



Drought-induced changes in xylem pH, ionic composition, and ABA concentration act as early signals in field-grown maize (*Zea mays* L.)

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Abstract

Early signals potentially regulating leaf growth and stomatal aperture in field-grown maize (*Zea mays* L.) subjected to drought were investigated. Plants grown in a field lysimeter on two soil types were subjected to progressive drought during vegetative growth. Leaf ABA content, water status, extension rate, conductance, photosynthesis, nitrogen content, and xylem sap composition were measured daily. Maize responded similarly to progressive drought on both soil types. Effects on loam were less pronounced than on sand. Relative to fully-watered controls, xylem pH increased by about 0.2 units one day after withholding irrigation (DAWI) and conductivity decreased by about 0.25 mS cm⁻¹ 1–3 DAWI. Xylem nitrate, ammonium, and phosphate concentrations decreased by about 50% at 1–5 DAWI and potassium concentration decreased by about 50% at 7–8 DAWI. Xylem ABA concentration consistently increased by 45–70 pmol ml⁻¹ at 7 DAWI. Leaf extension rate decreased 5 DAWI, after the changes in xylem chemical composition had occurred. Leaf nitrogen significantly decreased 8–16 DAWI in droughted plants. Midday leaf water potential and photosynthesis were significantly decreased in droughted plants late in the drying period. Xylem nitrate concentration was the only ionic xylem sap component significantly correlated to increasing soil moisture deficit and decreasing leaf nitrogen concentration. Predawn leaf ABA content in droughted plants increased by 100–200 ng g⁻¹ dry weight at 7 DAWI coinciding with

a decrease in stomatal conductance before any significant decrease in midday leaf water potential was observed. Based on the observed sequence, a chain of signal events is suggested eventually leading to stomatal closure and leaf surface reduction through interactive effects of reduced nitrogen supply and plant growth regulators under drought.

Key words: Abscisic acid, drought, nitrate, pH, root–shoot communication, xylem sap composition.

Introduction

Over the last two decades, there has been much discussion on the different factors regulating growth and physiological processes in plants subjected to drought. Maize responds to progressive drought with a reduction in assimilate production (Jones *et al.*, 1986; Hirasawa and Hsiao, 1999), a reduction in leaf extension (Ben and Tardieu, 1997) and ultimately with a reduction in grain yield (Balaños and Edmeades, 1993). Variations in soil moisture can change root physiology and thereby enable plants to sense soil water status and adapt to decreasing soil moisture content by reducing growth, transpiring leaf surface and stomatal aperture (Hartung and Jeschke, 1999; Dodd *et al.*, 1996). Those responses reflect decreasing plant available soil moisture and the plant's need to control and decrease its water use. The root system communicates changes in soil water availability to

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the shoot via xylem hydrostatic pressure (root water status) and non-hydraulic (chemical composition of the xylem sap) signals (Davies *et al.*, 1990, 1994; Tardieu and Davies, 1993).

In drying soil, nutrient availability and uptake to the root system may decrease and, in turn, alter the physicochemical properties of the xylem sap (Chapin, 1991; Bacon *et al.*, 1998; Schurr and Schulze, 1996). Mild drought stress may increase the xylem pH due to reduced nitrate uptake before it reaches the leaves, causing an increase in apoplastic pH (Gollan *et al.*, 1992; Schurr *et al.*, 1992). If acting as signals, these changes may play a role in mediating physiological responses to stress (Hartung and Radin, 1989; Wilkinson and Davies, 1997; Wilkinson *et al.*, 1998). Increased xylem pH was suggested to act as a drought signal (Netting, 2000) reducing leaf elongation rate via an abscisic acid (ABA) dependent mechanism (Bacon *et al.*, 1998), whereas nitrate availability may affect leaf expansion through changes in the cell wall properties (Palmer *et al.*, 1996). The ratio of cations to anions determines the xylem pH and, thus, influences the protonation of ABA and the cell wall biochemistry of growing cells (Thompson *et al.*, 1997).

There is a wealth of evidence for non-hydraulic root-to-shoot communication via xylem ABA regulating stomatal aperture and leaf growth (Davies and Zhang, 1991; Tardieu *et al.*, 1992a, b; Bano *et al.*, 1993; Asch *et al.*, 1995; Dodd and Davies, 1996; Ali *et al.*, 1998). In rice, wheat and lupin subjected to drought, root signals have been shown to regulate transpiration and leaf expansion before shoot water status is affected (Ali *et al.*, 1999; Bano *et al.*, 1993; Jensen *et al.*, 1989). In a second phase, hydraulic signals from the change of hydrostatic pressure may add to the control of the plant's physiological responses to the stress by, for example, modifying the stomatal sensitivity to ABA (Tardieu and Davies, 1992; Tardieu *et al.*, 1993) or reducing shoot growth (Saab and Sharp, 1989) and plant gas exchange (Davies *et al.*, 1994; Tardieu *et al.*, 1992a).

To date, little is known on the timely interaction of multiple signal events and the resulting responses triggered in field-grown plants. Field-grown plants experience minor drought events daily due to diurnal variation in top soil moisture. Early signals may help the plant to stabilize its metabolism in periods of limited water availability. Long-term drought is likely to trigger more permanent metabolic changes to adapt to sub-optimal conditions. The present paper focuses on identifying a possible sequence of multiple signals, non-hydraulic and hydraulic, ultimately controlling leaf growth and leaf conductance of field-grown maize in drying soil of different texture.

Materials and methods

Experimental site, crop establishment and growing conditions

The experiment was conducted in a field lysimeter at Højbakkegaard, 20 km west of Copenhagen (55°40' N; 12°18' E; 28 m above MSL). Maize (*Zea mays* L. cv. Loft) was sown on 8 June 1998 and the treatments were started on 26 July 1998. Three seeds were sown per seed position, with a spacing of 0.30 × 0.20 m between the plants and the rows. After thinning to one plant per seed position, the density was 16 plants m⁻².

Prior to sowing, the equivalent of 92 kg N ha⁻¹, 15 kg P ha⁻¹, and 50 kg K ha⁻¹ were applied. The lysimeter (as detailed by Kristensen and Aslyng, 1971), comprises 40 plots, each 2 × 2 m surface area and 1 m deep, positioned in two rows separated by a 2 m wide covered drainage tunnel. The plants were protected from rain by an automated mobile glass roof. Irrigation was provided by an automated trickle irrigation system (AMI 1000/5000, DGT Volmatic A/S, Odense N, Denmark). Nine experimental plots contained a loamy sand (termed 'sand') with 6.5% clay and 8% silt in the top soil containing 105 mm of water at field capacity and 20 mm at permanent wilting (-1.5 MPa) with 85 mm plant-available soil water. The other nine experimental plots contained a sandy loam soil (termed 'loam') with 16% clay and 17% silt in the top soil containing 260 mm water at field capacity and 115 mm at permanent wilting (-1.5 MPa) with 145 mm plant available soil water. Details of soil density, volumetric water content, and porosity of the soils were described previously (Jensen *et al.*, 1998).

