height was obviously reduced compared with the control, indicating a rapid response to drought by the plants. There was no significant difference in plant height for the control among the foliar treatments. Under drought conditions, however, plant height was higher for the tap water

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application compared with the other foliar applications. Under saline conditions, plant height with the application of tap water was lower than the height with the other foliar applications. When plants were harvested at day 23 after sowing, the reduction in plant height compared with the control plants was 23% for H_2O daily, 33% for 2×NPK, 26% for NPK daily and 25% for NK daily under drought and 32% for H_2O daily, 23% for 2×NPK, 25% for NPK daily and 15% for NK daily under salinity, respectively.

The shoot fresh weight under drought and salinity was significantly reduced by approximately 50% compared with the control plants ($P < 0.05$) (Fig. 3). Similarly, shoot dry weight was significantly reduced by both drought and salinity (Fig. 3). Fresh weight did not significantly differ between drought and salinity for the H_2O treatment, whereas it was higher for other foliar fertilization treatments under salinity than under drought. In contrast, shoot dry weight was higher for the H_2O treatment under drought than under salinity, whereas there was no significant difference between both stresses for the treatments with 2×NPK, NPK and NK daily.

The length of the fully developed blade of leaf 3 and the expanding blades of leaves 4 and 5 is presented in Fig. 4. In general, drought and salinity significantly reduced the length of all three leaves regardless of the foliar treatments. However, there was no significant difference in length of leaves 3, 4 and 5 for the H_2O spray application between drought and salinity treatments. For the other three foliar applications, the length of leaves 3 and 5 was higher under salinity than under drought, whereas the length of leaf 4 was lower under salinity. For a given condition (i.e. control, drought or salinity), there was no significant difference among the foliar fertilization applications, and this was similar for leaf fresh and dry weights (Fig. 5).

Drought and salinity significantly decreased both the fresh and dry weights of leaves 3, 4 and 5 regardless of the foliar fertilization treatments (Fig. 5). Compared to salinity, drought resulted in a higher reduction in the fresh weight of leaves 4 and 5 and in the dry weight of leaf 5.

Mineral nutrient concentrations in leaves 3 and 5

As there was a similar tendency in the concentrations of mineral elements (Na, K, Ca, Mg, P and N) (in mmol kg^{-1} dry weight) for leaves 4 and 5, the results for leaves 3 and 5 are presented in Fig. 6. Salt stress caused a significant ($P < 0.05$) increase in the Na concentration in the blades of leaves 3 and 5, and Na concentration was higher in leaf 3 than in leaf 5 regardless of the foliar fertilization treatments (Fig. 6). Salinity increased or did not change the concentration of nutrients (K, Ca, Mg, P and N) in leaf blades, whereas the application of foliar fertilization did not influence these tendencies. In contrast, drought reduced or did not significantly change the concentration of K, Ca, Mg and P in leaf blades. Similar to the salinity treatments, there was almost no effect of foliar fertilization application on the status of these elements in leaves. Although the N concentration in leaf 3 was not affected by drought for all foliar fertilization applications, N concentration in leaf 5 was increased by drought stress.

DISCUSSION

Drought and salinity significantly decreased leaf growth in terms of length and weight (Figs 3,4), confirming findings from earlier studies that leaf growth in the seedling stage is highly sensitive to these stresses (Rodriguez *et al.* 2004; Schmidhalter *et al.* 1998; Studer 1993).

