Root Osmotic Adjustment under Osmotic Stress in Maize Seedlings

1. Transient Change of Growth and Water Relations in Roots in Response to Osmotic Stress

Atsushi Ogawa¹ and Akira Yamauchi²

¹Department of Biological Production, Akita Prefectural University, Shimoshinjyou-nakano 241-7, Akita 010-0195, Japan; ²Graduates School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan

Abstract: The transient changes in seminal root elongation rate, osmotic potential, water potential, and turgor pressure in maize after the onset of stress treatments were examined using polyethylene glycol 6000 to evaluate the process of osmotic adjustment in root. Maize seedlings were exposed to different intensities of osmotic stress for 1, 3, 5, 10, and 20 minutes, and 2, 6, 12, and 24 hours. Seminal roots shrunk immediately after the onset of the −0.41 and −0.89 MPa stress treatments, due to dehydration. However, roots gradually resumed elongation from 5 minutes after the onset of the stress treatment. The osmotic potential in the root elongating zone dropped drastically with the onset of stress followed by a gradual decrease for 6 hours. We quantitatively analyzed the relative importance of the factors that contributed to the reduction of osmotic potential, and found that 46% reduction of osmotic potential was caused by tissue dehydration and 54% by solute accumulation 20 minutes after start of −0.89 MPa treatment. At hour 12, however, only 7% reduction of osmotic potential was caused by tissue dehydration and 93% by solute accumulation. In the root elongating zone, turgor pressure decreased immediately after the onset of the stress treatment due to the larger decline of water potential than that of the osmotic potential. However, from minute 20 onwards, turgor pressure started to recover due to osmotic adjustment. These results indicated that osmotic adjustment develops immediately after exposure to osmotic stress, especially in the root elongating zone, and substantially contributed to the maintenance of turgor pressure and root growth.

Key words: Elongating zone, Elongation rate, Osmotic adjustment, Osmotic potential, Root, Turgor pressure, Zea mays L.

Osmotic adjustment is a mechanism in plants to tolerate osmotic stress by lowering the osmotic potential by the accumulation of solutes (Radin, 1983), and to maintain the volume of protoplast and turgor pressure (Kaiser, 1982; Santakumuri and Berkowitz, 1991). Osmotic adjustment is a function of either an increase in the net osmoticum deposition rate in the growing region and/or a reduction in the rate of tissue volume expansion (Sharp et al., 1990). The former is more likely to represent an adaptive response that could contribute to growth maintenance (Sharp et al., 2004).

Osmotic adjustment occurs in leaves, hypocotyls, roots, floral apex and spikelets under osmotic stress conditions (Turner and Jones, 1980). In leaf mature zone, osmotic adjustment plays an important role for plant cell survival (Flower and Ludlow, 1986), facilitates higher stomatal conductance (Henson et al., 1983; Wright et al., 1983; Düring and Dry, 1995) and leaf expansion (Hsiiao et al., 1976; Westgate and Boyer, 1985) to sustain photosynthesis under water stress condition (Turner and Jones, 1980).

Turner and Jones (1980) stated that osmotic adjustment in the root has a different role from that in the shoot, and Serraj and Sinclair (2002) stated that osmotic adjustment in root might be important in relation to crop production. As in leaf growth, root elongation is related with the turgor pressure in root elongation zone (Greacen and Oh, 1972; Sharp and Davies 1979; Westgate and Boyer, 1985; Frensch and Hsiao, 1994). The role of osmotic adjustment in root elongating zone is to maintain turgor pressure to continue root elongation and root growth in drying soils, which enable the plant to maintain its transpiration by exploiting a greater volume of soil or utilize available water in a given soil volume more efficiently. The significance of osmotic adjustment in the elongated zone of the root is to promote desiccation tolerance and to initiate lateral roots that are responsible for efficient absorption and transport of water (Azhiri-Sigari et al., 2000; Bañoc et al., 2000a, 2000b).

Hence, improvement of crop performance by increasing osmotic potential-adjusting ability might be more significant in roots than other plant parts (Serraj and Sinclair, 2002), although the process in roots has not been studied as extensively as in leaves. There are few reports on osmotic adjustment in the root. In maize, Sharp and Davies (1979) showed that even when osmotic potential decreased under water...
stress conditions, turgor potential and root growth were maintained (Shrap and Davies, 1979; Westgate and Boyer, 1985). On the other hand in leaf (Shrap and Davies, 1979) and stem (Westgate and Boyer, 1985), extension rate and the development of leaf area were reduced by water deficit, due largely to the reduction in leaf turgor pressure. For the decrease of osmotic potential, the accumulation of compatible solute (Voetberg and Sharp, 1991) and the decrease in the rate of the water influx to the tissue and the tissue volume expansion (Sharp et al., 1990) were contributed in maize seminal root. Turgor was recovered by osmotic adjustment faster in the cells located deep inside the tissue compared with cells near the root surface, and this result showed that the phloem was the possible source of compounds for osmotic adjustment (Frensch and Hsiao, 1994).

