Sequence of drought response of maize seedlings in drying soil

Urs Schmidhalter, Michel Evéquoz, Karl-Heinz Camp and Christoph Studer


Leaf elongation in monocotyledonous plants is sensitive to drought. To better understand the sequence of events in plants subjected to soil drying, leaf elongation and transpiration of maize seedlings (Zea mays L.) of 4 cultivars were monitored continuously and the diurnal courses of the root and leaf water relations were determined. Results from this study indicate the following sequence of drought response: Leaf elongation decreased before changes in the leaf water relations of non-growing zones of leaf blades were detected and before transpiration decreased. Reductions in leaf elongation preceded changes in the root water potential ($\Psi_w$). Root $\Psi_w$ was not a very sensitive indicator of soil dryness, whereas the root osmotic potential ($\Psi_r$) and root turgor ($\Psi$) were more sensitive indicators. The earliest events observed in drying soil were a significant increase in the largest root diameter class (1720 to 1960 µm) and a decrease in leaf elongation ($P = 0.08$) 2 days after withholding water. Significant increases in root length were observed 2 days later. Soil drying increased the number of fine roots with diameters of <240 µm. Slight increases in soil strength did not affect leaf elongation in the drying soil.

Key words – Leaf elongation, leaf water relations, root diameter, root length, root osmotic potential, root water potential, root water relations, Zea mays.

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Introduction

The traditional view of how plants respond to a reduction in soil water availability was that they reduce their water uptake, resulting in a reduction in the water content, water potential ($\Psi_w$), or turgor ($\Psi_r$) in the leaves (e.g. Kramer 1988). There is no doubt that, under many circumstances, this does occur, but this is probably not always the case (Davies et al. 1990). Recent studies show that plants may react to drying soil well before their leaves wilt and even before there is a detectable change in leaf $\Psi_w$ (Bates and Hall 1981, Davies and Zhang 1991). The growth rate of the leaves may fall (Passioura 1988, Saab and Sharp 1989), and their stomata may close (Blackman and Davies 1985, Gollan et al. 1986), apparently in response to signals received from the roots in drying soil (Davies and Zhang 1991, Passioura 1994). Decreases in $\Psi_w$ of drying roots may trigger an early signal (Zhang and Davies 1989, Davies and Zhang, 1991).

There are few reports on the inhibition of leaf expansion, which occurs independently of changes in leaf water relations (Munns 1987, Passioura 1988, Saab and Sharp 1989, Augé et al. 1994). There are also only very few studies of changes in leaf elongation and transpiration of monocotyledonous plants in drying soils as compared with changes in root and leaf water relations (e.g. Sharp and Davies 1979). There are no studies comparing early events in drying soil among different cultivars.

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To better understand the sequence of events in plants subjected to soil drying an experiment was set up in which leaf elongation and transpiration of 4 maize cultivars were monitored continuously. The diurnal water relations of both roots and leaves were measured several times during the experiment. Root growth of the whole root system was measured and expressed as root length and specified for different root diameter classes. This study emphasizes the specific reaction of individual root diameter classes.

Leaf elongation is generally accepted to be sensitive in monocotyledonous plants subjected to soil drying. Direct sensing of the soil water status would have to occur in the roots. Abscisic acid (ABA) produced in dehydrating roots may enable the plant to ‘measure’ the water status of the soil (Zhang and Davies 1989, 1991). These authors speculated that dehydration of a few fine roots may promote the production of a chemical signal which will restrict not only leaf conductance, but also leaf expansion (Masle and Passioura 1987, Rosa da Costa et al. 1987), thereby increasing carbohydrate availability to the root system. At low soil $\Psi_w$, roots can shrink considerably (Huck et al. 1970). At early stages of soil drying or with mild drought, however, roots may not become substantially dehydrated. In contrast, decreases in leaf elongation can be observed at very early stages of soil drying. Decreases in root $\Psi_w$ as sensitive means of detecting the degree of early soil drying may therefore not constitute a signalling system. This hypothesis was tested in the present work with 4 different maize cultivars. As an alternative we investigated whether early soil drying might be more sensitively indicated in changes in $\Psi_s$ and $\Psi_p$ of whole roots.