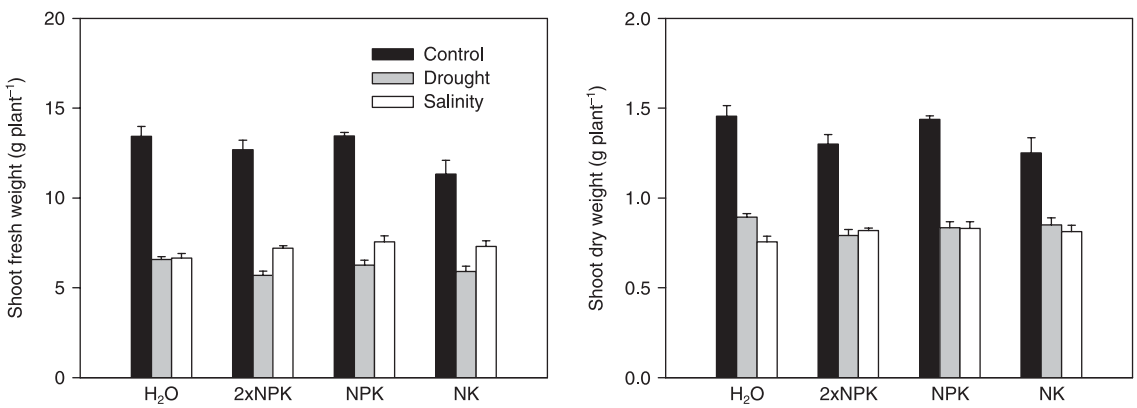

Figure 3 Shoot fresh weight and dry weight (g $plant^{-1}$) of maize seedlings at harvest under control, drought and saline conditions applied with four different foliar treatments: tap water (H_2O); NPK solution applied only twice (2×NPK); NPK solution daily (NPK); N and K solution (NK). Error bars represent standard errors ($n = 4$).

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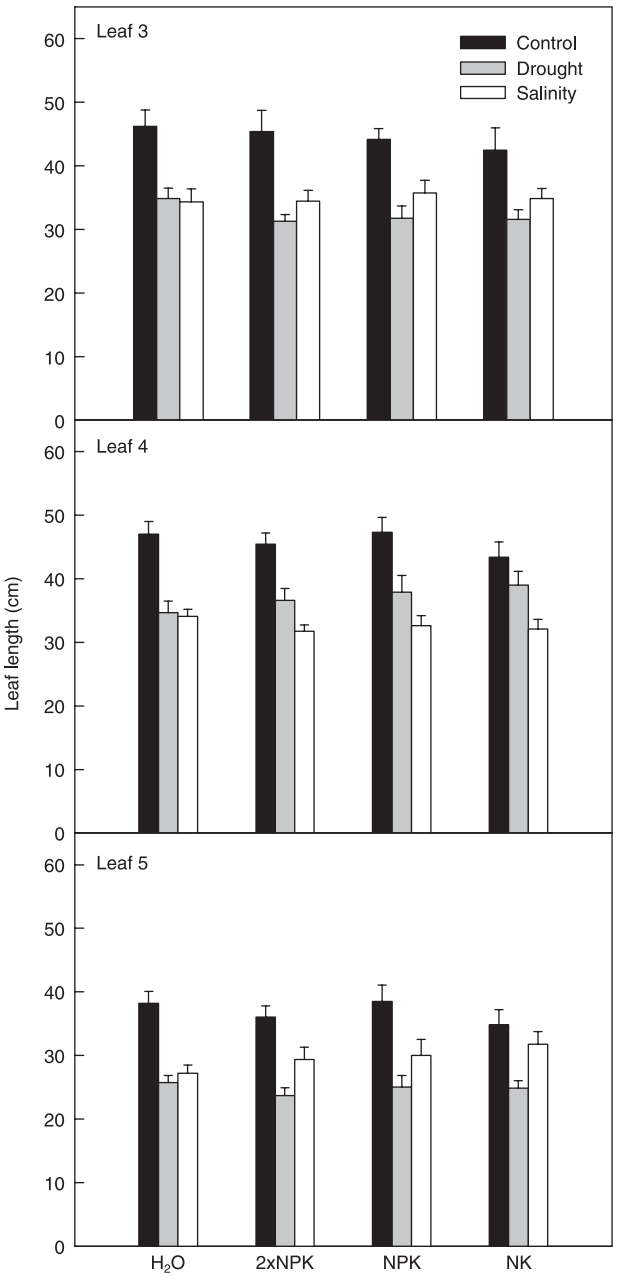

Figure 4 Length of blade of leaves 3, 4 and 5 of maize seedlings at harvest under control, drought and saline conditions applied with four different foliar treatments: tap water (H_2O); NPK solution applied only twice (2×NPK); NPK solution daily (NPK); N and K solution (NK). Error bars represent standard errors ($n = 4$).

