Sugar Accumulation along the Seminal Root Axis, as Affected by Osmotic Stress in Maize: A Possible Physiological Basis for Plastic Lateral Root Development

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Abstract: We assumed that allocation of photosynthate is one of the main factors that regulate lateral root development in root systems under water deficit conditions. Six-days-old maize seedlings were exposed to osmotic stress (-0.21 MPa). Then, the sugar content of the 10 mm segment of seminal roots sampled successively along the root axis was examined in relation to the development and elongation of lateral roots at 0, 1 and 2 after the start of the stress treatment. In the basal 0-40 mm region of the seminal root, lateral roots emerged before the stress treatment, but further initiation and elongation of lateral roots in this region was inhibited by the stress treatment. In contrast, in the region more than 40 mm distant from the base of the seminal root, lateral roots emerged after the start of stress treatment and their development was not influenced or was slightly promoted by the stress treatment as compared with the control plant. The concentrations of glucose and sucrose were determined for every 10 mm segment along seminal root axis. The concentrations of glucose and sucrose tended to increase acropetally, and were increased by the stress treatment, especially in the apical 50-mm portions in both roots sampled after 1 day and 2 days of the stress treatment. On these root portions, while lateral roots had not yet developed when the root was sampled, lateral roots were initiated and they elongated one or two days later. These facts suggest that the accumulation of glucose and sucrose promoted lateral root development, and as a result, canceled the inhibitory effects of stress treatment, and thereby sustained lateral root development under osmotic stress conditions.

Key words: Elongation, Glucose, Initiation, Lateral root, Osmotic stress, Plasticity, Sucrose, Zea mays.

Plants change their morphology to adjust to changes in their growth conditions. Therefore, their morphology is dramatically influenced by environmental signals (Malamy and Ryan, 2001). The growth and development of the plant root system is an excellent example of this developmental plasticity (O’Toole and Bland, 1987). Since the environment of the root system is highly inconstant and heterogeneous both in time and space, it would appear essential that root systems would have the ability to react to the changes in environment and to the heterogeneity; in other words, they should possess phenotypic plasticity (Fitter, 1996). The expression of plasticity under water deficit conditions is highly affected by the initiation and elongation of lateral roots because the plant root system consists largely of many lateral roots. Therefore, it is important to examine the developmental and physiological mechanisms controlling lateral root initiation and elongation under various conditions in order to understand the basis for the expression of plasticity.

There are a number of studies about the change in lateral root development, which takes place under non-uniform nutrient conditions. Under nutrient-limited conditions, lateral roots commonly proliferate in zones of ammonium, nitrate, phosphate and magnesium enrichment, but not in response to potassium (Drew, 1975; Scott and Robson, 1991; Brouder and Cassman, 1994; Robinson, 1994). The changes in lateral root development were triggered by changes in water status in the root zone, and these developmental changes were induced by genetic (Weaver and Zank, 1955; Hunt et al., 1987) and environmental factors. With regard to the environmental factors, Bañoc et al. (2000) showed that phenotypic plasticity in promoted lateral root development and that nodal root production was the key trait that ensured stable growth of rice plants grown under changing soil moisture levels. Taylor and Klepper (1974) reported that young cotton plants developed much deeper root systems under conditions of frequent superficial application of water to surface soil than those grown with sufficient water. This type of promotion of rooting depth by surface irrigation was reported for lupin (Devries et al., 1989;
Rodrิงguees et al., 1995), soybean (Finn and Brun, 1980; Mason et al., 1982; Hoogenboom et al., 1987), maize (Robertson et al., 1980; Sharp and Davies, 1985; Newell and Wilhelm, 1987), lettuce (Gallardo et al., 1996) and sorghum (Burch et al., 1978). These results showed that the change in lateral root development, i.e. in the plasticity of the root system, exhibited under water deficit conditions may play an important role in drought tolerance (Yamauchi et al., 1996). From an agronomical view, the knowledge about the lateral root development is useful for breeding of varieties with drought tolerance.

There are many studies on the relationship between drought stress and seminal root elongation, and it is considered that the turgor pressure affect the cell elongation and growth of plant (Lockhart, 1965). Frencш and Hsiao (1994) discussed the root elongation and turgor pressure in elongating zone of seminal root in maize seedlings under osmotic stress condition. They monitored the change of elongation in root elongation zone and the presses of turgor loss and recovery. For the turgor maintenance in the elongating zone of seminal root in maize seedlings, it is reported that osmotic adjustment plays an important role (Sharp et al., 1988; Sharp et al., 1990; Voetberg, and Sharp, 1991; Ogawa et al., 1996; Ogawa and Yamauchi, 1999; Verslues and Sharp, 1999). However, the mechanism of development of lateral roots is not yet fully understood. Many previous studies showed that auxin substantially affects the development of lateral roots (Blakely et al., 1982; MacIsaac et al., 1989; Malamy and Benfey, 1997; Lloret and Casero, 2002). However, auxin is a type of phytohormone, and only has a role as a signal that triggers the development of lateral root primordia.