It is difficult to measure water relations in growing tissues, but essential for a causal analysis of growth regulation. This was not attempted in this work. Our measurements of root and leaf water relations were restricted to whole organs. We postulate, however, that changes in the water relations of whole organs might also be reflected in changes in the water relations of actively expanding cells. Decreases in $\Psi_w$ and $\Psi_s$ of mature regions of roots and leaves, representing the major part of these organs, have been shown to be associated with decreased values in the respective actively expanding tissues (Westgate and Boyer 1985). Our investigation at the whole organ level should therefore allow to more specifically focus on relevant processes occurring at early stages of soil drying in actively expanding tissues.

Abbreviations – DAW, days after beginning of different watering treatments; LVDT, linear variable differential transducer; $\Psi_m$, soil matric potential; $\Psi_p$, turgor; $\Psi_s$, osmotic potential; $\Psi_w$, water potential.

Materials and methods

Description of experiments

Two experiments were conducted under the same experimental conditions. Details will be given in Cultivation of plants (below). In the first experiment, the influence of soil drying on leaf elongation, root and shoot biomass, and leaf and root water relations of 4 cultivars of Zea mays L. (Issa, Irrat 42, Irrat 32, Costeño de Culiacan) were determined. The cultivar Issa was used for the second experiment in which the influence of soil drying on soil strength and root growth was measured. Changes in soil strength may represent an early trigger of decreased shoot growth and were therefore determined in this work. Growth of root and shoot biomass in well-watered and drought treatments were comparable in both experiments. All treatments were replicated at least three times and all experiments were repeated with similar results.

Cultivation of plants

The experiments were conducted in a growth chamber under constant conditions (12/12 h day/night; 20/18°C day/night temperature; 420 µmol m$^{-2}$ s$^{-1}$ [PPFD]; 70% RH). Plants were grown in pots (20 cm in height, 10 cm in diameter) containing 1.32 kg of soil (dry weight basis). The soil used in this investigation was illitic-chloritic Charrat silt loam (fine mixed mesic Aquic Ustifluent). The soil was 9.1% clay, 39.5% silt, 31.4% sand, and 0.83% organic matter (Schmidhalter et al. 1994). The initial soil matric potential ($\Psi_m$) was based on a soil water retention curve, as described in the next section. Four seeds of Zea mays L., pre-germinated for 2 days, were sown in the moist soil of each pot at a depth of 2 cm. The top of the soil was covered with a layer of sand, 1 cm thick (grain size 1–2 mm) to reduce the compacting action caused by watering the plants and to reduce evaporation from the soil surface. Evaporation was further decreased in the second experiment by covering the pots with transparent polyethylene sheets with small holes through which seedlings grew and were watered. Half the pots were watered daily to give a $\Psi_m$ of −0.03 MPa, the rest were allowed to dry out from day 9 to day 17 (Exp. 1) and from day 9 to day 18 (Exp. 2).

Soil matric potential and soil strength

Gravimetric water contents were converted to $\Psi_m$ based on a previously determined water retention curve (Schmidhalter 1997). A needle penetrometer with a stainless steel needle of 2 mm diameter and 30° internal angle was used to measure soil strength. The needle was driven into the soil at 2 mm s$^{-1}$. Readings were recorded from a balance and con-
verted to penetrometer resistance values by dividing the force by the cone’s projected cross-sectional area. Penetration measurements were taken sequentially in the two treatments (control and drought), with 3 replicates per treatment. Each replicate represented one pot with 3 measurements averaged per pot. The sand layer was carefully removed prior to the measurements. Measurements in the soil were made at depths of 1, 4, 7, 10 and 13 cm. Measurements were made 2, 4, 6 and 7 DAW (days after beginning the different watering treatments).

**Dry weight of roots and shoots**

Dry weights of roots and shoots were determined in all experiments after drying the fresh material in an oven at 105°C for 48 h. Values represent pot averaged values of 4 pots from each treatment. Growth of root and shoot biomass in the control and drought treatments were comparable in all experiments and are reported for the second experiment (penetrometer resistance and root growth experiment). In this experiment, root and shoot biomass for both treatments were determined 2, 4, 6 and 7 DAW.