Top soil pH, conductivity and nutrient levels (ions) were determined for 0–0.15 and 0.15–0.40 m depths at the end of

Table 1. Soil nutrient composition for the soil types and treatments at the experimental site at two depths at the end of the experiment ± standard error of the mean, n = 3–4

Treatment		pH	EC (mS cm ⁻¹)	[NO ₃] ⁻ (mg kg ⁻¹)	[NH ₄] ⁺ (mg kg ⁻¹)	[PO ₄] ³⁻ (mg kg ⁻¹)	K ⁺ (mg kg ⁻¹)	Ca ²⁺ (mg kg ⁻¹)	Mg ²⁺ (mg kg ⁻¹)	Cl ⁻ (mg kg ⁻¹)
Soil depth 0.15 m										
Sand	FW ^a	7.7 ± 0.1	0.24 ± 0.02	2.0 ± 0.6	3.3 ± 0.7	4.5 ± 0.4	3.4 ± 0.5	114.7 ± 8.7	12.0 ± 1.1	4.0 ± 1.0
	DR ^a	7.5 ± 0.2	0.21 ± 0.02	8.7 ± 4.7	2.8 ± 0.3	4.8 ± 0.3	4.6 ± 0.4	131.0 ± 12.5	13.3 ± 0.8	3.0 ± 0.0
Loam	FW	7.5 ± 0.1	0.37 ± 0.05	38.8 ± 60.5	4.4 ± 0.1	8.5 ± 0.1	8.5 ± 1.8	218.3 ± 5.5	22.2 ± 1.1	6.0 ± 0.0
	DR	7.0 ± 0.3	0.35 ± 0.05	76.6 ± 45.7	3.5 ± 0.8	8.3 ± 0.9	10.7 ± 1.0	212.3 ± 9.3	22.2 ± 0.9	8.5 ± 5.5
Soil depth 0.4 m										
Sand	FW	7.7 ± 0.0	0.21 ± 0.02	1.3 ± 0.4	2.7 ± 0.3	2.7 ± 0.4	1.9 ± 0.4	98.7 ± 9.5	7.9 ± 0.7	2.5 ± 0.7
	DR	7.5 ± 0.1	0.20 ± 0.01	4.3 ± 1.3	2.0 ± 0.2	3.2 ± 0.8	2.2 ± 0.5	106.7 ± 14.4	9.2 ± 2.0	2.0
Loam	FW	7.4 ± 0.2	0.60 ± 0.30	2.4 ± 0.7	2.9 ± 0.1	7.5 ± 0.6	6.2 ± 0.1	220.0 ± 7.1	17.7 ± 1.6	4.7 ± 2.9
	DR	7.5 ± 0.1	0.30 ± 0.02	22.2 ± 7.3	1.7 ± 0.3	5.7 ± 0.6	6.1 ± 0.8	245.3 ± 2.3	18.3 ± 1.1	4.0 ± 1.0

^aFW, fully watered; DR, droughted.

the experiment (Table 1). Soil solution pH and conductivity were determined in a dispersion of 15 g soil in 30 ml water. The pH was measured with a pH meter (PHM95, pH/on meter, Radiometer Danmark A/S, Denmark) and conductivity was measured with a conductivity meter (CDN210, Conductivity meter, Radiometer Danmark A/S, Denmark). Soil potassium content was measured by flame photometry and Mg, Ca, NH_4 , NO_3 , and PO_4 content were measured by spectrophotometry (Plantedirektoratet, 1994).

Soil water status, weather conditions, and drought treatment

Soil water content was measured three times per week by neutron moderation method (Kristensen, 1973) at 10, 20, 40, 60, and 80 cm depths. For control plots, calculated water deficits were restored three times per week ensuring a soil water potential above 30 kPa. Soil water potential was recorded daily in the morning using tensiometers (ceramic cups no. 2131, Soil Moisture Equipment Corp. Santa Barbara, Ca, USA) at depths of 10, 30, 40, and 60 cm with four replicates. Climatic conditions were monitored by an automated meteorological station next to the experimental site. Relative air humidity, air temperature, irradiance, and potential evapotranspiration (calculated according to Penman, 1956) for the 1998 growing season data are given in Fig. 1. The drought treatment was started 28 d after emergence (26 July 1998) by withholding irrigation. That day was defined as 0 d after withholding irrigation (DAWI). The drought treatment was terminated by rewatering after near total depletion of available plant soil water. The experiments had three replicates of fully watered plots and four replicates of drought treatments on each soil type.

Gas exchange, leaf nitrogen, leaf water relations, and extension rate measurements

Predawn leaf water potential was measured daily with four replicates at about 5.30 a.m. GMT+1 on the youngest fully expanded leaves. Stomatal conductance and photosynthesis were measured on intact leaves with the LI-6200 portable photosynthesis system (LiCor Inc., Lincoln, NE, USA). Gas exchange at midday was measured 1.5 h before to 2 h after solar zenith at photosynthetic light saturation. The leaf was then enclosed in a polyethylene bag and detached at the leaf blade base for measurement of leaf water potential with a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA). The leaf was then immediately transferred into liquid nitrogen and stored at -80°C in a freezer for later analysis. Leaf osmotic potential was determined from expressed cell sap after 20 min of thawing. Filter paper discs soaked in cell sap were sealed into a C-52 chamber (Wescor Inc., Logan, UT, USA) and incubated at 22°C for 20 min before reading the osmotic potential of the sap using a dew point microvoltmeter (HR-33T from the same company). The leaf turgor potential was calculated from the difference between leaf water potential and osmotic potential of the cell sap.

The length of the youngest expanding leaf was measured daily in the morning with a ruler. The total nitrogen content of the third youngest expanding leaf was measured after oven drying by the Kjeldahl method (Bremner and Mulvaney, 1982).

Collection of xylem sap

Xylem sap was collected in the morning using root pressure. At dawn, the shoots were detopped 5–10 cm above the soil surface and immediately wiped with absorbent tissue to remove contaminants from cut cells. After discarding approximately

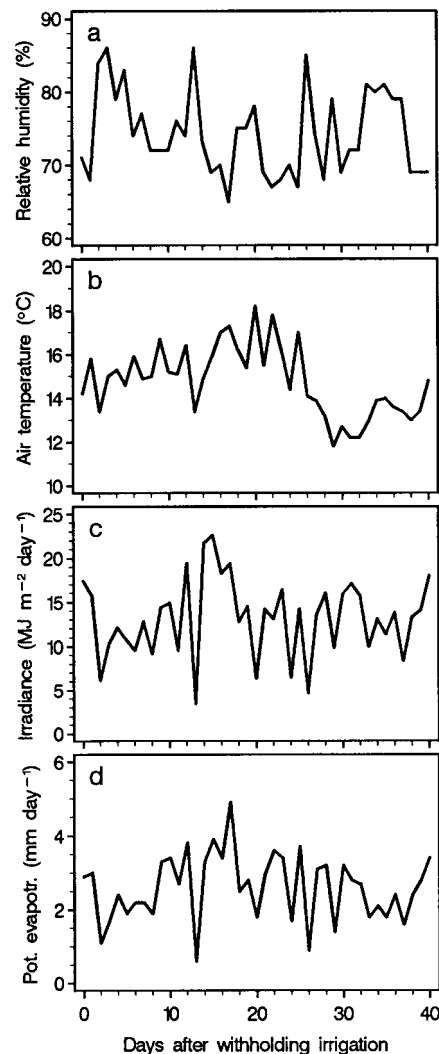


Fig. 1. Daily mean values of relative humidity (a), air temperature (b), irradiance (c), and potential evapotranspiration as recorded by an adjacent weather station.