According to the Sattelmacher and Tomas hypothesis (1989, 1991), proliferation of lateral roots occurs in response to an increase in phloem import generated by an increase in sink demand for carbohydrates. They argued that an increase in respiratory activity, and consequently in sink demands for carbohydrate, would increase lateral root initiation. Bingham et al. (1997, 1998) studied the pattern of lateral root initiation in seminal roots of wheat, and reported that the seminal root elongation was inhibited by the osmotic stress of lower than -0.21 MPa. Therefore, in this experimental, we added 100 g PEG per liter of nutrient solution, and no PEG to the control; the resulting water potential values were -0.21 and -0.08 MPa, respectively. Seedlings were harvested just before the treatment (Day 0), and on the next two days (Days 1 and 2). 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. In this way, it mimics the drying effects of a soil environment. In contrast, osmoticum such as salts, mannitol, or sorbitol penetrate the cell wall and the cells themselves, and therefore may alter the normal response to low water potential. There

Materials and methods

1. Plant culture

Seedlings of Zea mays L. (c.v. White Pop) were germinated in the dark at 28°C in petri dishes for 3 days. During this time, the seminal root elongated approximately 10 mm. These seedlings were transplanted onto nets floating on 8 L of nutrient solution in 10 L plastic containers (39 cm width, 22 cm depth and 16 cm height). Thirty seedlings were planted in one container for growth analysis and solute analysis. The nutrient solution contained 6.0 mM KNO₃, 4.0 mM Ca(NO₃)₂, 1.0 mM NH₄HPO₄, 2.0 mM MgSO₄, 26.8 µM Fe-EDTA, 4.6 µM MnCl₂, 23.1 µM H₃BO₃, 0.38 µM ZnSO₄, 0.16 µM CuSO₄ and 0.015 µM (NH₄)₆Mo₇O₂₄. The nets were covered with aluminum foil with holes to allow roots to grow in the dark. The nutrient solution was aerated sufficiently. The plants were exposed to a 12-hour photoperiod in a growth chamber (MLR-350H, SANYO). The photon flux density of photosynthetically activity radiation (PAR, 400-700 nm) was 520 µmol m⁻² s⁻¹. The chamber was maintained at 28°C with 80% relative humidity during the day and night. Seedlings were grown for 72 hours on the nutrient solution.

2. Osmotic stress treatment

After the plants grew on the net for 72 hours (6 days after seeing), polyethylene glycol (PEG) 6000 (CaH₂C₂O₄, Ltd., Japan) was dissolved in the nutrient solution to induce osmotic stress. Ogawa et al. (1996) investigated the seminal root elongation of 7-day-old maize seedling under various osmotic stress conditions, and reported that the seminal root elongation was inhibited by the osmotic stress of lower than -0.21 MPa. Therefore, in this experimental, we added 100 g PEG per liter of nutrient solution, and no PEG to the control; the resulting water potential values were -0.21 and -0.08 MPa, respectively. Seedlings were harvested just before the treatment (Day 0), and on the next two days (Days 1 and 2). 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. In this way, it mimics the drying effects of a soil environment. In contrast, osmoticum such as salts, mannitol, or sorbitol penetrate the cell wall and the cells themselves, and therefore may alter the normal response to low water potential. There

expressed in response to water stress is unknown. Our objective in this study, therefore, was to examine the relationship between the development of lateral roots and the accumulation of sugar under osmotic stress conditions. Specifically, the initiation and elongation of lateral roots were compared with the content of glucose, sucrose, and fructose along the seminal root axis of maize seedlings.
were no apparent toxic effects of PEG under the well-aerated condition (Verslues et al., 1998; Raymond and Smirnoff, 2002; Ober and Sharp, 2003).

3. Growth analysis

Five seedlings harvested from each treatment on Days 0, 1, and 2 were separated into shoots and roots. Plant length, seminal root length, and fresh weight of the shoot and root were measured, and these samples were desiccated at 80°C for 2 days. Dry weight of these samples was measured. The carbohydrate and nitrogen content of the desiccated samples were measured using a C/N coder (NC-900 Shimadzu). The seminal root system of three seedlings from each treatment and each sampling day were separated from the shoot and preserved in FAA solution (formalin, acetic acid, 70 % ethanol; 1:1:18 parts by volume) for obtaining root data. The seminal root samples were cut into 5 mm segments keeping the lateral roots intact. The number and the length of the lateral roots branching out of each segment of seminal root were determined. Five replications were used for these measurements.