**Development, length and diameter of roots**

Development of roots was recorded at harvest time. The roots were washed manually for root length measurements. The roots were stained with Pararosaniline (Sigma Chemical Company, St Louis, MO, USA) and stored until measurement at 4°C in water. The roots were cut into small pieces and evenly distributed in a shallow, transparent tray for image recording with the image processing system IMCO/S (Kontron Elektronik, Eching, Germany) and a CCD camera ProgRes 3000 (Kontron Elektronik). The maximum resolution was 80 μm. Roots were divided into 8 classes with diameters being less than 240, 480, 720, 960, 1 200, 1 440, 1 680 and 1 920 μm, respectively. Images were analyzed for length and diameter of roots using the computer program ROOT DETECTOR (Walter and Bürgi 1996).

**Water relations of leaves and roots**

Leaf and root water relations were determined on 4 plants from different pots for each treatment on the same days at 0, 2, 5 and 8 h during the light period, except on DAW 8 where measurements were performed once, immediately before the light period commenced. The youngest not yet fully developed leaf blade, cut one third from the leaf base, and the root system cut at the mesocotyl were used for the measurements. Water potential was determined with a pressure chamber (PMS Instrument Co., Model 1002, Corvallis, OR, USA) and osmotic potentials (Ψs) with a vapor pressure osmometer (Wescor 5500, Wescor Inc., Logan, UT, USA). Turgor was calculated as the difference between Ψs and Ψw. The same measurements of the water relations were made for whole roots using a recently adapted pressure chamber/osmometer technique (Schmidhalter et al. 1992). Measurements were first made on the detached leaf, leaving the remainder of the plant in the pot. Thereafter, only the bulk of the soil was gently shaken from the root/shoot system, and the intact root system, cut above the root/shoot junction, was used for the pressure chamber measurements. Further details have been reported elsewhere (Schmidhalter et al. 1998).

Pressure chamber measurements were not corrected for the Ψs of the xylem sap; previous investigations showed that it was higher than −0.05 MPa in leaves, whereas lower values may have occurred in roots. Xylem sap which was obtained by applying higher than balancing pressures to leaves and roots was contaminated with symplastic solutes. Uncorrected Ψs of the xylem sap were higher than −0.09 and −0.25 MPa for leaves and roots, respectively. Corrections applied for the measured contributions of glucose, fructose and sucrose increased these values to −0.05 and −0.16 MPa, respectively. We conclude that xylem sap Ψs of roots must have been markedly higher than −0.16 MPa. No significant difference was found in xylem sap Ψs between differently droughted and control roots. Leaf Ψw in these experiments were closely approximated by the measured balancing pressures, whereas root Ψw were significantly underestimated by the pressure chamber technique. Because comparable values of xylem sap Ψs were found in droughted and control roots changes in balancing and osmotic pressures can be used to characterize the sensitivity of the water relations to early soil drying.

After the balancing pressures were recorded leaf and root tissues were immediately sealed in plastic bags and kept in ice before transfer to the deep-freeze (−80°C). For Ψs determinations the tissues were plunged into liquid nitrogen and then equilibrated at room temperature. Sap was obtained from leaves and whole root systems with a specially designed press and Ψs from sap determined with an osmometer. Dilution of the sap due to apoplastic mixing was not taken into account. The xylar contribution to the dilution of root sap Ψs is comparatively small for normally fertilized plants in this experiment, whereas apoplastic liquids contained in the intercellular and cell wall space will increase root Ψs determined by osmometry. At the present there are no methods available to exactly quantify their contribution to apoplastic mixing. We assume that Ψs in control and droughted roots is similarly affected by the dilution due to apoplastic mixing.