50 μl of sap, the cut surfaces were wiped again with moist absorbent tissue to reduce contaminants and then silicon tubing was fitted to the stems. The tubes were sealed with parafilm and wrapped in aluminium foil to prevent evaporation and protect against dust, insects and photodegradation of susceptible compounds. Over 2–3 h, 100–1500 μl of sap were collected. Xylem sap was sampled daily for 30 d into the drying cycle. The flux of xylem sap in droughted plants at the beginning, middle and the end of collecting sap was approximately 0.8, 0.5, and 0.03 ml h^{-1} , with a constant flow rate of about 0.8 ml h^{-1} in well-watered plants. The sap was stored at -80°C for further analysis.

Abscisic acid determination and other physiochemical analyses

Leaf samples (mid-part) from leaves taken in parallel to those for water potential determination at predawn observations and the mid-part of the ones in which midday leaf water potential had been determined were freeze-dried, finely ground, extracted in glass-distilled water using about 1.2 ml per 40 mg dry weight,

boiled for 1–2 min, shaken up and again extracted on a shaker at 4 °C overnight. The extracts were centrifuged and the supernatants assayed in an enzyme linked immunosorbent assay (ELISA) for ABA using a monoclonal antibody for ABA (AFRC MAC252) (Hansen, 1996; Asch, 2000).

Frozen xylem sap was allowed to thaw for about 45 min before being assayed without further purification for ABA in the ELISA. No cross-reaction of the antibody with other compounds in either leaf extracts or xylem sap was detected when tested for according to Quarrie *et al.* (Quarrie *et al.*, 1988).

Xylem pH, conductivity and ionic composition was determined after the sap was allowed to thaw for about 45 min. Xylem pH and conductivity were measured using pH and conductivity meters as described above. Cations and anions in the xylem sap were determined by isocratic chromatography analysis (Husted *et al.*, 2000). Cations in the apoplast were analysed on an ion chromatograph (Waters, Milford, MA) equipped with a 3.9×150 mm cation M/D column. The mobile phase was

4.0 mM HNO₃, 0.1 mM EDTA and 2.5 mM 18-crown-6-ether (Sigma, St Louis, MO) which enables detection of small amounts of NH₄⁺ in the presence of high concentrations of Na⁺ and K⁺ by isocratic elution at 1.0 ml min⁻¹. Inorganic anions were analysed by a traditional isocratic procedure on the ion chromatograph (Technical Bulletin 091064TP-O, Waters, MA).

Results

Soil water content and potential during soil drying

Kinetics of relative soil moisture content and soil matrix potential are shown for the experimental period in Fig. 2. In unirrigated plots soil moisture content decreased over time to a minimum of 8% in the top soil and 25% at 0.60 m depth in sand, whereas in loam the top soil

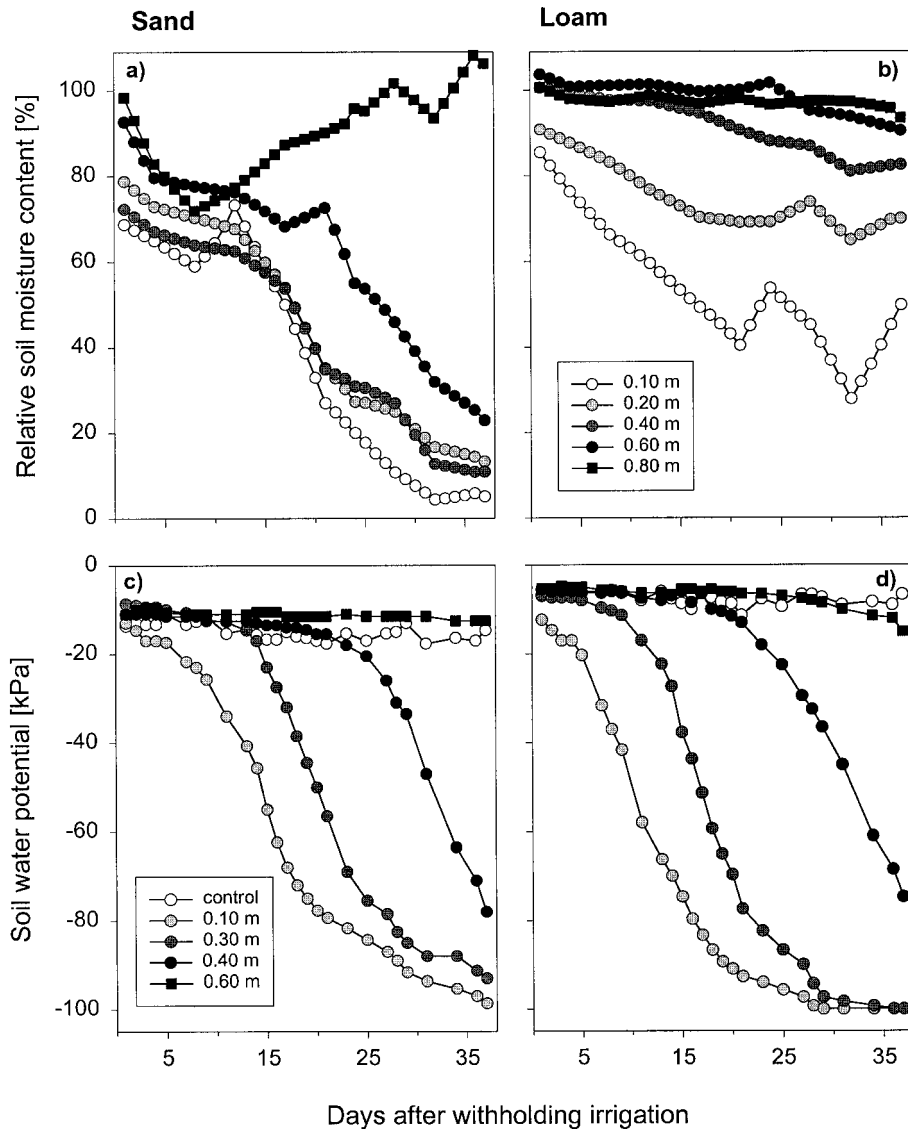


Fig. 2. Depth profiles of soil moisture content relative to fully watered control plots during the experiment (a, b) and soil water potential of droughted and non-droughted control plots during the experiment (c, d) for two soil types in the lysimeter plots. Data are means of three observations. Sand (a, c); loam (b, d).

retained about 50% of the soil moisture of well-watered plots and more than 90% at 0.6 m depth (Fig. 2a, b). In the well-watered treatment, soil water potential (SWP) was generally maintained close to field capacity (0 to -30 kPa) with an average value of -10 kPa for both soil types. In the drought treatment, SWP in loam soil (Fig. 2d) measured at 10 cm depth decreased rapidly to -76 kPa 15 d after withholding irrigation (DAWI). A similar decrease in SWP occurred at 30 and 60 cm depths at 20 and 37 DAWI, respectively. The decreases were slower in the deeper layers. SWP decreased faster in loam than in sand. In sand (Fig. 2c) at 18 DAWI, SWP at 10 cm depth decreased to -76 kPa, while SWP at 30 cm and 60 cm depth reached -76 kPa 26 and 36 DAWI, respectively.

