Root Osmotic Adjustment under Osmotic Stress in Maize Seedlings

2. Mode of Accumulation of Several Solutes for Osmotic Adjustment in the Root

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Abstract: The changes in the accumulation of compatible solutes in the seminal root and leaves of maize were examined under four osmotic stress conditions to elucidate the expression pattern of osmotic adjustment. 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 using polyethylene glycol 6000. Segments were obtained from the elongating zone of the root, elongated zone of the root and mature part of leaves. The concentrations of potassium ion and some amino acids under osmotic stress began to increase within 20 minutes after the onset of stress treatment in the root elongating zone. From hour 2, reducing sugars contributed mainly to the change of osmolarity. The amino acids that responded most quickly to the osmotic-stress treatment were Asp, Glu, Asn and Thr, which started to increase within 20 minutes, followed by Ser and Ala, which increased from hour 2 to 6, and Pro which increased from hour 12 to 24. Moreover, in the roots at hour 24, the proportion of potassium ion to total solutes decreased with the increase in the stress intensity. The proportion of amino acids to total solute in the root elongating zone was higher in the -0.13 MPa treatment group and that of the reducing sugars in the root elongated zone was higher in the -0.13 MPa and -0.41 MPa treatment groups than in the -0.08 and -0.89 MPa treatment groups. These results suggested that the kinds of solutes that contributed to osmotic adjustment differed depending on the duration and intensity of osmotic stress.

Key words: Amino acid, Osmotic adjustment, Osmotic stress, Potassium ion, Reducing sugars, Root, Zea mays L.

In osmotic adjustment, a mechanism for tolerating osmotic stress, the osmotic potential is decreased by net accumulation of solutes. There have been many reports about solutes related to osmotic adjustment. The kinds of solutes reported so far are ions, mainly potassium ion (Itoh et al., 1986; Itoh et al., 1987; Morgan, 1992; Gnanasiri et al., 1995), sugars (Jones et al., 1987; Wang et al., 1995; Yakushiji et al., 1996), and amino acids, mainly proline (Dingkuhn et al., 1991; Voetberg and Sharp, 1991; Morgan, 1992; Ober and Sharp, 1994; Verslues and Sharp, 1999). These solutes are called "compatible solutes" and do not interfere with normal metabolic functions even at higher concentrations. Gnanasiri et al. (1995) showed that a drought-tolerant grain sorghum line had higher capacity to accumulate potassium ion and maintained a relatively higher content and turgor pressure under the stress condition than a drought-susceptible line. Yakushiji et al. (1996) showed that the concentrations of sucrose, glucose and fructose in fruit sap increased under a water stress condition for osmotic adjustment in Satsuma mandarin (Citrus unshiu Marc.). Voetberg and Sharp (1991) and Sharp et al. (1988, 1990) showed that osmotic adjustment occurred due to increased proline deposition in the primary root growth zone in maize seedling, and it played an important role in the maintenance of root elongation at the low water potentials. Yoshiba et al. (1997) reviewed the regulation of proline content under water stress.

Energy is needed for the syntheses or transport of solutes for osmotic adjustment (Munns, 2002). Taking into consideration energy efficiency, it is predicted that the accumulation of ions, which is not needed in the metabolism and is of low molecular weight, is efficient for the osmotic adjustment, and that the ions can be accumulated quickly in response to osmotic stress (Yeo, 1983; Raven, 1985). However, the excessive accumulation of ions may disrupt the balance of the absorption and the function of other ions in the cell. When massive accumulation of compatible solute is needed under strong osmotic stress, it is predicted that sugars and amino acids, which do not affect the metabolism although they are high molecular weight and need to be synthesized, are used as compatible solutes. Thus, the relative contribution of each solute to osmotic adjustment would vary with the stress intensity.