**Leaf elongation and plant transpiration**

Instantaneous measurements of the growth of leaf 3 were made on 8 plants per treatment from different
pots by using linear variable differential transducers (LVDT) connected to the data logger. Measurements began when leaf 3 emerged above the enclosing sheath of the preceding leaf. The tip of the growing leaf was attached to the LVDT by a fishing line (0.22 mm diameter) which was attached to the leaf tip using a small clamp cushioned with mounting rubber to avoid damaging the leaf. The force on the fishing line was 10 g to minimize oscillations in the LVDT output; the force applied did not affect leaf elongation rate during measurement. LVDT readings corresponded to the elongation of leaf blades only. Leaf area expansion was derived from destructive leaf area measurements with a portable leaf area meter (LI-3000A, Li-Cor, Lincoln, NE, USA) on DAW 3, 5, 7 and 9. The diurnal course of transpiration was determined by weighing 8 pots per treatment every half hour and deducting evaporative losses from pots of soil without plants. Less frequent determinations of transpiration were made for the drought treatment before DAW 2. However, no difference in the average values of transpiration were found on DAW 1 between the two treatments. Values of transpiration have been expressed as amount of water lost per unit surface of the experimental pots (mm² mm⁻² h⁻¹) by assuming the density of water to be 1 g cm⁻³.

Statistical analysis

Values are presented as means with standard errors of 3 to 8 replications. Analysis of variance was carried out for root and shoot dry weight, total root length and root length by diameter class, leaf elongation, and transpiration rates for the first and second experiments. t-Tests were used for mean separations of the two treatments for the parameters leaf elongation, transpiration and diurnal water relations. Characterization of the drought response can be derived either from comparisons with the well-watered control or by examining the behaviour of the trait with increasing soil dryness. To allow both comparisons, the statistical analysis for leaf and root water relations was conducted on the basis of DAW and water supply treatments (with 5 levels: DAW 3 drought, DAW 5 drought, DAW 7 drought, DAW 4 well-watered and DAW 7 well-watered). Prior to the analysis of variance, tests indicated homogeneity of variances among these treatment levels. Differences in root and leaf $\Psi_m$ components between these treatment combinations and genotypes were analyzed in a combined analysis of variance using the SAS-GLM procedure (SAS/STAT 1990).

Results

Soil water status

$\Psi_m$ was maintained between $-0.03$ and $-0.06$ MPa in the well-watered treatment (control) (Fig. 1). A gradual decline in $\Psi_m$ to $-0.2$ MPa was observed in the stress treatment until DAW 5, and thereafter a steeper decline, lasting 3 days, to about $-0.7$ MPa.

Soil penetrometer resistance

Penetrometer resistance was generally low (Fig. 2). Values recorded were about 300 kPa in the well-watered treatment and 400 kPa in the drought treatment except in the uppermost soil layer at DAW 7 (900 kPa).

Fig. 1. Change in soil matric potential in a well-watered treatment and a soil drying treatment in which water was withheld 9 days after emergence of maize seedlings. Results are presented for experiments 1 and 2. Error bars indicate $\pm$ SE of the means of 4 observations.

Fig. 2. Penetrometer resistance and soil water content at different soil depths in pots containing maize seedlings which were either well-watered (closed symbols) or watered for the last time 9 days after emergence (open symbols). Points are means $\pm$ SE of 4 observations and are indicated for 4 and 7 days after withholding water (DAW) and at the end of the experiment for the well-watered treatment.
Development, length and diameter of roots

Root systems of the investigated seedlings consisted of the primary root and a tier of 4 shoot-borne roots. No difference in the number of shoot-borne roots was observed between DAW 1 and 7 in the two treatments. In accordance with root dry weight the total root length (expressed as root length density) was significantly increased under drought beginning on DAW 4 (Fig. 3). Root length and root dry weight were significantly correlated \( r = 0.77^{***} \). The distribution of root length by diameter class shows that increases in total root length were due mainly to roots having diameters less than 480 \( \mu \)m (Fig. 4). Increased growth of roots thicker than 1920 \( \mu \)m was most likely caused by a proliferation of shoot-borne roots on DAW 2 and 4.