The root is the first organ to be exposed to water deficit. Leaf growth is very sensitive to water stress, and may be inhibited by a slight reduction of water potential in the tissue (Hsiao and Xu, 2000). Therefore, under the water deficit condition, it is assumed that osmotic adjustment in the root occurs before that in the leaf to enhance turgor pressure for continued root growth and absorption of water and nutrients. Thus, the osmotic adjustment in the root is expected to delay the onset of water deficit in the shoot, which reduces the activity of stomatal conductance and photosynthetic activity. In order to elucidate the role of root osmotic adjustment in maintaining plant growth under a water deficit condition, it is important to examine the transient changes of solute concentration under stress. Most of the previous studies in this field evaluated osmotic adjustment by measuring osmotic potential and other water relations at fixed times after the onset of stress treatment. Although the transient change of turgor pressure after osmotic stress treatment was shown in the order of minutes using a pressure probe (Frensch and Hsiao, 1994, 1995), the information about the initial transient responses to the stress related to osmotic adjustment is not enough (Frensch and Hsiao, 1994). Besides, little attention has been paid to the differences in the responses to osmotic stress among various parts of the roots in a root system. Comparison between the responses in the root and shoot is also needed because of the difference in physiological roles of the root and shoot.

The objective of this study was to examine the transient processes of osmotic adjustment in maize seedling by determining root elongation and water relations of root and leaf subjected to osmotic stress. In this process, we paid special attention to the difference in the response to osmotic stress between plant tissues and parts, because there are characteristic functions of osmotic adjustment in each organ and each part. We also compared the transient processes of osmotic adjustment in root elongating zone, root elongated zone and leaf mature zone. Additionally the relative contribution of solute accumulation and dehydration to the change of osmotic potential under osmotic stress has not yet been well documented. In this study, the contribution of the two factors was quantitatively evaluated and the process of osmotic adjustment by the accumulation of solutes was also demonstrated.

Materials and methods

1. Plant culture

Seeds of Zea mays L. (cv. White Pop) were germinated in the dark at 28°C in petri dishes. Forty-eight hours after sowing the seeds, the seedlings with seminal roots (approximately 8 mm in length) were transplanted onto a net floating on 8 L of nutrient solution in 10 L plastic containers (39 cm width, 22 cm depth and 16 cm height) filled with 8 L nutrient solution. The nutrient solution contained 6.0 mM KNO₃, 4.0 mM Ca(NO₃)₂, 1.0 mM NH₄H₂PO₄, 2.0 mM MgSO₄, 26.8 µM Fe-EDTA, 4.6 µM MnCl₂, 23.1 µM H₂BO₃, 0.38 µM ZnSO₄, 0.16 µM CuSO₄ and 0.015 µM (NH₄)₆Mo₇O₂₄. The nutrient solution was aerated sufficiently. The nets were covered with aluminum foil with pinholes to allow roots to grow in the dark. Transplanted seedlings were exposed to a 12-hour...
photoperiod in a walk-in type growth chamber supplied with 320 µmol m⁻² s⁻¹ photon flux density of photosynthetically activity radiation (PAR, 400-700 nm) and maintained at 28 ± 0.5°C and relative humidity of 80 ± 5%. Seedlings were grown for 48 hours in nutrient solution.

2. Osmotic stress treatment

Polyethylene glycol 6000 (PEG) (CaHC Co., Ltd, Japan) was dissolved in the nutrient solution at concentrations of 0 (control), 50, 200 and 300 g per litter, to induce osmotic stress. The water potential of the nutrient solutions was determined using a vapor pressure osmometer (model 5300, Wescor), and was −0.08, −0.13, −0.41 and −0.89 MPa, respectively. High-molecular weight PEG was used as an osmoticum because it is virtually excluded from entering the root apoplast (Carpita et al., 1979), and thus removes water from the cell and cell wall space. PEG has no apparent toxic effects under a well-aerated condition (Verslues et al., 1998; Raymond and Smirnoff, 2002; Ober and Sharp, 2003). Hence, this method mimics the drying effects of a soil environment. In contrast, other osmotica such as salts, mannitol and sorbitol penetrate the cell wall and the cells themselves, and therefore may alter the normal response to low water potential.

The seedlings grown in the nutrient solution for 48 hours were transferred to PEG solutions for osmotic stress treatment. At 0 (before stress treatment), 1, 3, 5, 10, 20 minutes (minute 0, 1, 3, 5, 10 and 20), 2, 6, 12,
and 24 hours (hour 2, 6, 12 and 24) after the onset of PEG treatment, the seedlings were sampled for various measurements as described below.