Reduced plant growth caused by drought and salinity may be attributed to a disturbance in the nutrients, resulting in the decreased uptake of K, Ca, Mg, P and N. To test this hypothesis, variation in the nutrient status of plants grown under control and stress conditions and the effect of supplying nutrients on plant growth and nutrient status in plants would exclude or include the nutrient effect over a short-term period of drought or salt stress. In contrast to the increased nutrient concentration under saline conditions, lowered K, Ca and Mg concentrations in leaves 3 and 5 and P concentration in leaf 5 were observed for drought conditions. However, applying foliar fertilization did not enhance either the growth or the ion uptake of maize plants. Although there are many studies on the effect of nutrients on plant growth under drought conditions, the results are contradictory. For example, Bennett *et al.* (1986) and Sadras (2004) reported a positive effect of N supply on plant growth, whereas Morgan (1986) reported that a higher N supply negatively affected plant growth under drought conditions. Maintained turgor of roots under drought stress obtained with an optimal nutrient supply results in better root growth and apparently promoted overall plant growth in maize seedlings (Studer *et al.* 2007). An early study of the relationships between water availability and N fertilizer responses from Smika *et al.* (1965) demonstrated that fertilizer N will not increase yield without sufficient water being available to the plant. This is also true for other nutrients, such as K and P (Hu and Schmidhalter 2005). Aside from lowering nutrient availability in soil, a reduced plant nutrient uptake may also be attributed to a decreased transpiration rate to transport nutrients from roots to shoots (Tanguilig *et al.* 1987). When plants suffer from a nutrient deficiency, the efficacy of foliar fertilization is higher than that of soil fertilization. The reasons for this are because of the supply of the required nutrient directly to the location of demand in the leaves and its relatively quick absorption (e.g. 0.5–2 h for N and 10–24 h for K), and independence of root activity and soil water availability (Römheld and El-Fouly 1999). Under non-stress conditions, the application of foliar nutrients has been recommended to more effectively improve plant growth at the early stages of development because foliar absorption rates for younger leaves are more favorable than for older leaves (Wittwer and Teubner 1959). Foliar application of N, P, K and S at the four–five-leaf stages significantly increased the N and P contents of maize seedlings and resulted in an increased final grain yield (Giskin and Efron 1986). No effect of the foliar application of fertilizer on plant growth and ion concentrations in maize leaves in the present study may be because of sufficient nutrients in the soil. Similarly, Murillo-Amador *et al.* (2006) recently showed that foliar $Ca(NO_3)_2$ sprays had little effect on cowpea plant growth and ion content in plants under saline conditions. Compared with the nutrient disturbance for maize seedlings in a short period of drought, no effect of foliar fertilization on either the growth or the ion uptake of maize plants may