Leaf and root water relations

The aim of this paper is to report the development of leaf and root \( \Psi_w \) in drying soil based on 4 maize

Shoot and root dry weight production

Withholding water caused a decrease in shoot dry weight and an increase in root dry weight (Fig. 3), resulting in an increased root to shoot dry weight ratio. Decreases in shoot dry weight were highly significant on DAW 6 and 7. Root dry weight was increased on DAW 4, 6 and 7 \( (P < 0.05, P < 0.08, P < 0.07) \). Whole plant dry weight was increased on DAW 4 and was significantly decreased on DAW 6 and 7 in the drought treatment as compared with the well-watered treatment.

Fig. 3. Shoot and root dry weight production and root length of soil-grown maize seedlings, which were either well-watered or watered last (drought) on day 0 (9 days after emergence). Points are means ± SE of 4 observations. *, ** and *** indicate significant difference at \( P \leq 0.05, 0.01 \) and 0.005, respectively.

The water content of the droughted pots was reduced fairly homogeneously along the profile. Gravimetric water content was by less than 2% lower in the upper third of the pot than in the lower parts of the pot. The moderately higher penetrometer resistance recorded in 1 cm soil depth probably reflects a higher soil dryness and crusting effects caused by watering the plants before withholding water.

Fig. 4. Root length by diameter class of maize seedlings grown in well-watered soil and in soil that was watered last on day 0. Points are means ± SE of 4 observations. Results are shown for days 2 to 7 after withholding water (DAW). * and ** indicate significant difference at \( P < 0.05 \) and 0.01, respectively.
Tab. 1. Leaf water potential ($\Psi_w$), leaf osmotic potential ($\Psi_s$) and leaf turgor ($\Psi_p$) of maize seedlings which were well-watered or subjected to drought by withholding water 9 days after emergence. Measurements were conducted, 0 (predawn), 2, 5 and 8 h after the light period commenced, on days 3, 4, 5 and 7 after beginning the different watering treatments (DAW). Four maize cultivars were included in the experiments. Means within each water potential component not followed by the same letter are significantly different at $P \leq 0.05$ according to the Student-Newman-Keuls test.

<table>
<thead>
<tr>
<th>Leaf parameter</th>
<th>DAW Water supply treatment</th>
<th>Light period (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>$\Psi_w$ (MPa)</td>
<td>Drought</td>
<td>-0.14b</td>
</tr>
<tr>
<td>3</td>
<td>Drought</td>
<td>-0.15b</td>
</tr>
<tr>
<td>5</td>
<td>Drought</td>
<td>-0.27c</td>
</tr>
<tr>
<td>7</td>
<td>Well-watered</td>
<td>-0.09a</td>
</tr>
<tr>
<td>$\Psi_s$ (MPa)</td>
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<tr>
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</tr>
<tr>
<td>5</td>
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</tr>
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<td>$\Psi_p$ (MPa)</td>
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<td>Drought</td>
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<td>Well-watered</td>
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</tr>
<tr>
<td>4</td>
<td>Well-watered</td>
<td>0.62a</td>
</tr>
</tbody>
</table>

Cultivars representing a broad genetic background. Therefore, results of the combined analysis of leaf and root water relations are presented in Tabs 1 and 2 with emphasis on the general response to soil drying, although some genotypic differences were found among the 4 maize cultivars (M. Evéquoz. 1993. Thesis, Swiss Federal Institute of Technology, Zurich, Switzerland).

Except at predawn (shortly before commencing the light period), leaf and root $\Psi_w$ on DAW 3 in the drought treatment were comparable to the corresponding values in the well-watered treatment (DAW 4 and 7). Leaf $\Psi_w$ in the drought treatment decreased from DAW 3 to 7. In contrast, root $\Psi_w$ was only decreased on DAW 7. Root $\Psi_w$ decreased slightly during the light period. A marked decrease was observed on DAW 7, 8 h after the light period commenced. Two hours after beginning the light period transpiration decreased to very low levels. Root $\Psi_w$ were lower or very similar to the bulk $\Psi_m$ on DAW 3 and 5 (Tab. 2, Fig. 1, Exp. 1). A remarkable exception to this was observed on DAW 7, where the decrease in the root $\Psi_w$ lagged behind the decreasing bulk $\Psi_m$, except after 8 h where both values became again similar.