The stress treatment started after a 12-hour dark period, but the dark condition was further maintained for the initial 20 minutes to examine the initial response to osmotic stress without the effect of light. The seedlings were sampled inside the growth chamber.

3. Measurement of water relations

Leaf segments 10 mm from the tip of the second leaf (leaf maturated zone) (Michelena and Boyer, 1982) and seminal roots 0–6 mm from the tip (root elongating zone) and 14–20 mm from the tip (root elongated zone) were excised. At the sampling, the third leaf was just emerging.

The turgor potential of the tissue was calculated as the difference between water potential and osmotic potential measured. The water potential of leaf and root was measured by thermocouple psychrometry using sample chambers (model C-52, Wescor) and a microvoltmeter (HR-33T, Wescor). The sample chambers had been calibrated with NaCl solution of a known molarity in advance. In preliminary measurements, we had confirmed the amount and the time for the equilibrium. The plant specimens in the −0.08 MPa and the −0.89 MPa treatment groups at hour 24 were sealed in the C-52 psychrometer chambers at 25°C (Fig. 1). The equilibrium was reached at hour 0.5 in root segments of the 0-6 mm part and at hour 2 in the leaf segment in both treatments. The amount of specimen did not affect the time for equilibration in either part. Based on this result, one leaf or root segment was sealed in one sample chamber to measure

![Figure 4](image-url)
Ogawa and Yamauchi — Transient Change of Growth and Water Relations under Osmotic Stress

...the water potential. It took 2.5 hours to reach the equilibrium. After hour 2.5 for equilibration, the C-52 sample chamber was connected with HR-33T microvoltmeter and it was ascertained that the value of zero offset was below 0.3 µV. The measurement was carried out by psychometric method with a thermocouple cooling time of 20 seconds.

Osmotic potential was measured using a vapor pressure osmometer (model 5300, Wescor). Five leaf segments and 16 root segments were sealed in plastic tubes and plunged into liquid N₂. The samples were stored in a freezer at −30ºC prior to measurement of osmotic potential. The frozen tissues were thawed for over 30 minutes at room temperature, and then homogenized with a glass rod. Then the samples were centrifuged for 20 minutes at 11,000 g. The supernatant was used for osmotic potential measurement. Osmotic potential ($\Psi_s$) was calculated as;

$$\Psi_s = -nRT$$  \hspace{1cm} (1)

where: $n$ is the osmolarity of solutions, $R$ is the gas constant, and $T$ is the absolute temperature.

To examine the contribution of dehydration to osmotic potential, we calculated absolute water content based on the following formula;

Absolute water content = \frac{(fresh\ weight - dry\ weight)}{fresh\ weight} \times 100  \hspace{1cm} (2)

Additionally, we calculated the osmotic potential assuming that its decrease was caused only by dehydration of the tissue due to lowered external water potential. The calculated osmolarity after stress treatment (time $t$; $N_t$) was given as;

$$N_t = N_0 \times \frac{W_o}{W_t} = N_0 \times \frac{WC_0 \times F_o}{WCl \times Ft}$$  \hspace{1cm} (3)

Fig. 5. Changes in osmotic potential of leaf (a), root elongating zone (b) and root elongated zone (c) under −0.08 MPa (■; control), −0.13 MPa (◆), −0.41 MPa (▲) and −0.89 MPa (▲) osmotic stress. Closed bars show dark period and open bar shows light period. Data are means ± standard errors (n=3).
where \( N_0 \) is the osmolarity before stress treatment (minute 0), \( W_0 \) and \( W_t \) are the amount of water in the tissue before (minute 0) and after stress treatment (time \( t \)), \( W_{C0} \) and \( W_{Ct} \) are the absolute water content at minute 0 and at time \( t \), and \( F_0 \) and \( F_t \) are fresh weight at minute 0 and at time \( t \), respectively. The calculated osmotic potential after stress treatment (time \( t \)) was given using the value of \( N_t \) and the formula (1).

Three replications were used in the measurement of water relations.

4. Measurement of root elongation

At minute 0, 3, 5, 10 and 20 and hour 2, 6, 12 and 24, a seminal root was horizontally placed in a transparent plastic box with 15 cm in length, 10 cm in width and 2 cm in depth, previously fixed on the stage of the inverted microscope (IX70; OLYMPUS). The seminal root was gently fixed on the bottom of the box by adhesive tape (T-112, KOKUYO, Japan) with 1.2 cm width at 4-5.2 cm from the tip. The change of elongation rate at 0-4 cm from the root tip was then measured. The box was filled with the nutrient solution for each stress treatment. The stage with plastic box was covered with black curtain, and root zone in the nutrient solutions was kept in the dark to minimize the effect of light on root elongation. However, when the seminal tip position was recorded, it was exposed to weak light to observe with an inverted microscope. The shoot was outside of the plastic box and was kept in the light on the microscope stage when seedlings were sampled in the light condition (hour 2, 6 and 12), and were kept in the dark when seedlings were sampled in the dark condition (minute 0, 3, 5, 10 and 20, and hour 24). The root tip position of the seminal root was recorded for 2 minutes with a charge couple device (CCD) camera (C5985; Hamamatsu Photonix), and image data was captured.
using FISH Imaging Software (Hamamatsu Photonix). The change of root tip position was measured from the image data using NIH Image (Version 1.6) installed in a Macintosh computer (Power Macintosh 8500/1200). The seminal root elongation rate was calculated as the difference of root tip position. Five replications were used for the measurement of root elongation.