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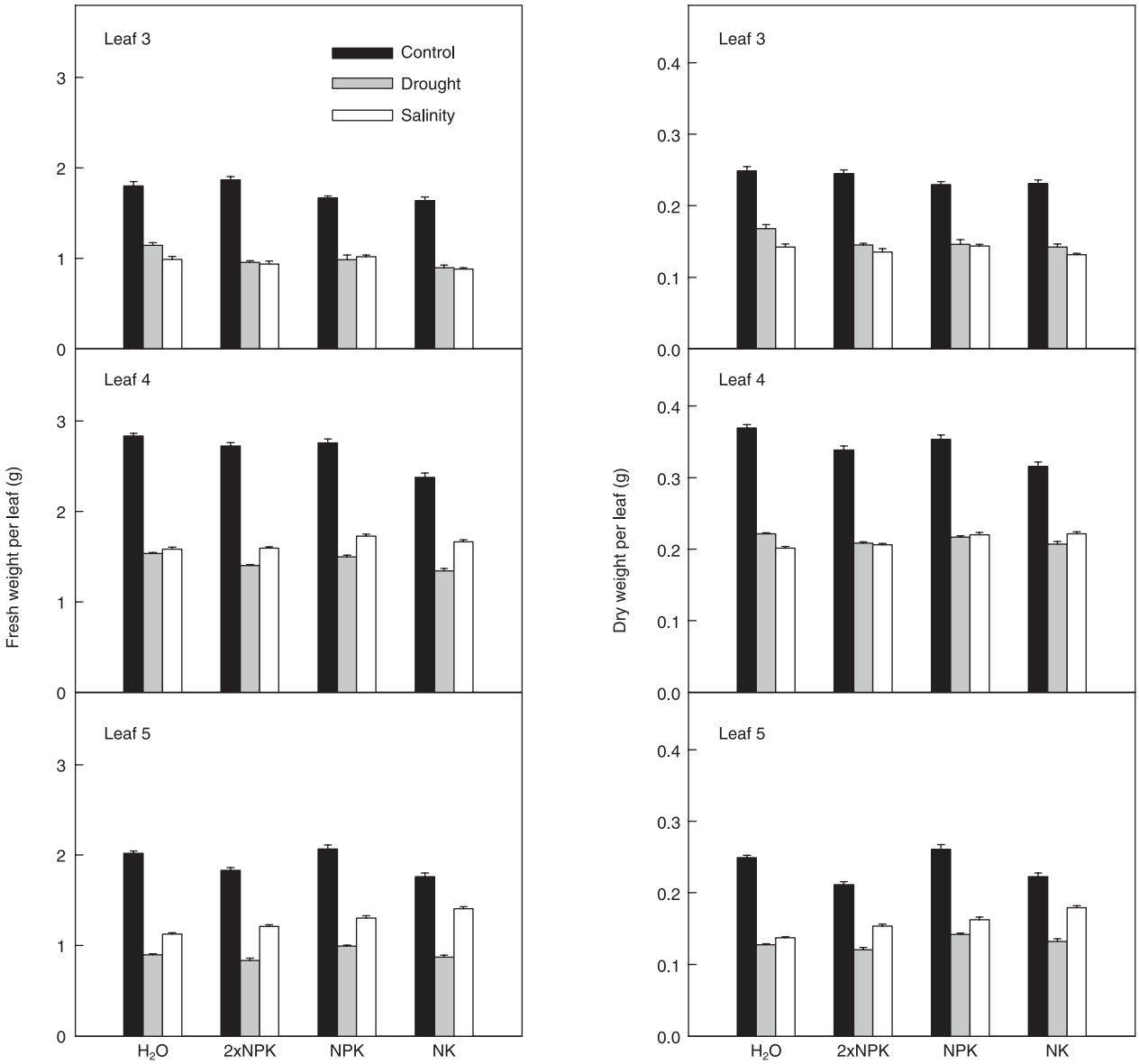

Figure 5 Fresh weight (g) and dry weight (g) of the blades of leaves 3, 4 and 5 of maize seedlings at harvest under control, drought and saline conditions applied with four different foliar treatments: tap water (H_2O); NPK solution applied only twice (2×NPK); NPK solution daily (NPK); N and K solution (NK). Error bars represent standard errors (n = 4).

indicate that the reduction in the seedling growth might be because of the limitation of water.