It is hypothesized that the degree of contribution of each solute to osmotic adjustment would vary with the time after the onset of stress treatment and...
stress intensity. Most of the previous studies evaluated osmotic adjustment by measuring osmotic potential and concentrations of solutes at fixed times after the onset of stress treatment. The change in the concentration of ions under the stress condition was examined in the order of hour (Rodrígues et al., 1997), that of sugar content in the order of hour (Rodrígues et al., 1997) and month (Yakushiji et al., 1996), and that of proline concentration in the order of hour (Verslues and Sharp, 1999), the information about the initial rapid changes in the concentrations of these solutes and their contribution of osmotic stress under different intensities were not examined (Frensch and Hsiao, 1994).

In this study, we examined the change in the accumulation of solutes including potassium ion, reducing sugars and amino acids, in maize under osmotic stress. The measurements were done in a wide time range of minutes to hours after the onset of stress. Significant differences in the response to osmotic stress may exist between plant tissues and parts, due largely to specific functions of osmotic adjustment in each organ and each part. Hence, we compared the transient processes of osmotic adjustment in root elongating zone, root elongated zone and leaf mature zone.

**Materials and methods**

Seeds of *Zea mays* L. (cv. White Pop) were germinated, transplanted onto nets floating on nutrient solution, and grown for 48 hours, as previously described (Ogawa and Yamauchi, 2006) and then transferred to PEG solutions for osmotic stress treatment. 0 (control), 50, 200 and 300 g of polyethylene glycol 6000 (PEG) (CaHC Co., Ltd, Japan) per liter was dissolved in the nutrient solution to induce osmotic stress. The water potential of the nutrient solutions determined using a vapor pressure osmometer (model 5300, Wescor), and was −0.08, −0.13, −0.41 and −0.89 MPa, respectively. After 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 analysis of solutes, the segments of the second leaf at 10 mm from the tip (leaf mature zone) (Michelena and Boyer, 1982) and seminal roots at 0 −6 mm from the tip (root elongating zone) and 14−20 mm from the tip (root elongated zone) (Ogawa and Yamauchi, 2006) were excised. At the sampling, the third leaf was just emerging. Five leaf segments or 16 root segments were sealed in a plastic tube and plunged into liquid N₂. The samples were stored in a freezer at −30°C for measurement. The frozen tissue samples were thawed for over 30 minutes at room temperature, and homogenized with a glass rod. Then the samples were centrifuged for 20 minutes at 11,000 g. The 1/5000 dilutions of the extracted cell sap were used to determine concentrations of potassium ion and reducing sugars.

The concentration of potassium ion was measured using an atomic absorption spectrophotometer (Shimadzu type AA-6400) by flame photometry using KCl as the standard. Reducing sugars were assayed by the improved neopropin method (Chaplin and Kennedy, 1995). Forty g of sodium carbonate, 16 g of glycine and 450 mg of copper (II) sulfate pentahydrate were dissolved in one liter of water (Solution A), and 150 mg of neopropin hydrochloride monohydrate was dissolved in 100 ml of water (Solution B). Two ml each of the 1/5000 dilutions of the extracted cell sap, Solution A and Solution B were mixed and heated at 100°C for 13 minutes and then cooled on ice. The increase in A 340 of the solution was recorded using a UV-VIS spectrophotometer (Shimadzu type UV mini1240), and the concentration of reducing sugars was calculated using D-glucose as the standard. The concentration of reducing sugars did not include the sucrose concentration. Sucrose was assumed to be the source of reducing sugars (Sharp et al., 1990), but to check whether sucrose affects the change of osmotic potential, sucrose concentration was measured separately.

Three leaf or ten root segments were sampled by the same method as that for potassium and reducing sugars measurement. For amino acid and sugar analyses, these samples were extracted with ethanol. Protein in the extracts was removed by 0.1N HCl and chlorophyll by diethyl ether. Amino acids were analyzed by ninhydrin method, using an amino acid analyzer (JEOL type 500/v). Glucose, fructose and sucrose were analyzed using a Shimadzu HPLC system composed of an isocratic pump (LC-10AD), a refractive index detector (RID-6A) and a column (Shim-pack CLC-NH2 (M)). Three replications were used for each measurement of solutes.