To determine root elongating zone, we excised 10 mm roots from the tip at minute 0, fixed in FAA solution, dehydrated in a water-ethanol-butanol series and embedded in paraffin wax. Longitudinal sections were made at 15 µm thickness using a slide microtome and stained with hematoxylin. After that, each cortex cell length of the third layer was measured basipetally from the tip under the microscope. Three root segments were used for the detection of root elongating zone.

Results

1. Determination of cell elongating zone in root

Cell elongation started at 1.7 mm from the apical meristem, and then cell length increased basipetally. At about 8.0 mm, cell elongation stopped, and cell length attained a constant value of approximately 180 µm (Fig. 2). Based on this result, we defined 0-6 mm from the root tip as the elongating zone and 14-20 mm as the elongated zone.

Since there is no elongation in the 0-10 mm part from the leaf tip (Michelena and Boyer, 1982), we defined this part as the maturated zone.

2. Changes in root elongation after the onset of stress treatment

The seminal root elongation rate decreased within one minute after the onset of osmotic stress treatment, with a greater inhibition at a higher PEG...
concentration (Fig. 3). In the −0.13 MPa treatment group, the elongation rate was about 40 µm min⁻¹ and similar to that in the control. By contrast, in the −0.41 and −0.89 MPa treatment groups, seminal root elongation was inhibited and the root shrank substantially. The elongation rate at minute 1 was −12 µm min⁻¹ in the −0.41 MPa treatment group and −66 µm min⁻¹ in the −0.89 MPa treatment group. From minute 5, the degree of inhibition of the root elongation decreased gradually. The elongation rate was increased to 20 µm min⁻¹ in the −0.41 MPa treatment group at hour 24, which was almost the same as that of the control. In the −0.89 MPa solution, however, the elongation rate did not recover to the control level and it was 5 µm min⁻¹ even at hour 24.

3. Changes in water relations after the onset of stress treatment

Absolute water content was 89% in leaf, 96% in the root elongating zone, and 97% in the root elongated zone before stress treatment (Fig. 4). After the onset of stress treatment, water content of root lowered with time, and that of the elongating zone was lower than that of the elongated zone. For the first 20 minutes, the same degree of decline in the water content was observed in the −0.13 MPa and −0.41 MPa treatment groups, but in the −0.89 MPa treatment group the decline was more marked than in the other two treatments. In the −0.13 MPa treatment group, the lowered water content recovered to the control level, but those in the −0.41 and −0.89 MPa treatment groups did not return to the control level during the experimental period. On the other hand, the water content of leaf hardly changed throughout the experimental period except for the −0.89 MPa treatment.

Osmotic potential was −0.88 MPa in the leaf, −0.50 MPa in the root elongating zone, and −0.55 MPa in the root elongated zone before stress treatment (Fig. 5). Osmotic potential in the root elongating zone it dropped significantly within the initial minute and then continued to decrease gradually in all treatment groups except for the −0.13 MPa treatment group. In the −0.13 MPa treatment group, the decrease of osmotic potential stopped after one minute. The osmotic potential at hour 24 was −0.73 MPa, −1.15 MPa, and −1.77 MPa in the −0.13, −0.41 and −0.89 MPa treatment groups, respectively. In the elongated zone, the patterns of osmotic potential changes were similar to those in the elongating zone, although the net changes were much smaller. The changes in the osmotic potential in the leaf were smaller than
that observed in the root (Fig. 5). In the −0.89 MPa treatment group, the osmotic potential of the leaf began to decrease at hour 6 and it was −1.24 MPa at hour 24. In other treatments, the osmotic potential in the leaf did not change or increased about 0.2 MPa during the experimental period.

The water potential was −0.2 MPa in the leaf and −0.04 MPa in both the elongating and elongated zones of root before stress treatment (Fig. 6). Root water potential drastically decreased up to 20 minutes after the onset of stress treatments. The rate of decrease in water potential in the elongating zone was greater than that in the elongated zone. Among the three treatments the highest PEG concentration caused the greatest decrease in water potential in both zones. Changes of the water potential in the leaf were smaller than that in the root during the initial period of 20 minutes. In the leaf, only the −0.89 MPa treatment decreased the water potential compared with the control.