4. Solute analysis

The seminal roots were excised from the seedlings and were cut into 10 mm segments. The lateral roots branching out of each segment were excised. Ten root segments were sealed in the same plastic tube and plunged into liquid N2. Three tubes, each with ten segments sealed within, were used for osmotic potential measurement, and three tubes for sugar analysis. The sample tubes were stored in a freezer at -30°C prior to measurement.

The glucose, fructose and sucrose contents of the samples were assayed by the coupled enzymatic assay method, which measures the increase in A340, as described by Guglielminetti et al. (1995). The fresh weight of the frozen samples was measured, and then the samples were extracted with 80% ethanol at 80°C. The samples and standards were incubated at 37°C for 30 minutes, and then mixed with the reaction mixture (1 ml). The mixture was then incubated at 37°C for 30 minutes, and the increase in A340 was recorded. The composition of the reaction mixture for the glucose assay was Tris-HCl (120 mM, pH 7.6), MgCl2 (3 mM), ATP (2 mM), NADP (0.6 mM), hexokinase (1 unit), and glucose 6-phosphate dehydrogenase (1 unit). Fructose was assayed using the reaction mixture of the glucose assay plus phosphoglucose isomerase (2 units). Sucrose was hydrolyzed using inverase (85 units in 15 mM sodium acetate, pH4.6), and then the glucose and fructose were assayed as described above.

For osmotic potential measurement, the frozen tissue samples were thawed for over 30 minutes at room temperature, after which they were homogenized with a glass rod. Then the samples were centrifuged for 20 minutes at 11,000 g. The supernatants were used for measuring osmotic potential. Osmotic potential was measured using a vapor pressure osmometer (Model 5300, Wescor). Three replications were used for these solute analyses.

5. Statistical analysis

The significance of the differences in growth between the control and the PEG treated samples of Day 1 and Day 2 plants was determined using a t-test.

Results

1. Plant growth

Plant growth was influenced by osmotic stress (Table 1). Shoot growth on Day 2 was inhibited by stress, but that on Day 1 was not. Plant length of the stressed plants, measured on Day 2, was 3.9 mm shorter than that of the control plants (P < 0.001). Between Day 0 and Day 1, the total root length increased by 133 mm in the control, but only 48.7 mm in the stressed plants; the difference (84.5 mm) was significant at P < 0.001.

| Table 1. Plant growth, water relations, and the distribution of carbon and nitrogen under osmotic stress conditions. Each value is the mean of five replications. |
|---|---|---|
| Days after stress treatment | Control | Stress |
| Water content in shoot (%) | 91.8 | 91.1*** | 91.2*** |
| Water content in root (%) | 95.0 | 95.9*** | 94.6** |
| Shoot dry weight (mg) | 15.6 | 37.8 NS | 50.0 NS |
| Root dry weight (mg) | 5.60 | 13.8 NS | 18.8 * |
| Shoot/Root ratio | 2.91 | 2.75** | 2.67 * |
| Plant length (cm) | 6.54 | 11.2 NS | 17.0 *** |
| Total root length (cm) | 27.8 | 161 *** | 353 *** |
| Total root number | 64.2 | 236 * | 357 NS |
| Carbon content in shoot (mg) | 16.1 NS | 20.9 NS |
| Carbon content in root (mg) | 2.04 | 5.35 * | 7.11 ** |
| Nitrogen content in shoot (mg) | 0.970 | 2.26 NS | 2.96 ** |
| Nitrogen content in root (mg) | 0.245 | 0.737 NS | 1.13 NS |

Abbreviations: *, **, ***; and NS indicate significant differences at P = 0.05, P = 0.01, P = 0.001, and no significant difference compared with the control values, respectively.
However, between Day 1 and Day 2, the difference was smaller (48.5 mm per day). Similarly, between Day 0 and Day 1, the increase in the total number of roots of the stressed plants was significantly smaller than that of the control plants. However, between Day 1 and Day 2 there was no statistically significant difference between the control and the stressed plants. These results show that osmotic stress first affected root growth, followed by resumption of root growth and then, inhibition of shoot growth.