Leaf water status

Predawn leaf water potential in droughted plants started consistently to decrease, compared to well-watered plants, 22 DAWI and 23 DAWI for both soil types (Fig. 3a, b). A significant difference in midday leaf water potential (mlWP) between well-watered and droughted plants in sand and loam was observed at 11 DAWI and 16 DAWI,

respectively. In sand, mlWP decreased from about -0.5 MPa in well-watered plants to -1.5 MPa in droughted plants, while in loam mlWP decreased from about -0.5 MPa to -1.0 MPa (Fig. 3c, d). Leaf turgor at midday was consistently lower in droughted plants than in well-watered plants from 20 to 21 DAWI (Fig. 3e, f).

Xylem sap constituents: ABA, pH, conductivity, anions, and cations

In both sand and loam, 1 DAWI an increase in xylem sap pH (4.91 cf. 5.10 and 4.99 cf. 5.08; Fig. 4c, d) and a decrease in xylem sap conductivity (Fig. 4e, f) were observed in droughted plants compared to well-watered plants. Likewise, in both soil types, at 7 DAWI a consistent increase in xylem [ABA] was observed in droughted as compared to well-watered plants [125 cf. 170 pmol ml $^{-1}$ (Fig. 4a), and 130 cf. 200 pmol ml $^{-1}$ (Fig. 4b) in sand and loam respectively].

Concentration differences for nitrate, ammonium and phosphate concentrations between well-watered and droughted plants of about 50% for sand and loam were first observed at 1–5 DAWI, respectively (Fig. 5a–f).

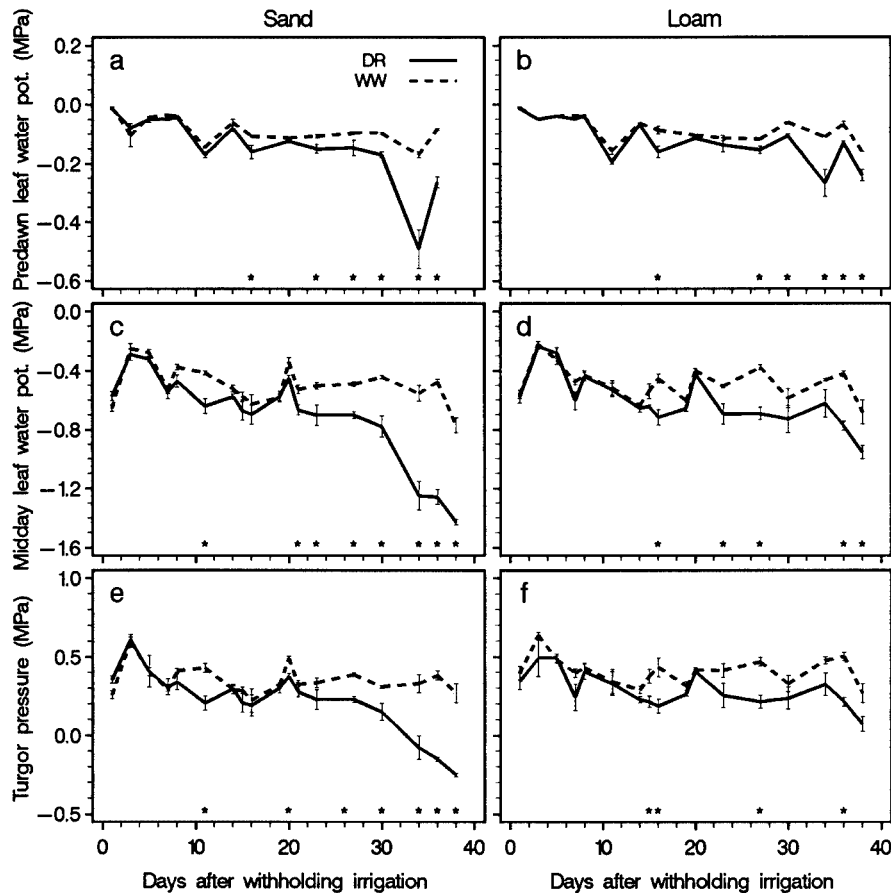


Fig. 3. Time-courses of predawn leaf water potential (a, b), midday leaf water potential (c, d) and turgor pressure (e, f) during the experiment for well-watered (dashed line) and droughted plants (solid line). Error bars = standard error of mean ($n = 3-4$). *, Indicates mean values different at least at the 95% level according to Student's distribution.

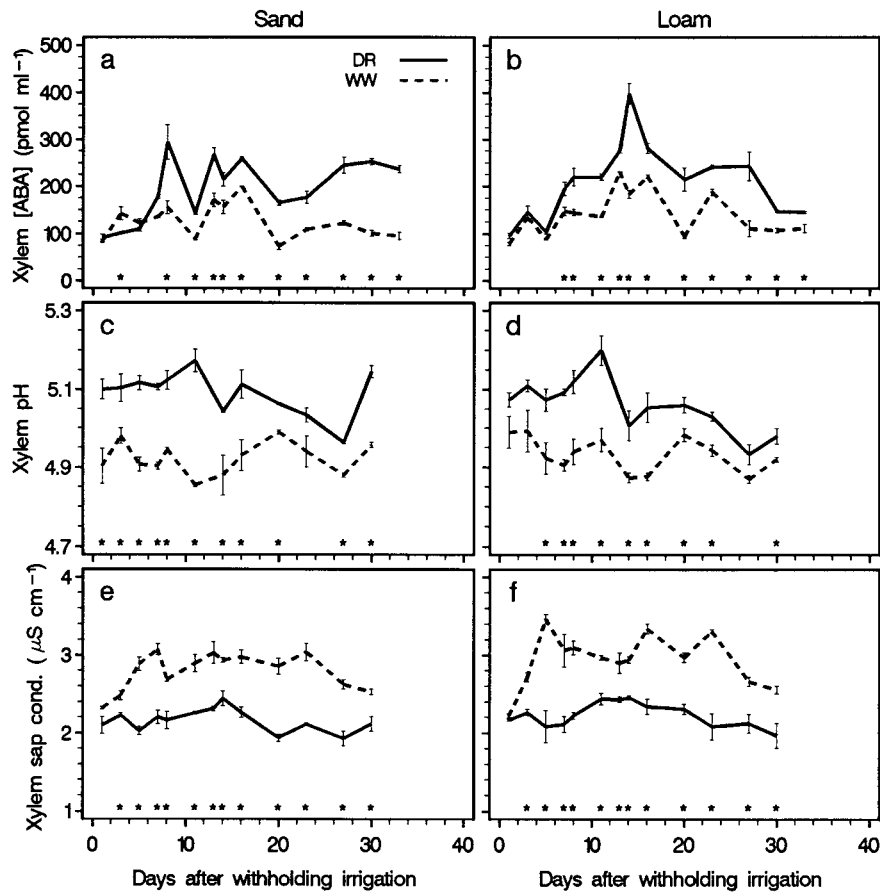


Fig. 4. Time-courses of xylem [ABA] (a, b), xylem pH (c, d), and xylem conductivity (e, f) during the experiment for well-watered (dashed line) and droughted plants (solid line). Error bars = standard error of mean ($n=3-4$). *, Indicates mean values different at least at the 95% level according to Student's distribution.