Turgor pressure (difference between the water potential and osmotic potential) was 0.65 MPa in the leaf, 0.47 MPa in the root elongating zone and 0.52 MPa in the root elongated zones before the stress treatment (Fig. 7). After stress treatment, however, root turgor pressure in the elongating zone was either maintained or increased by about 0.1 MPa in the −0.13 MPa treatment group during the experimental period. In the −0.41 and −0.89 MPa treatment groups on the other hand, turgor pressure decreased, and at minute 20, it was nearly 0 MPa. The decreased turgor pressure started to recover from hour 2. The recovery rate was faster in the −0.41 MPa treatment group than in the −0.89 MPa treatment group. At hour 24, turgor pressure was 0.37 MPa in the −0.89 MPa treatment group and 0.40 MPa in the −0.41 MPa treatment group. These turgor pressures were about 0.16 MPa lower than the control. The leaf turgor pressure was decreased by about 0.15 MPa during the initial one minute after the onset of stress treatment. Thereafter, leaf turgor pressure fluctuated between 0.35 and 0.65 MPa throughout the experimental period, except for hour 2 and hour 24 in the −0.89 MPa treatment group.

Discussion

1. **The root elongation under osmotic stress**

   In this study, the seminal root was fixed on the bottom of plastic box with adhesive tape, when seminal root elongation rate was measured. In our previous study, the change of whole seminal root length was measured for one day at the same growth stage in the −0.08 MPa solution (control) using the water culture system. The elongation rate did not differ from that in the present study (data not shown). Hence, we concluded that fixing the root with adhesive tape on the bottom of the box did not affect seminal root elongation.

   Root elongation was strongly affected by the water potential of external solution (Fig. 3). Seminal root elongation rate decreased within one minute after the onset of osmotic stress treatment, with a greater inhibition at higher PEG concentrations. In the −0.13 MPa treatment group, the elongation rate was similar to that of the control, despite the decline of absolute water content in root elongating zone (Fig. 4-B). Sharp et al. (1988) examined maize seminal root elongation under low water potential condition. In maize seedlings grown under −0.20 MPa in vermiculite, elongation in the elongating zone was comparable to that in the control, although the stressed root was thinner. Although we did not examine the radial changes of seminal root under osmotic stress, we speculated that root turgor pressure was maintained and consequently root elongation was sustained under the mild osmotic stress condition (−0.13 MPa treatment), despite the fact that stressed root was thinner with reduced absolute water content.

   Under −0.41 and −0.89 MPa treatments, root elongation was severely inhibited and the root shrunk substantially. When elongation rate was being measured, the seminal root at the 4 cm zone from the root tip including both the elongating and the elongated zone was fixed onto the bottom of container. It can be speculated that the shrinking was caused by the dehydration in the elongated zone of seminal root, in addition to the inhibition of elongation in the elongating zone. Kuzmanoff and Evans (1981) showed that the overall elongation of lentil root was inhibited and the root shrunk within 10 to 15 minutes after the onset of osmotic stress at −0.1 MPa to −0.5 MPa but it recovered gradually thereafter. Frensch and Hsiao (1994) also discussed root elongation and turgor pressure in root elongating zone of maize under osmotic stress condition. They monitored the change of elongation and turgor in the elongating zone and their recovery. They showed that the elongation of seminal root elongating zone was inhibited just after the start of osmotic stress treatment. The elongation was resumed only after the recovery of turgor pressure and the reduction of the yield threshold after 10 minutes of the onset of osmotic stress treatment. In this study, the inhibition of root elongation was cancelled gradually (Fig. 3) associated with the turgor recovery in root elongating zone after minute 20 (Fig. 7), the reduction of the decline rate in absolute water content in root elongated zone after minute 5 (Fig. 4) and the reduction of yield threshold by osmotic stress treatment (Frensch and Hsiao, 1994).

2. **Physiological process of osmotic adjustment**

   The osmotic potential in the root elongating zone drastically decreased within a minute after the onset of stress treatment, and the rate of decrease gradually declined thereafter. In the elongated zone,
the change in osmotic potential was similar to that in the elongating zone although the net changes were relatively smaller than in the elongating zone. On the other hand, the leaf osmotic potential slightly changed during the first two hours of the stress treatment, and increased thereafter in all treatments except for the \(-0.89\) MPa treatment.

There is only one report on the initial transient change within a few minutes of root osmotic potential in response to osmotic stress. Kuzmanoff and Evans (1981) found that 1) the whole root osmotic potential continued to decrease at a constant rate until 10 minutes after the onset of stress treatment, and thereafter kept a steady value; while 2) the osmotic potential of the root tip decreased drastically within the initial two minutes, after that kept a constant value, and decreased again after 10 minutes. These results coincide with the results obtained in this study (Fig. 5).