**Results**

1. **The transient change in the accumulation of solutes**

Fig. 1 shows the changes in the concentrations of potassium ion (Fig. 1-A), reducing sugars (Fig.1-B), and total amino acids (Fig.1-C) in root elongating zone and leaf during the osmotic stress treatments.

In the root elongating zone, the potassium ion concentration responded to all the treatments within 1 minute. The potassium ion concentrations in the three stress treatments were 20-30 mM higher than that in the control throughout the course of measurements. The concentration of reducing sugars did not change until hour 2 and increased thereafter. The degree of increase in the concentration of reducing sugars corresponded to the intensity of stress. The
The concentration of total amino acids began to increase from minute 10 and markedly increased from hour 2 in the −0.41 MPa and −0.89 MPa treatment groups, but not in the −0.13 MPa treatment group.

Although the pattern of change in solute concentration in the elongated zone of root were similar to those in the elongating zone, the rate of increase of each solute concentration was lower in the elongated zone than in the elongating zone (data not shown).

In the leaf, the response to osmotic stress was slower compared with that in the root. The concentration of potassium ion in the control did not differ from that in the stressed leaf, except for the −0.89 MPa at hour 24. The concentrations of reducing sugars in the leaf increased by about 20 mM at minute 1, in the −0.41 MPa and −0.89 MPa treatment groups compared with the control. The concentration of total amino acids in the leaf in the −0.89 MPa began to increase after hour 2, although the rate of increase was lower than that in the root. The concentration in the −0.89 MPa treatment group was about 40 mM higher than the control value at hour 24. The other treatments did not have marked effects on the amino acid concentration.

The sucrose concentration measured separately, was lower than that of the measured glucose (data not shown), which agreed with those reported by Sharp et al. (1990). Therefore, we assumed that the sucrose concentration did not affect osmotic potential.

2. The contribution of each solute to osmotic adjustment

Fig. 2 shows the concentrations of total solutes and component solutes in the root elongating zone and leaf in stress treatments relative to those in the control (−0.08 MPa).

In the root elongating zone, the total solute concentration at minute 20 increased linearly with increasing intensity of osmotic stress (Fig. 2-A). The concentration of potassium ion was about 15 mM higher than that in the control in all treatments while the concentration of reducing sugars in −0.13 and −0.41 MPa treatment groups was lower than that in the control, and that in the −0.89 MPa treatment group was nearly the same as that in the control. Thus, the potassium ion accounted for 8%, reducing sugars 1% and total amino acids 2% of the total solute concentration in −0.89 MPa treatment groups. At hour 24, the concentrations of total solutes increased linearly with increasing stress strengths. Reducing sugars mainly contributed to the increase of total solute concentrations in all treatments (Fig. 2-C). In addition, the concentrations of potassium ion and total amino acids in −0.13 and −0.41 MPa treatment groups were higher than those in the control. In the −0.89 MPa treatment group, the potassium ion accounted for 19%, reducing sugars 35% and total amino acids 12%
of the total solute concentration.

In the leaf, total solute concentrations were not affected by osmotic stress treatment at minute 20 (Fig. 2-B). At hour 24 however, total solute concentration increased with increasing osmotic stress intensity (Fig. 2-D). The rate of increase of total solute concentration was primarily associated with the increase in the concentrations of reducing sugars particularly in the −0.41 and −0.89 MPa treatment groups, although other solutes also substantially contributed to the change of osmolarity in the −0.89 MPa treatment group.