The potassium concentration was about 50% lower in droughted plants for sand at 7–8 DAWI; the decrease was less clear for loam (Fig. 5g, h). Kinetics of xylem sodium, calcium, magnesium, sulphate, and chloride concentrations over the growing seasons partly reflected plant development and partly atmospheric conditions, but were not altered by progressive drought (data not shown). Neither did the cation:anion ratio change (Fig. 5i, j) except for 3–6 DAWI in sand with low values in well-watered plants due to low K^+ values (Fig. 5g).

Drought effects on leaf properties

Under well-watered conditions predawn leaf [ABA] varied between 200 and 550 $ng\ g^{-1}\ DW$ until about 16 d into the experimental period when it peaked at about 700 $ng\ g^{-1}\ DW$ for a few days (Fig. 6a, b). Midday leaf [ABA] varied between 200 and 550 $ng\ g^{-1}\ DW$ (Fig. 6c, d). Leaf nitrogen content was at about 3.5% decreasing to less than 3% over the experimental period (Fig. 6e, f).

Under conditions of progressive drought predawn leaf [ABA] significantly increased relative to control plants at

7 DAWI and stayed about 100–200 $ng\ g^{-1}\ DW$ above control levels until 32 DAWI (Fig. 6a, b). After 33 DAWI midday leaf [ABA] increased to over 900 $ng\ g^{-1}\ DW$ under drought conditions (Fig. 6c, d). Leaf nitrogen content significantly decreased by about 1.2% over the experimental period (Fig. 6e, f) in accordance with the significant decrease in xylem nitrate concentration (Fig. 5a, b). Leaf nitrogen content was correlated with xylem nitrate concentration: For droughted plants in sand: leaf nitrogen content = $2.14 + \text{xylem } [NO_3] \times 0.279$; $S_a = 0.167$; $S_b = 0.0647$; $r^2 = 0.52$; $P > 0.001$; $n = 20$. For droughted plants in loam: leaf nitrogen content = $2.29 + \text{xylem } [NO_3] \times 0.252$; $S_a = 0.153$; $S_b = 0.0866$; $r^2 = 0.33$; $P > 0.001$; $n = 19$.

Two to four DAWI was cloudy and rainy resulting in high humidity, low evaporative demands, and slow soil drying (Figs 1, 2). Therefore, gas exchange data were scarce during this period. In sand, midday stomatal conductance (g_s) was consistently reduced in droughted plants from 16 DAWI (Fig. 7a, b). At 33 DAWI, g_s decreased from about 0.30 $mol\ m^{-2}\ s^{-1}$ in well-watered to 0.20 $mol\ m^{-2}\ s^{-1}$ in droughted plants in sand; the differences were less clear in loam (Fig. 7a, b).

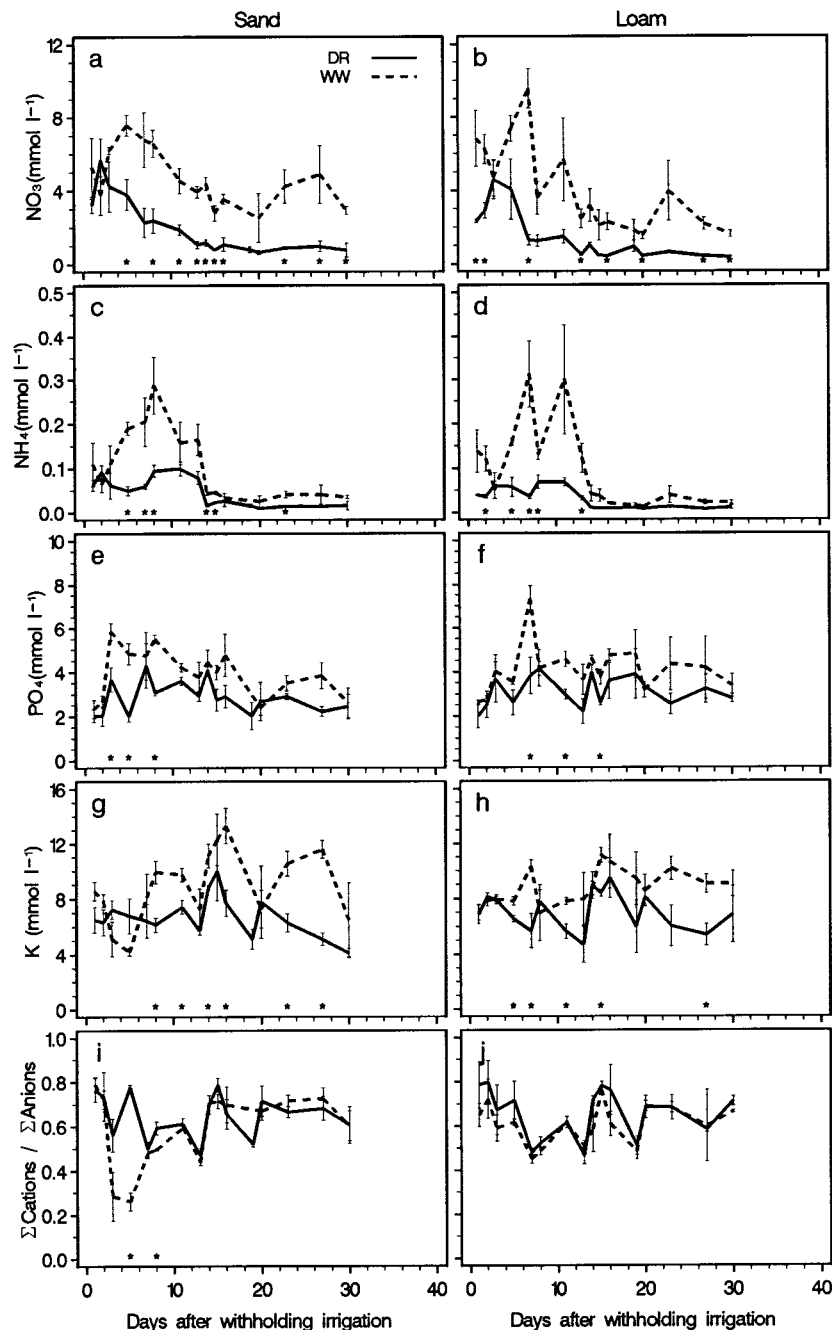


Fig. 5. Time-courses of xylem sap nitrate (a, b), ammonium (c, d), phosphate (e, f), and potassium (g, h) concentrations and cations : anions ratio of xylem sap (i, j) during the experiment for well-watered (dashed line) and droughted plants (solid line). Error bars = standard error of mean ($n = 3-4$). *, Indicates mean values different at least at the 95% level according to Student's distribution.