As described earlier, the osmotic potential is adjusted by solute accumulation and dehydration of tissues. Therefore, we evaluated the contribution of these factors to the changes in leaf and root osmotic potential. We calculated the osmotic potential changes based on the difference between the measured osmotic potential (Fig. 5) and absolute water content (Fig. 4), assuming that the decrease in osmotic potential was caused only by dehydration of the cells and tissues due to lowered external water potential (Fig. 8). The differences in osmotic potential between the control and calculated values indicate the change in osmotic potential caused by dehydration and the difference between the calculated and measured values indicates the change in osmotic potential caused by accumulation of solutes.

In the root elongating zone, the calculated osmotic potential was higher than the measured osmotic potential. This means that two factors were involved in the decrease of osmotic potential in elongating zone. One factor is dehydration of the cells, and the other is the increase of solute concentration resulting from the import of solutes into cells and/or the synthesis of solutes within the cells. It should be emphasized that the latter factor begins to function within a very short time (only one minute) after the onset of stress treatment. Our calculation indicates that 46% of osmotic potential reduction was caused by dehydration and 54% by solute accumulation at minute 20 in the condition of the \(-0.89\) MPa treatment. At hour 12, however, 7% was caused by dehydration and 93% by solute accumulation. Both solute accumulation and tissue dehydration contributed to the change of osmotic potential only in very early responses within a few minutes, and solute accumulation in later responses.

On the other hand, Sharp et al. (1990) stated in their study on the seminal root elongating zone of maize that the osmotic potential changed without osmoticum accumulation because the stressed roots became thinner by dehydration. This result was inconsistent with our results that both solute accumulation and dehydration are involved in osmotic potential reduction. Frensch and Hsiao (1994) showed that the turgor potential of maize seminal root started to recover from the inner cortex towards the outer cells. They discussed that solute movement from the stele toward the epidermis caused the osmotic adjustment and that the phloem is the source of the solutes.

In the leaf, the calculated value of osmotic potential was lower than the measured value during the initial 20 minutes. The decrease of osmotic potential may have been caused by dehydration and solute export from the tissues due to osmotic stress treatment. At hour 6, the calculated value was between the control and measured values, suggesting that osmotic adjustment began to function.

There are characteristic functions of osmotic adjustment in each organ and each part, i.e. osmotic adjustment plays a role in the root elongation in the root elongating zone (Sharp and Davies, 1979; Turner and Jones, 1980; Westgate and Boyer, 1985; Frensch and Hsiao, 1994; Serraj and Sinclair, 2002) and the maintenance of high stomatal conductance and photosynthetic rates (Henson et al., 1983; Wright et al., 1983; Düring and Dry, 1995; Nepomuceno et al., 1998; González et al., 1999), and of leaf expansion in the leaf mature zone (Hsiao et al., 1976; Westgate and Boyer, 1985). In this study, we paid special attention to the difference in the response to osmotic stress between different plant tissues and parts. We found that that osmotic adjustment by accumulation of solutes in the root began to function within much shorter time and more distinctly than that in the leaf. Therefore, these facts show that in the roots, osmotic adjustment respond to external water stress more distinctly than in leaves. In the roots, the elongating zone responded more distinctly than the other parts, mainly due to easier change in the elongation rate. Hsiao and Xu (2000) also suggested that the growth zone of roots adjusts osmotic potential rapidly after sudden reduction in water potential, whereas the leaf adjusts the potential slowly or not at all, which could be attributed to the lower sensitivity of root growth to water deficits, compared to that of leaf growth. These results implied that osmotic adjustment in the root under a water-deficit condition commenced earlier than that in the shoot, to maintain root growth and the absorption of water and nutrients, and thus to delay the occurrence of water deficit in the shoot which reduces leaf stomatal conductance and photosynthesis. However, in this study, the transient osmotic adjustment in the leaf elongating zone was not examined. Further studies are needed to elucidate the significance of osmotic adjustment at each growth...
stage of the plants under a water-deficit condition.

We also calculated turgor pressure based on the difference between water potential and osmotic potential. However, in the growing zone, which was excised to measure water potential, the water was not supplied from other parts, leading to relaxation of the cell wall (Matyssek et al., 1988). As a result, the measured value of water potential was underestimated by about 0.1 MPa compared with the water potential of intact tissue of soybean hypocotyls (Matyssek et al., 1991). In our study, although the calculated turgor pressure in the elongating zone of the root was also underestimated by about 0.1 MPa compared with the actual value, it was considered negligible to affect our conclusions.

Generally, the value of water potential in elongating zone is lower than that in the mature zone. However, in this study, there was no difference in water potential between root elongating and elongated zones in the control (Fig. 5). Rodríguez et al. (1997) reported that the value of water potential in the tissue including elongating zone was similar to that of the elongated zone in maize seminal root when water potential of the culture solution was very high, near 0 MPa. When the water potential of solution decreased, the difference between the two portions becomes apparent. Hence, it is very difficult to estimate the difference of the water potential between root elongating zone and root elongated zone at the tissue level when the water potential of the medium is near 0 MPa as in the control of this study.