3. The changes of each amino acid concentration

Table 1 shows the changes of each amino acid concentration in the root elongating zone during the stress treatment. The amino acid that responded most rapidly to the osmotic stress treatment was Asp in all treatment groups, with an increase within 20 minutes, followed by Ser, Glu, Ala, and Asp (in the −0.41 and −0.89 MPa treatment groups) that increased from hour 2 to 6, and Pro (in the −0.13 and −0.89 MPa treatment groups) that increased from hour 12 to 24. The sum amount of Ala, Asn and Pro accounted for 58% of the total amount of increased amino acids at hour 24 in the −0.89 MPa treatment (47.6 mM) group. Thus, these three amino acids mainly contributed to the increase of total amino acids concentration at hour 24 in the −0.89 MPa treatment group. The concentrations of Ala, Asp and Pro increased 11.2, 20.4 and 7.7 mM, respectively, during a 24-hour stress treatment. In the root elongated zone, the amino acids which mainly increased were Ala and Asn, in all treatments, and these increases were observed 2−6 hours after the onset of stress treatment (data not shown).

The increase in the concentrations of amino acids (Ser, Ala and Asn) in the leaf was smaller than that in the root (data not shown). At hour 24, particularly in the −0.89 MPa treatment group, the concentrations of Ser, Ala and Asn were 3.0, 7.1 and 2.6 mM, respectively, which were higher than those before stress treatment. The total amount of these three amino acids accounted for 56% of the total amount of
Table 1.  The transient change of the concentrations (mM) of each amino acid and total amino acids concentration in root elongating zone during 24 hours after stress treatments. Data are means of 3 replications.