At 30 DAWI, photosynthesis at high light intensity ($\text{PAR} > 780 \mu\text{mol m}^{-2} \text{s}^{-1}$) on sand significantly decreased from about $28 \mu\text{mol m}^{-2} \text{s}^{-1}$ in well-watered to $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ in droughted plants and on loam 34 DAWI from about $32 \mu\text{mol m}^{-2} \text{s}^{-1}$ in well-watered to $24 \mu\text{mol m}^{-2} \text{s}^{-1}$ in droughted plants (Fig. 7c, d). The leaf extension rate in droughted plants was lower than that in well-watered plants 5 DAWI on both soil types;

27 DAWI at severe soil drying the difference was further enhanced at both soil types (Fig. 7e, f).

Discussion

The present study had two major objectives: (1) to investigate physiological responses of field-grown maize

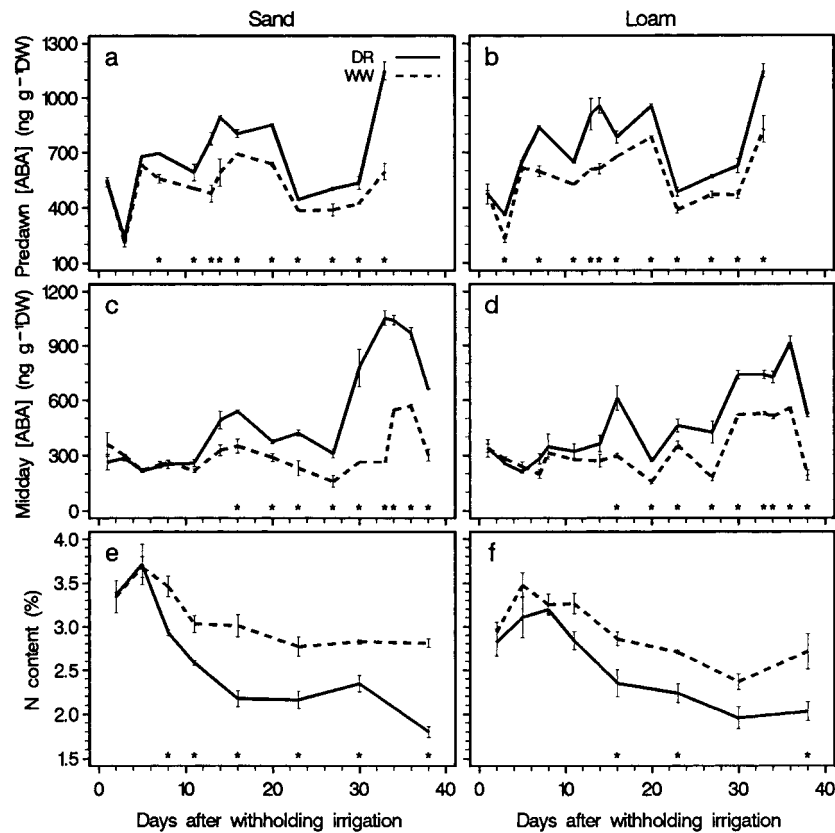


Fig. 6. Time-courses of predawn leaf [ABA] (a, b), midday leaf [ABA] (c, d), and leaf nitrogen content (e, f) during the experiment for well-watered (dashed line) and droughted plants (solid line). Error bars = standard error of mean ($n = 3-4$). *, Indicates mean values different at least at the 95% level according to Student's distribution.

to progressive drought on two different soil types and (2) to study the likely chain of physiological events eventually leading to stomatal closure and leaf growth reduction in field-grown maize under conditions of progressive drought. Two factors most efficiently control plant water losses: (1) the actual transpiring surface and (2) stomatal aperture (Kramer and Boyer, 1995). It has been shown that increasing drought rapidly leads to an inhibition of leaf extension (Ali *et al.*, 1998; Bacon *et al.*, 1998; Tardieu *et al.*, 2000); thus, an active reduction in the transpiring leaf surface. Stomatal closure as a response to decreasing soil moisture availability has been observed even before the plant water status was affected (Tardieu *et al.*, 1992a; Ali *et al.*, 1999). Since the root system is the first plant organ to sense a decrease in soil moisture availability, any reaction to drought in above-soil plant parts is likely to be caused by changes in the root properties (Zhang and Davies, 1990a, b; Dodd *et al.*, 1996). Changes in chemical composition of the xylem sap and changes in the plant water status have been shown to act as signals in root–shoot communication (McDonald and Davies, 1996; Schurr *et al.*, 1992). In recent years, in addition to the well-known drought effects on the ABA concentrations in both xylem sap and leaves (Thompson *et al.*, 1997), other parameters such as xylem nutrient status, xylem mineral

composition (Gollan *et al.*, 1992) and xylem pH (Hartung and Jeschke, 1999), have been shown to have the potential to act as signals in root–shoot communication under conditions of soil water deficits (McDonald and Davies, 1996). In the present study, maize plants were subjected to progressive drought under field conditions and xylem and leaf properties were followed for more than 30 d into the drying cycle. In the following the relevance of the results presented here is discussed in relation to a possible chain of signal events ultimately leading to a reduction in overall transpiration due to reductions in both leaf expansion and stomatal conductance.

The relevance of relative changes in predawn xylem sap constituents

The concentration of cations, anions, ABA, and other substances decrease hyperbolically when flux rates increase from pure root exudation up to values typical for transpiring plants (Schurr and Schulze, 1995). Ideally, xylem sap should be collected at the same flow rate as would be in the transpiration stream. Unfortunately, this is not possible under field conditions. Only the collection of guttate predawn is possible here (Dodd *et al.*, 1996). However, in maize predawn xylem