Furthermore, the turgor pressure in the leaf, particularly in the mature zone (Michelena and Boyer, 1982), showed a negative value in −0.89 MPa treatment at hour 2 and hour 24 (Fig. 7). Even if the error of measurement with C-52 psychometric chamber (0.01 MPa) and the error caused by the value of zero offset (maximum 0.3 µV; 0.063 MPa at 25°C) are considered, the turgor pressure still showed negative values. In previous studies, turgor pressure of the leaf cells under a water-deficit condition calculated based on the difference between water potential and osmotic potential showed negative values (Ali et al., 1999; Bahrurn et al., 2002). Beckett (1997) also reported that turgor pressure calculated from the PV isotherm showed negative value up to −0.3 MPa. These results supported our result that turgor pressure was negative in the leaf under a severe water-deficit condition.

In summary, in maize seedling roots, the accumulation of solutes occurred and osmotic adjustment began to function within a very short time after the onset of osmotic stress, particularly in the elongating zone of the root. Owing to the rapid osmotic adjustment in the elongating zone, the roots can keep growing and absorbing water and nutrients under water stress conditions and maintain the physiological activity of the shoots.

Acknowledgement

We are grateful to Mr. Roel R. Suralta for a critical reading of this manuscript. This research was partially supported from the Japan Society for the Promotion of Science by a Grant-in Aid for Scientific Research (No. 16580015).

References

Ali, N., Jensen, C.R., Mogensen, V.O., Andersen, M.N. and Henson, I.E. 1999. Root signalling and osmotic adjustment during intermittent soil drying sustain grain yield of field-grown wheat. Field Crop Res. 62 : 35-52.
Azhiri-Sigari, T., Yamauchi, A., Kamoshita, A. and Wad, L.J. 2000. Genotypic variation in response of rainfed lowland rice to drought and rewatering. II. Root growth. Plant Prod. Sci. 3 : 180-188.
Bahrurn, A., Jensen, C.R., Asch, F. and Mogensen, V.O. 2002. Drought-induced changes in xylem pH, ionic composition, and ABA concentration act as early signals in field-grown maize (Zea mays L.). J. Exp. Bot. 53 : 251-263.
Bańoc, D.M., Yamauchi, A., Kamoshita, A., Wad, L.J. and Pardales, J.R. Jr. 2000a. Dry matter production and root system development of rice cultivars under fluctuating soil moisture. Plant Prod. Sci. 3 : 197-207.
Bańoc, D.M., Yamauchi, A., Kamoshita, A., Wad, L.J. and Pardales, J.R. Jr. 2000b. Genotypic variations in response of lateral root development to fluctuating soil moisture in rice. Plant Prod. Sci. 3 : 335-343.
Beckett, R.P. 1997. Pressure-volume analysis of a range of poikilohydric plants implies the existence of negative turgor in vegetative cells. Ann. Bot. 79 : 145-152.
Bewley, J.D. 1979. Physiological aspects of desiccation tolerance. Ann. Rev. Plant Physiol. 30 : 195-238.
Carpita, N., Sabularse, D., Montezinos, D. and Delmer, D.P. 1979. Determination of the pore size of cell walls of living plant cells. Science 205 : 1144-1147.
Düring, H. and Dry, P.R. 1995. Osmoregulation in water stressed roots: responses of leaf conductance and photosynthesis. Viitas 34 : 15-17.
Flower, D.J. and Ludlow, M.M. 1986. Combination of osmotic adjustment to the dehydration tolerance of water-stressed pigeonpea (Cajanus cajan (L.) mill sp) leaves. Plant Cell Environ. 13 : 33-40.
Frensch, J. and Hsiao, T.C. 1994. Transient responses of cell turgor and growth of maize roots as affected by changes in water potential. Plant Physiol. 104 : 246-254.
Frensch, J. and Hsiao, T.C. 1995. Rapid response of the yield threshold and turgor regulation during adjustment of root growth water stress in Zea mays. Plant Physiol. 108 : 303-312.
González, A., Martín, I. and Ayerbe L. 1999. Barley yield in water-stress conditions. The influence of precocity, osmotic adjustment and stomatal conductance. Field Crops Res. 62 : 29-34.
Greacen, E.L. and Oh, J.S. 1972. Physics of root growth. Nat. New Biol. 235 : 243-35.
Henson, I.J., Alagarswamy, G., Mahalakshmi, V. and Bidinger, F.R. 1983. Stomatal response to water stress and its relationship to bulk leaf water status and osmotic adjustment.
in pearl millet (*Pennisetum americanum* (L.) Leeke). J. Exp. Bot. 34 : 442-450.