Carbon content in the shoot showed no significant difference between the control and stressed plants during the 2 days after the start of stress treatment, while carbon content in the root was increased by the stress treatment. Nitrogen content in the shoot declined as a result of the stress treatment. In contrast, in the root there was no statistical difference in the nitrogen content between the control and stressed plants during the experimental period. These results show that under osmotic stress conditions, allocation of carbon to the root and that of nitrogen to the shoot increased.

2. The developmental characteristics of lateral roots

The detailed developmental characteristics of the lateral roots along the seminal root under osmotic stress conditions were examined (Fig. 1). At the onset of the stress treatment, the 40 mm basal portion of the seminal root had already branched out lateral roots, whereas the rest of the apical part had not.

Fig. 1-A shows the relationship between the number of lateral roots per 5 mm segment of seminal roots sampled successively along the seminal root axis. In the portion 20 mm from the base of the seminal root, the number of lateral roots of the stressed plant on Day 2 was 49% of that of the control plant. On the other hand, in the portion 40 mm from the base, the number of lateral roots was scarcely affected, or was slightly promoted by the stress treatment.

The average length of the lateral roots showed a tendency similar to the number of lateral roots (Fig. 1-B). The elongation of lateral roots in the 0-40 mm portion was substantially inhibited by the stress treatment. The inhibition of lateral root elongation was large, particularly at the basal part of the seminal root. In the portion 5 mm from the base of the seminal root, the length of the lateral roots on Day 2 was 16% of that of the control. On the other hand, in the portion 40 mm from the base, there was only a slight difference in the length of the lateral roots between the control and stressed plants.

Multiplication of the number (Fig. 1-A) and the length (Fig. 1-B) of the lateral roots produces the total length of the lateral roots branching out from each part of the seminal root (Fig. 1-C), where the development of the lateral roots under osmotic stress conditions is clearly shown. In the 0-40 mm portion, the development of lateral roots was remarkably inhibited by the stress treatment, compared with the control. However, in the portion 40 mm from the base, there was no difference in lateral root development between the control and stressed plants.

3. Sugar content of the seminal root axis

The concentration of glucose, sucrose and fructose in the 10 mm segments of the seminal root sampled
successively along the root axis was examined under osmotic stress conditions (Fig. 2). Sugar concentrations were changed drastically by the osmotic stress treatment, and were different along the seminal root axis. The changes in sucrose concentration (Fig. 2-A) and glucose concentration (Fig. 2-B) along the seminal root axis were similar, although the content of glucose was about two times that of sucrose. The concentrations of both sugars increased acropetally. The concentrations of both sucrose and glucose were increased by the stress treatment, especially in the apical 50-mm portions of both roots sampled on Days 1 and 2. These parts were where the lateral roots had been predetermined to branch out and elongate from the following day onward, although lateral roots had not branched out yet at that time. On Day 1 in the apical 50-mm portions, sucrose concentrations in the stressed plant were 1.1 to 3.1 times that in the control plant, and glucose concentrations were 1.7 to 4.1 times that in the control. On Day 2 in the apical 50-mm portions, sucrose concentrations in the stressed plant were 1.5 to 7.2 times that in the control plant, and glucose concentrations were 1.9 to 4.9 times that in the control.

Little fructose was detected (data not shown).

4. The change of osmolarity along the seminal root axis

Fig. 3 shows the change of osmolarity along the seminal root axis under osmotic stress conditions. Osmolarity increased acropetally. In the stress treatment, osmolarity along the seminal root axis was about 20-50 mM higher than in the control on Day 1, and about 20-70 mM higher on Day 2. The differences between the stress treatment and the control were large in the region 5-10 mm from the root apex, and osmolarity in this portion of the stressed root was 52 mM higher on Day 1 and 77 mM higher on Day 2 than that in the control. The difference was smaller in the basal part.

Discussion

In this study, we assumed that allocation of materials, mainly photosynthate, is one of the main factors that regulate lateral root development in root systems under water deficit conditions. The distribution of sugars along the seminal root axis of maize seedlings in relation to the initiation and elongation of lateral roots was examined under osmotic stress conditions.

The lateral roots that had emerged before stress treatment showed responses to water stress differently from those emerged after the stress treatment (Fig. 1). In the parts where lateral roots had emerged before the stress treatment (the 0-40 mm portion from the base of the seminal root), lateral root development was inhibited by the stress treatment. On the other hand, in the parts where lateral roots emerged after the stress treatment (the portion more than 40 mm distant from the base of seminal root), lateral root development was
scarcely affected by the stress treatment. These results suggest that lateral roots that had already emerged before the stress treatment could not grow because they did not adapt to the osmotic stress. In contrast, lateral roots that emerged after the stress treatment were adapted to osmotic stress, and could grow further under stress conditions, in a way which was similar to the control plants.