Leaf $\Psi_s$ and $\Psi_p$ on DAW 3 in the drought treatment were comparable to the corresponding values in the well-watered treatment throughout the experiment. In contrast, leaf $\Psi_s$ and $\Psi_p$ were markedly lower in droughted plants by DAW 7 once the plants were illuminated. Root $\Psi_s$ between DAW 3 and 7 was very stable in the well-watered treatment but declined by about 0.4 MPa over the 4 days in droughted plants. Root $\Psi_p$ showed an almost commensurate rise over the same period. The rise in root $\Psi_p$ should be contrasted with sharp decreases in leaf $\Psi_p$.

**Transpiration and leaf elongation**

Leaf area expansion increased linearly between DAW 3 and 9. Plant leaf area between the two treatments was not significantly different before DAW 5. No difference was to be expected in describing the sensitivity of transpiration to soil drying during DAW 0 to 5 by expressing gravimetric transpirational losses either per unit plant leaf area or per unit experimental surface. The latter has been preferred because frequent gravimetric measurements of transpiration were performed whereas only few measurements of plant leaf area were available throughout the course of the experiment. Decreases in transpiration were observed after DAW 5, 14 days after emergence (Fig. 5). In contrast, slight reductions in leaf elongation were observed after DAW 1 and were clearly manifest after DAW 2 (Fig. 5). Withholding water slowed down elongation to a greater extent during the light period than during the dark period. Mean values of transpiration and leaf elongation measured from the third to the fifth hour of the light period are represented in Fig. 6 for the drought and control treatment. Statistically significant differences in leaf elongation were observed on DAW 3 ($P < 0.05$) in the stress treatment as compared with the control treatment. A less significant difference had already been observed on DAW 2 ($P < 0.1$). Only on DAW 5 was transpiration significantly decreased ($P < 0.01$) in the stress treatment. This figure clearly shows that leaf elongation started to decrease well before transpiration was reduced. Statistically significant differences in dark period elongation were observed after DAW 6 ($P < 0.05$). The decline in leaf elongation in the control treatment was due to the leaf's normal development, where after a linear growth phase (DAW -1 to 2) a declining rate of leaf elongation is observed. Treatment
effects in our study were not confounded by changes due to development.

Discussion

Does increased soil strength represent an early trigger of decreased leaf elongation?

A decrease in leaf expansion rate has been interpreted as a response to changes in mechanical impedance as a consequence of soil drying in two studies (Masle and Passioura 1987, Passioura 1988). Soil strength can be increased in rapidly drying surface soil as shown by Weeich et al. (1992) and is also indicated in our study. Mechanical impedance greater than 2 MPa is likely to reduce total root length and root elongation rate by at least 50% (Atwell 1993) and root elongation ceases at root penetration resistances of about 1 MPa (Bengough and Mullins 1990). The low values of soil strength in our study lead to the conclusion that changes in soil strength did not reduce leaf elongation. Results of this study are not unexpected, because the low soil bulk density of 0.9 kg m\(^{-3}\) made it unlikely that soil strength interfered with shoot elongation. This study shows that decreases in leaf elongation in drying soil do not necessarily involve soil strength as a primary factor, whereas this may be different in other soils with higher soil bulk densities.

Water status of non-growing leaf blades and leaf elongation

Previous reports have shown that elongation reacted very sensitively to slight reductions in leaf \(\Psi_w\) (Boyer 1970, Acevedo et al. 1971). Leaf elongation decreased very drastically at leaf \(\Psi_w\) lower than \(-0.2\) to \(-0.3\) MPa in a dark and humid growth chamber (Boyer 1970, Acevedo et al. 1971). Both studies indicated that leaf elongation nearly ceased when the leaf \(\Psi_w\) reached \(-0.7\) to \(-0.8\) MPa. In our study, predawn leaf \(\Psi_w\) was \(-0.14\) MPa when elongation decreased significantly and predawn and daytime leaf \(\Psi_w\) were \(-0.7\) and \(-1.2\) MPa, respectively, when leaf elongation stopped. Lower elongation rates during the night as compared to the day, in spite of significantly higher leaf \(\Psi_w\), suggest other limiting factors. A higher leaf meristem temperature during daytime might partly account for the higher elongation rate as compared to nighttime, but this remains speculative because meristem temperatures were not determined. Decreases in daytime leaf elongation between and within treatments do not seem to be closely related to the leaf \(\Psi_w\) in non-growing leaf blades. There are several studies which suggest changes in growth rate without corresponding changes in \(\Psi_w\) (Matsuda and Riazi 1981, Michelena and Boyer 1982, Shackel et al. 1987).