Sodium toxicity is one of the major factors limiting plant growth (see Greenway and Munns 1980 for a review); however, the Na concentration was only 20–25 mmol kg^{-1} dry weight in salinized expanded and expanding leaves, which is lower than that in growing leaves of maize grown in hydroponics (Neves-Piestun and Bernstein (2005). This may be because of the buffering effect on soil salinity compared with hydroponics that was also found for wheat leaves by Hu and Schmidhalter (1997, 1998) or different genotypes of maize. However, Na concentration in leaves is far below the level of Na toxicity (e.g. 1,000–3,000 mmol kg^{-1} dry weight in mature leaves of wheat; Hu and Schmidhalter 1997). Although Cl concentration in growing leaves of maize is not available, our preliminary study under a similar condition showed that Cl concentration can be three–fourfold higher than Na concentration of maize leaves, which may not cause Cl toxicity either (Hu and Schmidhalter 1998; Neves-Piestun and Bernstein 2005). Furthermore, salinity increased the concentration of nutrients (K, Ca, Mg, P and N) in leaves and foliar fertilization application did not influence plant growth

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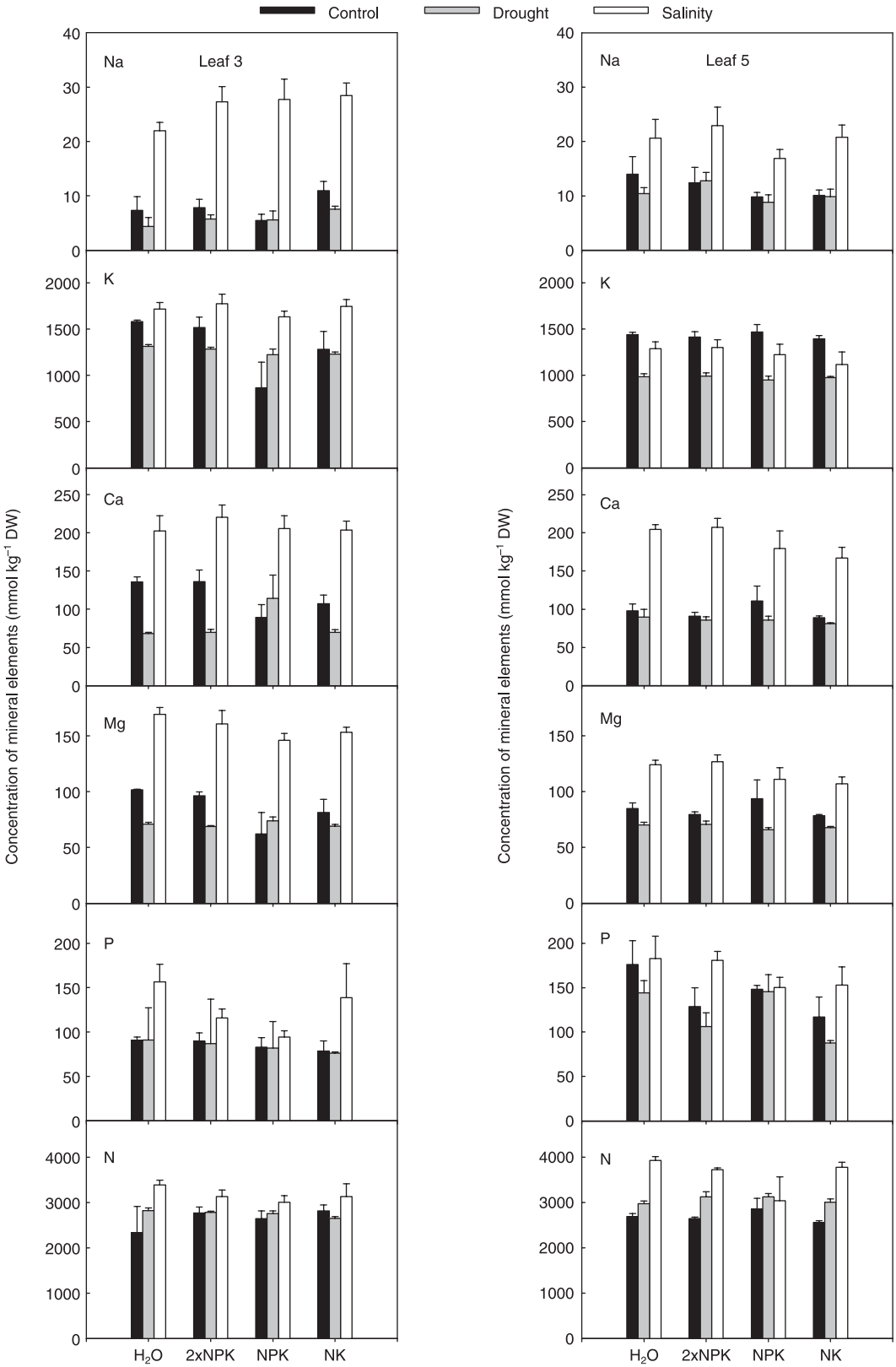