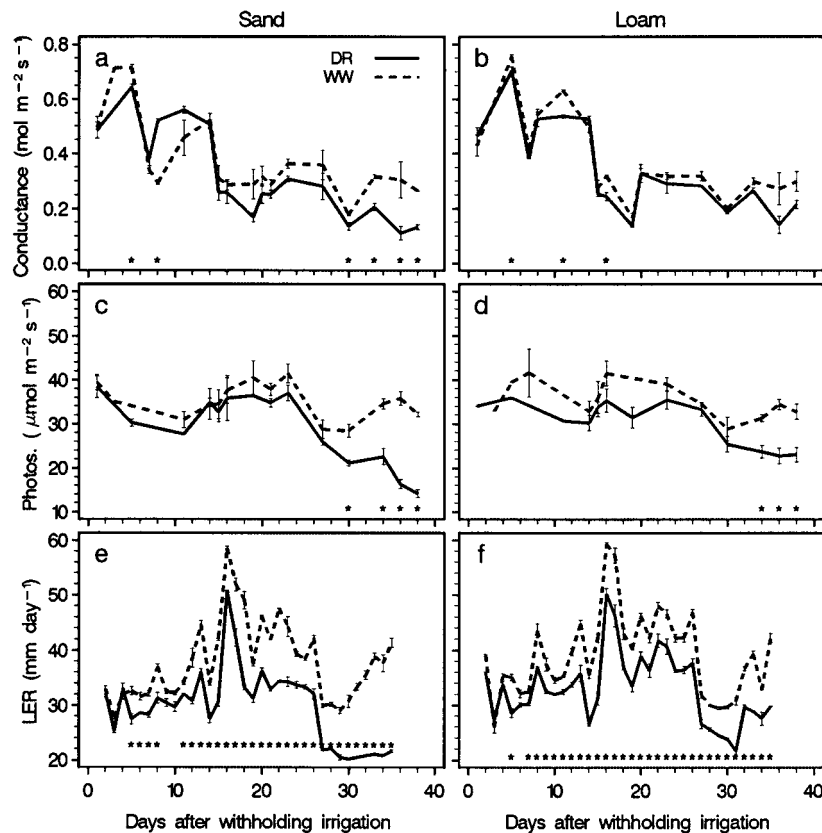


Fig. 7. Time-courses of leaf conductance (a, b), photosynthesis (c, d), and leaf extension rate (e, f) during the experiment for well-watered (dashed line) and droughted plants (solid line). Error bars = standard error of mean ($n=3-4$). *, Indicates mean values different at least at the 95% level according to Student's distribution.

ABA concentration correlates with decrease in soil water content or predawn LWP (Tardieu and Davies, 1992) in accordance with findings in the present investigation (Fig. 8b) indicating that the rate of ABA synthesis in the roots is linked to the root or soil water status. Similarly, xylem concentration of ions is affected by soil drying (Gollan *et al.*, 1992). Therefore relative changes in predawn sap constituents seem to be meaningful indicators of possible signalling mechanisms during soil drying (Fig. 8).

Drought effects in relation to soil type

Maize responses to progressive drought were related to increasing relative soil moisture deficit (Fig. 8a-d). In general, maize responded similarly to progressive drought on both soil types. Effects on loam were always less pronounced than on sand. This may have been due to the greater water-holding capacity of the loamy soil (Kramer and Boyer, 1995) and the relatively slower rate of depletion of relative available soil water in the lower soil layers (Jensen *et al.*, 1998). Relative soil water deficit in loam never exceeded 50% (not shown). Therefore, the drought stress maize plants experienced on loam was much less severe than on sand (90% soil moisture deficit at the end

of the experiment) and was established at a slower rate (Fig. 2). Most probably, maize responses to drought on loam reflect the potential of maize to adapt to decreasing soil water potentials, rather than responses to an actual lack of water. The later responses to drought by plants on loam correspond generally to those observed in sand in the early drought phase with the exception of xylem pH and xylem conductivity (Fig. 4d, f). Therefore, the following discussion of physiological responses of maize to progressive drought focuses on plant responses observed on sand rather than on loam.

Plant responses to increasing soil moisture deficit: a cascade of events

Increasing soil moisture deficit was accompanied by relative changes in xylem nitrate concentration, xylem ABA concentration, early morning leaf water potential and stomatal conductance (Fig. 8a-d). Additionally, withholding irrigation immediately caused a small but significant increase in xylem pH (Fig. 4c, d). Xylem pH of droughted plants was on average 0.2 units more alkaline than that of control plants throughout the drying cycle, but it was not significantly correlated with increasing soil moisture deficit.

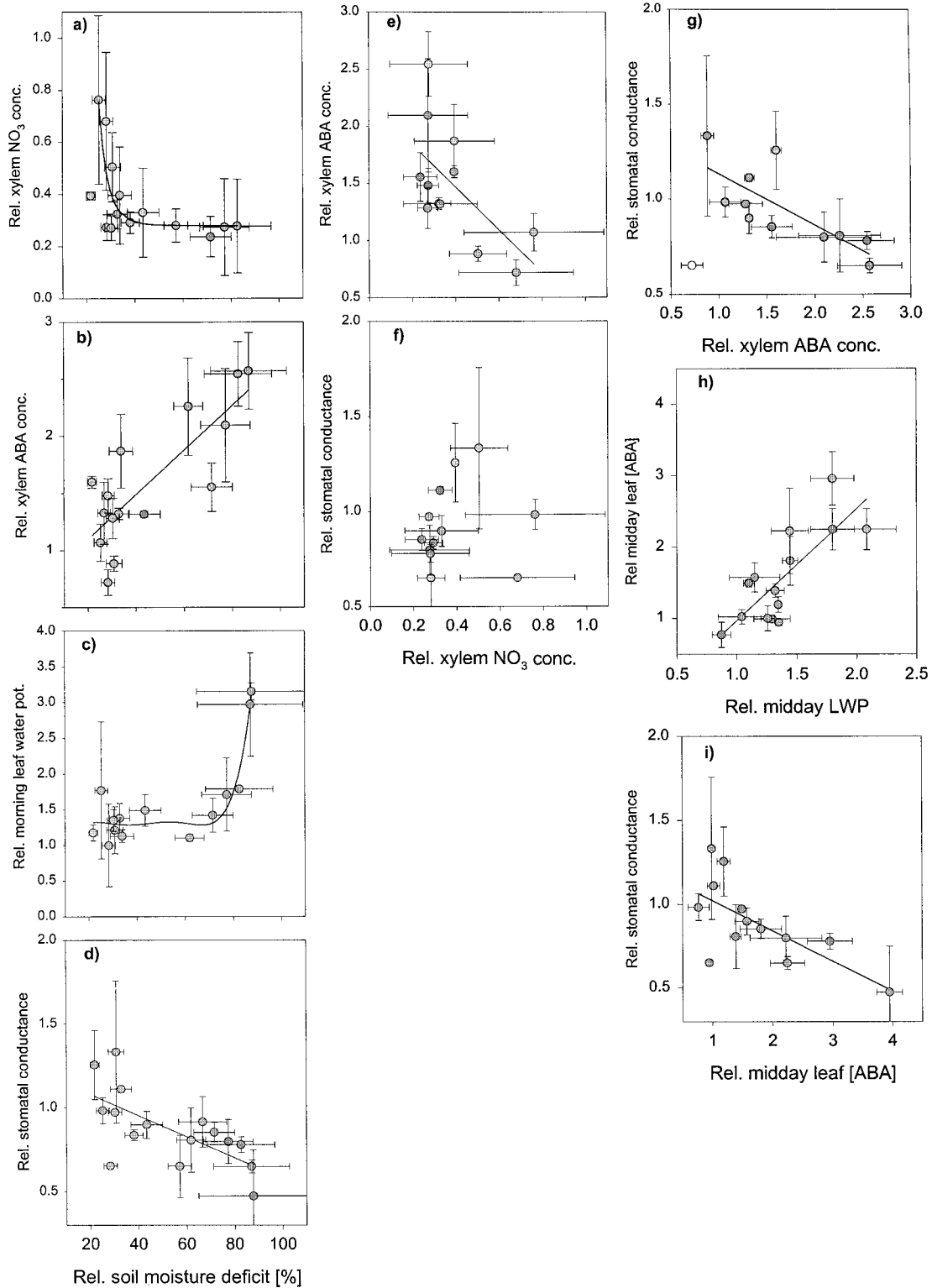


Fig. 8. Relations and correlations between biophysical parameters measured in maize plants grown on sand. Values are given relative to fully watered controls as [drought/fully watered] except rel. soil moisture deficit which is given as [soil moisture deficit of droughted plots–soil moisture deficit of fully watered plots]. LWP, leaf water potential; ABA, abscisic acid; conc., concentration; rel., relative. Error bars = standard error of mean ($n = 3-4$).