### Tab. 2. Root water potential (\(\Psi_w\)), root osmotic potential (\(\Psi_s\)) and root turgor (\(\Psi_p\)) of maize seedlings which were well-watered or subjected to drought by withholding water 9 days after emergence. Measurements were conducted, 0 (predawn), 2, 5 and 8 h after the light period commenced, on days 3, 4, 5 and 7 after beginning the different watering treatments (DAW). Four maize cultivars were included in the experiments. Means within each water potential component not followed by the same letter are significantly different at \(P \leq 0.05\) according to the Student-Newman-Keuls test.

<table>
<thead>
<tr>
<th>Root parameter (\Psi_w) (MPa)</th>
<th>DAW treatment</th>
<th>Water supply</th>
<th>Light period (h)</th>
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increased root tip $\Psi_p$ and $\Psi_w$ may also have been caused by cell wall relaxation of still growing root tissue in this study. Furthermore low values can result if non-living root tips were sampled from very dry topsoil. Higher root $\Psi_s$ and lower root $\Psi_p$, which were observed only at an early stage of stress (drought, DAW 3 vs well-watered, DAW 4), might be a result of the increased root growth, as demonstrated in the second experiment (Fig. 3). Increased growth will cause dilution of imported solutes. The main result, however, is a continuous and significant decrease in root $\Psi_s$ and an increase in root $\Psi_p$ of the droughted plants.

The suitability of pressure chambers to estimate root water status should be questioned because of an anomaly in our study on DAW 7. Higher root $\Psi_w$ as compared to the bulk $\Psi_m$ in our study were most likely due to an uneven depletion of water in the bottom of the pots in the first experiment which is not represented by the bulk $\Psi_m$. This was different in the second experiment where we could achieve fairly homogeneous soil water distributions throughout the soil profile. Bulk root $\Psi_w$ may be biased towards the water status of the wettest part of the soil. This is also indicated by signifi-

Root water status and leaf elongation

In our study, root $\Psi_w$ was hardly affected by early soil drying in contrast to leaf elongation. Decreasing root $\Psi_s$, whereby root $\Psi_p$ was maintained or increased, may be a more sensitive indicator of early soil dryness. Decreases in $\Psi_s$ may result from decreased leaf growth and thereby decreased sink strength in leaves, thus leading to a shift of assimilates from leaves to roots and to increased root growth as observed in this study. Our measurements showed that $\Psi_p$ was maintained in the whole root system. Continued root growth suggests maintenance of $\Psi_p$ above the $\Psi_p$ threshold value in growing root tips. This casts some doubt on the belief that decreases in root $\Psi_w$ and/or decreased root tip $\Psi_p$ are early events indicating soil dryness. We suggest instead that this role may be played by decreased root $\Psi_s$ and increased root $\Psi_p$ resulting from reduced leaf growth and continued assimilation. Maintenance of root $\Psi_p$ in drying soil was shown by other researchers as well (Sharp and Davies 1979, Westgate and Boyer 1985), whereas decreases in $\Psi_p$ were only found in severely stressed plants (Spollen and Sharp 1991); de-

Fig. 5. Diurnal and nocturnal values of transpiration and leaf elongation of maize seedlings in a well-watered treatment and a drought treatment in which water was withheld 9 days after emergence. Light/dark periods were 12 h. Nocturnal values of transpiration and elongation are reflected in markedly lower values as compared with diurnal values. Lines represent mean values of 8 observations per treatment.