|                   | Control Time after the onset of stress treatment | −0.41MPa treatment Time after the onset of stress treatment | −0.13MPa treatment Time after the onset of stress treatment | −0.89MPa treatment Time after the onset of stress treatment |
|-------------------|-------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|
|                   | 0min. 20min. 2h 6h 12h 24h                      | 0min. 5min. 20min. 2h 6h 12h 24h                        | 0min. 5min. 20min. 2h 6h 12h 24h                        | 0min. 5min. 20min. 2h 6h 12h 24h                        |
| Asp               | 3.76 3.77 3.79 3.45 3.62 2.77                    | 3.76 4.22 6.64 5.00 4.57 2.73                            | 3.76 4.83 5.14 3.58 5.69 3.99                            | 3.76 4.83 5.14 3.58 5.69 3.99                            |
| Glu               | 3.70 3.90 4.09 4.45 4.07 3.33                    | 3.70 3.69 4.00 5.53 8.12 4.89                            | 3.70 4.91 3.70 6.19 8.21 5.30                            | 3.70 4.91 3.70 6.19 8.21 5.30                            |
| Thr               | 4.17 4.15 4.45 4.33 4.17 3.22                    | 4.17 4.44 6.97 5.68 7.03 3.30                            | 4.17 5.04 4.58 5.36 4.13 2.37                            | 4.17 5.04 4.58 5.36 4.13 2.37                            |
| Asn               | 4.58 4.45 3.20 4.30 3.91 2.23                    | 4.58 3.83 5.13 6.42 9.69 5.86                            | 4.58 5.08 5.27 4.13 15.37 17.56                          | 4.58 5.08 5.27 4.13 15.37 17.56                          |
| Ala               | 3.15 3.09 3.36 3.78 3.24 2.75                    | 3.15 3.29 4.09 4.92 9.12 6.04                            | 3.15 3.81 3.58 6.50 11.29 9.18                           | 3.15 3.81 3.58 6.50 11.29 9.18                           |
| Ser               | 2.32 2.30 2.44 2.38 2.83 2.16                    | 2.32 2.04 2.82 3.12 5.54 4.14                            | 2.32 1.95 2.88 2.14 3.25 3.98                           | 2.32 1.95 2.88 2.14 3.25 3.98                           |
| Pro               | 0.66 0.66 0.53 0.92 0.58 0.25                    | 0.66 0.73 1.04 0.97 3.23 1.95                            | 0.66 0.65 0.56 0.79 1.06 0.19                           | 0.66 0.65 0.56 0.79 1.06 0.19                           |
| Gly               | 0.75 0.75 0.78 0.65 0.79 0.42                    | 0.75 0.56 0.82 0.88 1.40 0.50                            | 0.75 0.56 0.82 0.88 1.40 0.50                            | 0.75 0.56 0.82 0.88 1.40 0.50                            |
| Cys               | 0.04 0.04 0.04 0.04 0.04 0.03                    | 0.04 0.04 0.05 0.04 0.06 0.06                            | 0.04 0.04 0.05 0.04 0.06 0.06                            | 0.04 0.04 0.05 0.04 0.06 0.06                            |
| Val               | 1.57 1.50 1.48 1.89 1.54 1.65                    | 1.57 1.58 2.24 2.21 3.59 2.12                            | 1.57 1.58 2.24 2.21 3.59 2.12                            | 1.57 1.58 2.24 2.21 3.59 2.12                            |
| Met               | 0.07 0.08 0.09 0.11 0.06 0.08                    | 0.07 0.09 0.14 0.08 0.12 0.09                            | 0.07 0.09 0.14 0.08 0.12 0.09                            | 0.07 0.09 0.14 0.08 0.12 0.09                            |
| Ile               | 0.64 0.60 0.57 0.93 0.81 1.03                    | 0.64 0.77 0.99 0.90 1.40 0.92                            | 0.64 0.77 0.99 0.90 1.40 0.92                            | 0.64 0.77 0.99 0.90 1.40 0.92                            |
| Leu               | 1.08 1.10 1.07 1.67 1.70 2.27                    | 1.08 1.50 1.65 1.76 2.11 1.53                            | 1.08 1.50 1.65 1.76 2.11 1.53                            | 1.08 1.50 1.65 1.76 2.11 1.53                            |
| Tyr               | 0.57 0.55 0.54 0.67 0.68 0.53                    | 0.57 0.59 0.82 0.68 1.24 0.68                            | 0.57 0.59 0.82 0.68 1.24 0.68                            | 0.57 0.59 0.82 0.68 1.24 0.68                            |
| Phe               | 0.74 0.77 0.69 0.81 0.77 0.71                    | 0.74 0.79 1.07 0.99 1.64 0.86                            | 0.74 0.79 1.07 0.99 1.64 0.86                            | 0.74 0.79 1.07 0.99 1.64 0.86                            |
| His               | 1.58 1.56 1.40 1.70 1.77 1.18                    | 1.58 1.11 1.94 1.63 2.77 1.48                            | 1.58 1.11 1.94 1.63 2.77 1.48                            | 1.58 1.11 1.94 1.63 2.77 1.48                            |
| Lys               | 0.07 0.07 0.09 0.10 0.07 0.05                    | 0.07 0.07 0.11 0.08 0.16 0.07                            | 0.07 0.07 0.11 0.08 0.16 0.07                            | 0.07 0.07 0.11 0.08 0.16 0.07                            |
| Arg               | 0.07 0.07 0.04 0.11 0.10 0.06                    | 0.07 0.10 0.12 0.10 0.13 0.10                            | 0.07 0.10 0.12 0.10 0.13 0.10                            | 0.07 0.10 0.12 0.10 0.13 0.10                            |
| Total             | 29.47 29.36 28.63 32.31 30.76 24.73             | 29.47 29.42 41.07 40.98 61.92 37.33                       | 29.47 29.56 39.90 39.18 66.46 59.88                       | 29.47 29.56 39.90 39.18 66.46 59.88                       |
increased amino acids (23 mM). Other kinds of amino acids increased neither in the root nor leaf during the course of measurements.