Figure 6 Concentrations of Na, K, Ca, Mg, P and total N in the blades of leaves 3 and 5 of maize seedlings at harvest under control, drought and saline conditions applied with four different foliar treatments: tap water (H_2O); NPK solution applied only twice (2×NPK); NPK solution daily (NPK); N and K solution (NK). Error bars represent standard errors ($n = 4$). DW, dry weight.

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and nutrient statues in leaves (Figs 2–6). There are at least three reasons why ion concentrations in maize leaves were not reduced by salinity. One reason is that the higher concentrations may be because of the poor growth of plants under salinity, that is, the rate of the reduction in leaf growth may be higher than that of the ion uptake by roots. The second reason may be that the experiments only ran for a short period in this study. Over a longer period of stress, the uptake of ions by roots may be more affected, resulting in low inorganic nutrient content in the shoots. The third reason could be that the soil used in this experiment contained sufficient nutrients, especially N, P and Ca. Taken together, the results clearly suggest that the actual inhibition of growth under saline conditions is mainly a result of the associated water deficit, a finding that supports the two-phase response of plant growth to salinity proposed by Munns (1993). Higher Ca concentration in leaves of wheat under saline conditions was also found in our previous study because of the high Ca content of the soil (Hu and Schmidhalter 1998).

Furthermore, the higher accumulation of macronutrients in the leaves of salinized maize plants might be because of the physiological mechanisms involved in osmoregulation. According to the two-phase model proposed by Munns (1993), plant growth is inhibited under salinity during the first phase by the associated water deficit. In general, osmoregulation under saline conditions can use ions from the soil, whereas in the absence of salinity under drought, the necessary solutes, such as sugars, have to be produced within the plant (Hsiao *et al.* 1976). Analyses show that osmotic adjustment via ion uptake is also more energy efficient than that through the production of organic solutes (Wyn-Jones 1981). Thus, plants adapting to salinity should preferentially uptake more ions from the soil for osmotic adjustment, a conjecture that is supported by the increased concentrations of most ions we observed in the leaves. In addition, a study on the contribution of sugars to osmotic adjustment in the elongating and expanded zones of wheat leaves under drought (Munns and Weir 1981) showed that sugars were responsible for 55–88% of the osmotic adjustment. Under saline conditions, by contrast, sugars accounted for only approximately 13% in the expanding zone of wheat leaves, whereas cations and anions accounted for approximately 21–30% and 15–21%, respectively (Hu and Schmidhalter 1998).

In conclusion, although there was a reduction in evapotranspiration and maize growth, such as shoot and leaf fresh and dry weights, under drought and salinity, foliar fertilization application did not improve plant growth. Drought reduced the uptake of K, Ca, Mg and P, which may be attributed to a decrease in transpiration and dried soil causes low diffusion rates of K, Ca, Mg and P. An increase or no change in the nutrient concentration in leaves under saline conditions may be because of a greater reduction in plant growth.

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