An increase in xylem pH has been observed in several plant species under drought conditions (Hartung and Radin, 1989; Gollan *et al.*, 1992; Wilkinson *et al.*, 1998). For plants grown under controlled conditions, it has been proposed that increased xylem pH acts as a drought signal to reduce leaf expansion rate (LER) via an ABA-dependent mechanism (Bacon *et al.*, 1998). In the present field study, LER was significantly reduced under drought conditions compared to fully watered controls (Fig. 7e, f), but regression analyses revealed that the main determining factor for LER in both treatments was air temperature (data not shown). Because of the strong influence of air temperature on LER (explaining more than 60% of the variance) possible additional influences of pH combined with xylem [ABA] under drought conditions in the field could not be quantified. Xylem pH may be influenced by the ionic composition of the xylem sap. Gollan *et al.* found in droughted sunflower plants that a reduction in xylem nitrate concentration correlated with an increase in xylem pH, and an excess of cations in the xylem sap (Gollan *et al.*, 1992). In the present study, xylem concentrations of macro nutrients decreased under progressive drought (Figs 5, 8a). However, the cation:anion ratio was almost unaffected (Fig. 5i, j) (except for 3–6 DAWI in sand caused by low K^+ values). Limited nutrient availability is frequently observed in field-grown plants under drought conditions (Bacon *et al.*, 1998), most probably due to air replacing water in the top soil pores and thereby reducing ion diffusion and mass flow of nutrients to the root surface (Chapin, 1991; Barber, 1984). Nitrogen for cell expansion of younger leaves is mainly supplied via xylem nitrate import. In the present study, xylem nitrate concentration of droughted plants was reduced to about 50% of well-watered plants 5 DAWI (Fig. 5a, b) parallel to a significant decrease in LER (Fig. 7e, f) and a significant increase in xylem ABA concentration (Fig. 4a, b). Limited nitrogen supply can lead to a reduction in LER due to reductions in cell turgor (Radin and Boyer, 1982), however, leaf turgor pressure was not affected by the treatments in the present study (Fig. 3e, f) despite strong decreases in leaf nitrogen content in droughted plants (Fig. 6e, f). Since there was no change in leaf water relations at this early stage of drought (Fig. 3) the reductions in LER may have been the result of reduced nitrogen availability and changes in concentrations of plant growth regulators as observed for ABA in the present study and for cytokinins (Palmer *et al.*, 1996; Van der Werf and Nagel, 1996). Changes in the hormonal balance due to reduced nitrate availability may have reduced the rate of protein biosynthesis and cell division (Van der Werf and Nagel, 1996) and thus LER.

Xylem nitrate concentration was the only ionic xylem sap component that was significantly (non-linearly) correlated to increasing soil moisture deficit (Fig. 8a). It

has been argued (Gollan *et al.*, 1992) that a decreasing xylem nitrate concentration, if not acting as a signal itself, may play a modulating role in root–shoot communication, particularly through its influence on xylem pH. Wilkinson and Davies have shown that the effect of a drought-induced ABA signal on stomatal conductance is enhanced by relatively more alkaline apoplastic conditions (Wilkinson and Davies, 1997). Interactions between xylem nitrate, pH and ABA have been discussed for their relevance in root–shoot communication (Gollan *et al.*, 1992; Schurr *et al.*, 1992) but, according to McDonald and Davies, there is still little understanding of how ABA and nitrate can interact to influence stomata (McDonald and Davies, 1996).

Under field conditions it was found that drought first substantially reduced xylem nitrate concentration (Fig. 8a) followed by a linear increase of xylem ABA relative to fully watered controls (Fig. 8b; $r^2=0.648$, $P<0.001$), long before the leaf water status changed (Fig. 8c), whereas stomatal conductance decreased as a function of soil moisture deficit (Fig. 8d; $r^2=0.44$, $P<0.01$). Relative stomatal conductance was not correlated with relative xylem nitrate concentration (Fig. 8f). However, relative xylem ABA concentration was significantly ($r^2=0.39$, $P<0.05$) correlated with both relative xylem nitrate concentration (Fig. 8e) and relative stomatal conductance (Fig. 8g; $r^2=0.62$, $P<0.001$). Both, relative morning leaf water potential (Fig. 8c) and relative midday leaf water potential (data not shown) were correlated with relative soil moisture deficit in the same non-linear function. Leaf water potential started to decrease relative to fully watered controls at soil moisture deficits below 70%. The decrease in midday leaf water potential was significantly ($r^2=0.62$, $P<0.001$) linearly correlated with midday leaf [ABA] (Fig. 8h). Finally, both relative morning xylem ABA and relative midday leaf [ABA] were linearly correlated with relative stomatal conductance (Fig. 8g, i; $r^2=0.46$, $P<0.01$).

Based on these results the following sequence of events leading to transpirational control in field-grown maize under drought conditions is proposed: in response to a minor decrease in available top soil moisture, a rapid decrease in xylem nitrate concentration and a shift in xylem pH trigger the *de novo* synthesis of ABA in the roots and its release into the xylem (Slovik *et al.*, 1995). The shift in pH, the reduction in nitrate availability and the release of ABA into the xylem may have a direct effect on LER, which could not be quantified in this study, but which is likely to occur according to other reports (Bacon *et al.*, 1998; Palmer *et al.*, 1996; Wilkinson *et al.*, 1998). The ABA release into the xylem causes a decrease in stomatal conductance before the leaf water potential decreases (Fig. 8g, c and as observed in rice by Bano *et al.*, 1993; in wheat by Ali *et al.*, 1998; and in maize by Zhang and Davies, 1989a, b) which may be amplified by a

decrease in leaf nitrogen content. Increasing soil moisture deficit eventually decreased the leaf water potential which triggered a *de novo* synthesis of ABA in the leaves. Reduced stomatal conductance in the later stress stages was most probably an effect of both leaf and xylem ABA and reduced leaf nitrogen concentration. In contrast to work reported earlier (Tardieu *et al.*, 1993) no increased stomatal sensitivity for ABA through decreasing leaf water potentials was observed in the present study as the relationship between relative midday leaf ABA and relative stomatal conductance (Fig. 8i) was linear. Had the stomatal sensitivity to ABA increased with progressive drought the amount of ABA needed to decrease stomatal conductance should have decreased. More research is needed on the applicability of results obtained under controlled conditions with young plants in pots to field conditions. Field-grown plants experience drought in cycles and the severity of the stress effect might well depend on the swiftness of the plant's adaptation to fluctuations in top soil moisture. Xylem nitrate concentration and xylem pH seem to be fairly sensitive parameters which may allow the plant to fine tune transpirational control under non-lethal stress conditions.

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