The stress treatment produced a dramatic increase in concentration of sucrose and glucose in the distal 50 mm-portions on Day 1 and Day 2 (Fig. 2). The changes in sugar concentrations were compared with the change of osmolarity under the osmotic stress treatment (Fig. 3). Osmolarity under the stress treatment was higher than that in the control at any portion along the seminal root axis. In particular, the differences were large in the portion 5-10 mm from the seminal root apex, which is the root elongation zone. This result agrees well with previous studies (Sharp et al., 1990; Ogawa and Yamauchi, 1999), which indicated that a change of osmolarity functioned as an osmotic adjustment in all parts of the seminal root under osmotic stress conditions and that osmotic adjustment was functioned especially in the root elongating zone. In this study, however, the glucose and sucrose concentrations also increased in the apical part of the elongated and matured zone (10-50 mm from the seminal root apex), adjacent to the elongating zone (Fig. 2). In addition, the increased levels of glucose and sucrose concentration in the apical 50-mm portions were apparently higher than that caused by osmotic adjustment.

In the apical 50-mm portions of the seminal root, where lateral roots had not yet developed, lateral roots were predetermined to emerge and elongate one or two days later. In addition, lateral roots in the basal 0-40 mm portion were inhibited in initiation by the stress and elongation (Fig. 1). In contrast, lateral roots in the portion more than 40 mm distance from the base were not affected or were slightly promoted by the stress in initiation and elongation. These results suggest that glucose and sucrose were allocated to predetermined lateral root initiation sites in the apical 50-mm portions of the root in response to the osmotic stress treatment, and that they provided osmotic adjustment, promoted lateral root development, and cancelled the inhibition of lateral root growth caused by the stress treatment.

Sugars contributed to both initiation and elongation. Auxin has been found to play an important role in the initiation of lateral roots (Lloret and Casero, 2002). In recent investigations, it has been confirmed that sugars are also important for lateral root initiation (Bingham et al., 1997, 1998; Takahashi et al., 2003). Bingham et al. (1997) showed that when glucose was fed in root zone of wheat, primordia frequency of lateral root was increased within 15 hours, and additional primordia were initiated in the tissue located 0-20 mm behind the apex at the start of treatment. And when glucose was fed to a part of the root system using the split root system, lateral root primordia also increased in the tissue located 0-30 mm behind the apex (Bingham et al., 1998). Their proposition that proliferation of lateral roots might be signaled by increased sugar content in the tissue supports our results involving lateral root developed under osmotic stress conditions was supported by the increased sugar content. Also, the present results showed directly that endogenous sugars were distributed to the lateral root initiating zone. Furthermore, in our study the maintenance of lateral root growth (Fig. 1) and increased sugar accumulation (Fig. 2) were found 24 hours after the stress treatment (Day 1). This result is in good agreement with the findings by Bingham et al., (1997, 1998) that additional lateral root primordia were found 15 hours after glucose was fed to the root zone.

Elongation of lateral roots requires sugars as an energy resource and as a substrate for synthesis of components of the cell and the cell wall. In addition, under osmotic stress conditions, they are used as a compatible solute for osmotic adjustment in roots (Sharp et al., 1990; Rodrigues et al., 1997; Ogawa and Yamauchi, 1999). Although there are no studies about osmotic adjustment in lateral roots, Ogawa and Yamauchi (1999) investigated the transient changes in maize seminal root elongation and water relations after stress treatments, to evaluate the process of osmotic adjustment in roots. They reported that osmotic adjustment began to function within a few minutes after osmotic stress treatment, especially in the root elongation zone, and substantially contributed to the maintenance of turgor pressure and to elongation. After 2 hours of stress treatment the accumulation of reducing sugar contributed mainly to the change of osmolarity in the maize seminal root elongation zone.

Consequently, it is deduced that sugars substantially contributed to the initiation and elongation of lateral roots. In this study, we examined the distribution of sugars along the seminal root axis of maize seedlings in relation to the initiation and elongation of lateral roots under osmotic stress conditions. We concluded that the accumulation of glucose and sucrose could cancel the inhibitory effects of stress treatment, and thereby could maintain lateral root growth. The expression of plasticity in crop root systems is one of the most important drought tolerance mechanisms under water deficit conditions. The initiation and elongation of lateral roots is the main contribution to the expression of plasticity since plant root systems largely consist of lateral roots. We suggested the possibility that sugar distribution contributed substantially to the expression of plasticity in the root system.
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