**Discussion**

1. **The relation between solute concentration and stress-treatment duration**

In this study, we measured the solute concentrations at various times after the onset of stress imposition in the order of minutes to hours, paying special attention to the difference in the response to osmotic stress between plant tissues and parts. The results showed that the same kinds of solutes accumulated more rapidly in the root than in the leaf, but the time required for accumulation varied with the kind of solute (Fig. 1). In the expression process of osmotic adjustment, the time course of solute accumulation, namely the reaction time, varied with the solute. These results supported our hypothesis that the degree of contribution of each solute for osmotic adjustment would vary with the time after the onset of stress treatment. At the onset of osmotic stress, the potassium ion and several kinds of amino acids contributed largely to the root osmotic adjustment since they are readily available and easily transported from cell to cell. However, the accumulation of these solutes should be limited, since excessive accumulation of these solutes may interfere with enzymatic function and normal metabolic activity. Reducing sugars and some kinds of amino acids, which are considered as the compatible solutes and will not interfere with normal metabolic function, accumulated from hour 2. Trouverie et al. (2004) reported the existence of a time lag between the increase of ABA concentration and accumulation of sugars. In maize, the vacuole invertase activity reached the maximum at 4 hours in roots and at 8 hours in leaves after ABA treatment, and the hexoses content increased after 8 hours.

Proline, one of the major compatible solutes, accumulated slowly under osmotic stress as compared with other amino acids (Dingkuhn et al., 1991; Voetberg and Sharp, 1991; Morgan, 1992; Ober and Sharp, 1994). Hare and Cress (1997) indicated that under a water stress condition, proline is synthesized in “effector cells” and transported to the “target tissue”, namely elongating zone, using energy. Furthermore, Verslues and Sharp (1999), using a tracer method, showed that proline accumulation under stress was caused not by the increase of proline synthesis nor decrease of proline catabolism, but rather by the increase of transported proline. Frensch and Hsiao (1994) showed that solute movement from the stele toward the epidermis caused the osmotic adjustment and phloem was the primary source of the solutes. These findings suggest that the response of proline to osmotic stress may not occur in the order of minutes.

Our results showed that the increment of the proline concentration in the root elongating zone was first detected at hour 24 in −0.13 and −0.89 MPa treatment groups (Table 1). In the experiment of Verslues and Sharp (1999), proline accumulation rate increased distinguishably from hour 15 to 35 under −1.6 MPa osmotic stress treatment, and the increase continued until hour 60. Although the proline concentration in our experiment was low and did not play a significant role in osmotic adjustment as a compatible solute, its concentration is expected to increase and contribute to osmotic adjustment when the experimental period

| Water potential of external solution (MPa) | −0.08MPa | −0.13MPa | −0.41MPa | −0.89MPa |
|------------------------------------------|----------|----------|----------|----------|
| Root elongating zone                     |          |          |          |          |
| Potassium ion (%)                        | 46.54    | 43.93    | 24.09    | 19.40    |
| Reducing sugars (%)                      | 32.77    | 38.51    | 36.05    | 34.46    |
| Total amino acids (%)                    | 10.55    | 15.78    | 7.17     | 11.99    |
| Root elongated zone                      |          |          |          |          |
| Potassium ion (%)                        | 53.50    | 31.74    | 27.10    | 23.68    |
| Reducing sugars (%)                      | 35.66    | 47.87    | 45.89    | 35.52    |
| Total amino acids (%)                    | 11.14    | 12.96    | 1057     | 1029     |
| Leaf                                     |          |          |          |          |
| Potassium ion (%)                        | 46.37    | 34.28    | 33.98    | 32.73    |
| Reducing sugars (%)                      | 44.10    | 45.70    | 56.70    | 49.55    |
| Total amino acids (%)                    | 6.19     | 10.63    | 5.82     | 8.93     |
The potassium concentration in the root elongating zone and mature part of leaf began to increase from minute 20 to hour 12 when light was turned on in all the treatments (Fig. 1-A). Similar results have been reported by other researchers. For example, the rate of uptake of potassium ion in timothy and meadow fescue that were grown in the dark increased when light was turned on (Macduff et al., 1997). Mühling and Läuchli (2000) examined the light-induced change of potassium ion concentration in the apoplast of the intact leaves of *Vicia faba* and *Helianthus annuus*. The apoplastic K⁺ concentration in the intact leaves declined during the light period, which indicates that K⁺ uptake by leaf cells was induced by light.

Further studies on the leaf growing zone will be needed to understand the physiological process of osmotic adjustment in plant.