Fig. 6. Mean values of transpiration and leaf elongation of maize seedlings measured between 3 and 5 h after beginning the 12-h light period in a well-watered (closed symbols) and a drought treatment (open symbols) in which water was withheld 9 days after emergence. Points are means $\pm SE$ of 8 observations. *, ** and *** indicate significant difference at $P \leq 0.05$, 0.01 and 0.005, respectively. Significance symbols compare differences between treatments on the same day.
cantly higher root $\Psi_w$ as compared with the surrounding soil found in dry pots in split-root experiments (Saab and Sharp 1989, Augé et al. 1994). On DAW 7, a sudden decrease in the leaf $\Psi_w$ accompanied by a strongly decreased transpiration was observed 2 h after beginning the light period. Eight hours after commencing the light period a substantial drop in the root $\Psi_w$ was observed. Continued water losses from the plant, albeit at a very much reduced rate, may have resulted in dehydration of roots. Values of root $\Psi_w$ obtained by pressure chamber measurements were 'logical' throughout most of the time in this investigation. Together with the above plausible explanation we conclude that this technique can reliably be applied to measure balancing pressures of roots.

**Root growth and leaf elongation**

Root growth and leaf elongation seemed to react comparatively early to drying soil (Figs 3 and 6). Promotion of root growth and decreases in leaf growth under such conditions support previous reports. Low soil $\Psi_w$ promoted lateral root initiation and elongation in *Lolium perenne* plants, the total length of lateral roots being between 3 and 5 times that of control plants (Jupp and Newman 1987). Increased proliferation of lateral roots responding to soil drying have been reported for woody plants, wheat and soybean plants (Read and Bartlett 1972, Sharma and Ghildyal 1977, Osonubi and Davies 1978, Huck et al. 1983). Early increases in the length of thick, most likely basal roots are puzzling and may raise the question as to whether these roots react especially sensitively. This study emphasizes the specific reaction of individual root diameter classes. Most studies investigating primary effects of water deficit have focused on the growth of fairly large primary roots in maize plants. Although this may be representative of an early stage of growth, this study shows that withholding water mainly increased the length of fine roots. Consequently, further studies will have to address these diameter classes specifically.

**Sequence of drought response in maize seedlings**

The earliest events observed in the water deficit treatment were decreases in leaf elongation and increases in the length of the largest root diameter class (1 720 to 1 960 $\mu$m) followed by increases in root length of fine roots and in root dry weight. Except at predawn, leaf and root $\Psi_w$ reacted less sensitively and only after changes in root $\Psi_s$ and $\Psi_p$ had been observed. The least sensitive traits were transpiration and shoot dry weight. Results of this study agree with the observation of other authors that the inhibition of leaf elongation precedes decreases in transpiration (Acevedo et al. 1971, Davies et al. 1981, Passioura 1988, Saab and Sharp 1989) and in the water status of non-growing leaf blades (Saab and Sharp 1989). Decreases in root $\Psi_w$, however, as sensitive means of detecting the degree of soil drying, as suggested previously, are contrasted by our study and therefore do not seem to constitute a signalling system.

**Conclusions**

Reductions in leaf elongation can occur before significant changes in the water relations of non-growing leaf blades are observed. However, this is not necessarily the case for growing organs. Therefore, it is premature to conclude that growth reductions occur without parallel changes in the water relations of the growing cells. Reductions in leaf elongation precede changes in root $\Psi_w$. In contrast to root $\Psi_w$, changes in root $\Psi_s$ and $\Psi_p$ are more sensitive indicators of changes in drying soil and might constitute an early signalling system. Further work is required to determine whether reductions in leaf elongation precede decreases in root $\Psi_s$, which may be accompanied by increased root $\Psi_p$ values, or whether early changes in the water relations of the leaf growth zone precede inhibition of leaf elongation. Early increases in the length of fine roots and the thickest roots represent a particularly interesting subject for further investigations. This may allow us to determine whether decreases in leaf elongation and increases in root length occur simultaneously or not. In this study soil strength can be excluded as an inhibiting factor.

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**References**