Hsiao, T.C., Acevedo, F., Fereres, E. and Henderson, D.W. 1976. Stress metabolism, water stress, growth, and osmotic adjustment. Philosophical Transaction of the Royal Society, London, Series B 273 : 479-500.

Hsiao, T.C. and Xu, L.K. 2000. Sensitivity of growth of root versus leaves to water stress: biophysical analysis and relation to water transport. J. Exp. Bot. 51 : 1595-1616.

Kaiser, W.M. 1982. Correlation between changes in photosynthetic activity and changes in total protoplast volume in leaf tissue from hygro-, meso- and xerophytes under osmotic stress. Planta 154 : 538-545.

Kuzmanoff, K.M. and Evans, M.L. 1981. Kinetics of adaptation to osmotic stress in lentil (*Lens culinaris* Med.) roots. Plant Physiol. 68 : 244-247.

Matyssek, R., Maruyama, S. and Boyer, J.S. 1988. Rapid wall relaxation in elongating tissues. Plant Physiol. 86 : 1163-1167.

Matyssek, R., Maruyama, S. and Boyer, J.S. 1991. Growth-induced water potential may mobilize internal water for growth. Plant Cell Environ. 14 : 917-923.

Michelena, V.A. and Boyer, J.S. 1982. Complete turgor maintenance at low water potentials in the elongating region of maize leaves. Plant Physiol. 69 : 1145-1149.

Nepomuceno, A.L., Oosterhuis, D.M. and Stewart, J.M. 1998. Physiological responses of cotton leaves and roots to water deficit induced by polyethylene glycol. Environ. Exp. Bot. 40 : 29-41.

Ober, E.S. and Sharp, R.E. 2003. Electrophysiological responses of maize roots to low water potentials: relationship to growth and ABA accumulation. J. Exp. Bot. 54 : 813-824.

Radin, J.W. 1983. Physiological consequences of cellular water deficit: Osmotic adjustment. In H.M. Taylor, W.R. Jordan and T.R. Sinclair eds., Limitations to Efficient Water Use in Crop Production. American Society of Agronomy, Madison. 267-276.

Raymond, M.J. and Smirnoff, N. 2002. Proline metabolism and transport in maize seedlings at low water potential. Ann. Bot. 89 : 813-823.

Rodríguez, H.G., Roberts, J.K.M., Jordan, W.R. and Drew, M.C. 1997. Growth, water relations, and accumulation of organic and inorganic solutes in roots of maize seedlings during salt stress. Plant Physiol. 113 : 881-893.

Santakumari, M. and Berkowitz, G.A. 1991. Chloroplast volume: cell water potential relationships and acclimation of photosynthesis to leaf water deficits. Photosyn. Res. 28 : 9-20.

Serraj, R. and Sinclair, T.R. 2002. Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant Cell Environ. 25 : 333-341.

Sharp, R.E. and Davies, W.J. 1979. Solute regulation and growth by roots and shoots of water-stressed maize plants. Planta 147 : 43-49.

Sharp, R.E., Silk, W.K. and Hsiao, C.T. 1988. Growth of the maize primary root at low water potentials. I Spatial distribution of expansive growth. Plant Physiol. 87 : 50-57.

Sharp, R.E., Hsiao, C.T. and Silk, W.K. 1990. Growth of the maize primary root at low water potentials. II Role of growth and deposition of hexose and potassium in osmotic adjustment. Plant Physiol. 93 : 1337-1346.

Sharp, R.E., Poroyko, V., Hejlek, L.G., Spollen, W.G., Springer, G.K., Bohnert, H.J. and Nguyen, H.T. 2004. Root growth maintenance during water deficits: physiology to functional genomics. J. Exp. Bot. 55 : 2343-2351.

Turner, N.C. and Jones, M.M. 1980. Turgor maintenance by osmotic adjustment: A review and evaluation. In N.C. Turner and P.J. Kramer eds., Adaptation of Plant to Water and High Temperature Stress. John Wiley & Sons Inc., New York. 87-103.

Verslues, P.E., Ober, E.S. and Sharp, R.E. 1998. Oxygen relations and root growth at low water potentials. Impact of oxygen availability in polyethylene glycol solutions. Plant Physiol. 116 : 1403-1412.

Voetberg, G.S. and Sharp, R.E. 1991. Growth of the maize primary root at low water potentials. III. Role of increased proline deposition in osmotic adjustment. Plant Physiol. 96 : 1125-1130.

Westgate, M.E. and Boyer, J.S. 1985. Osmotic adjustment and the inhibition of leaf, root, stem, and silk growth at low water potentials in maize. Planta 164 : 540-549.

Wright, G.C., Smith, R.C.G. and Morgan, J.M. 1983. Differences between two grain sorghum genotypes in adaptation to drought stress. III. Physiological responses. Aust. J. Agri. Res. 34 : 637-651.