2. The relation between solute concentration and stress intensity

Table 2 shows the percentage of each solute concentration to the total solute concentration at hour 24 in the root elongating zone, root elongated zone, and leaf mature part calculated from the data shown in Fig. 1. In both root elongating and elongated zones, the proportion of potassium ion to the total solutes decreased with the increase in the stress intensity. However, the proportions of total amino acids in the root elongating zone was higher in the −0.13 MPa treatment group than in the other treatment groups and that of the reducing sugars in the root elongated zone was higher in the −0.13 and −0.41 MPa treatment groups than in other treatment groups. In the leaf mature part, the proportion of potassium ion to that in the control was higher than that of reducing sugars in all treatment groups and the proportions of total amino acids in −0.13 and −0.89 MPa treatment groups were higher than in the control. These facts show that the composition of solutes contributing to osmotic adjustment substantially varies with the stress intensity.

These results confirmed our hypothesis that the degree of contribution of each solute to osmotic adjustment would vary with the stress intensity. Taking into consideration the energy budget for osmotic adjustment, the accumulation of potassium ion is efficient for the osmotic adjustment under low osmotic stress, because it need not be synthesized and is easily transported due to low molecular weight. However, the over accumulation of potassium ion may affect the balance of the absorption and the metabolism of other cations in the cells because of its effect on enzymatic functions. Under such conditions, the accumulation of compatible solutes (i.e. sugars and amino acids) to counteract the strong effect of osmotic stress is needed. Such compatible solutes must be synthesized by the cell and have a high molecular weight but do not interfere with normal metabolic functions even at higher concentrations.

We found that mild water stress, such as −0.13 MPa treatment, affected solute accumulation and water relations (Ogawa and Yamauchi, 2006) in maize seminal root, as compared with the control (−0.08 MPa). Büssis and Heineke (1998) also showed that the amino acid concentration of young leaf of *Solanum tuberosum* was increased by osmotic stress induced by adding 10% (w/v) polyethyleneglycol 6000 (approximately −0.2 MPa) to external hydroponics solution. The concentration of ABA in maize root increased in the same strength of osmotic stress treatment (Jia et al., 2001). Moreover, the morphology of root system was changed by the mild drought stress (Kramer and Boyer, 1995). These results indicate that physiological and morphological changes in root can also be induced by mild osmotic stress treatment.

The sum of the concentrations of potassium, reducing sugars and total amino acids differs from the total solute concentrations calculated from the osmotic potential (Ogawa and Yamauchi, 2006). The difference at minute 20 was 0.5 mM, 28, 124, and 176 mM in the control, −0.13 MPa, −0.41 MPa and −0.89 MPa treatment groups, respectively. At hour 24, the difference was 24, 6, 145 and 234 mM in the control, −0.13 MPa, −0.41 MPa, and −0.89 MPa treatment groups, respectively. Several studies on the change of osmotic potential and solute concentrations under osmotic stress conditions are available although most of them showed only the change of either osmolarity or the concentration of solutes. In other words, there are a few studies showing the concentration of unmeasured solutes calculated from the difference between the total solute concentration and the sum of the concentrations of measured solutes (Voetberg and Sharp, 1991). Thus, the transient changes in the concentrations of the remaining solutes are not yet known. It is predicted that various ions, sugar alcohols, organic acids and so on, are involved, but no information from the past study is available, with which we could evaluate the accuracy of our results.

In conclusion, we found that the root responds rapidly to osmotic stress treatments through osmotic adjustment. The response was more rapid and more pronounced in the root than in the leaf, particularly in the elongating zone than in the elongated zone. Early osmotic adjustment depends on the free potassium ions and some kind of amino acids. The compatible solutes like sugars and amino acids accumulate slowly. The difference between the rapid and slow accumulation of compatible solutes in response to osmotic stress may be attributed to the differences may be caused by the difference in the processes of synthesis and/or transport of the solutes under the stress conditions.
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