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Aluminum-Induced Rapid Root Inhibition and Changes in Cell-Wall Components of Squash Seedlings[1]

Hoa Le Van, Susumu Kuraishi, and Naoki Sakurai*

Department of Environmental Studies, Faculty of Integrated Arts and Sciences, Hiroshima University, Higashi-Hiroshima 724, Japan

Growth of squash (*Cucurbita maxima* Duch.) roots was significantly inhibited by 1 mM $AlCl_3$ as early as 1 h after the treatment. The growth inhibition was confined to the elongating zone (1–6 mm from the root tip). Chemical analysis of cell-wall polysaccharides from roots revealed that aluminum increased pectin, hemicellulose, and cellulose contents after 3 h of treatment. The effect of aluminum on pectin content was found in the elongating zone including the root tip, whereas change in cellulose content was confined to only nonelongating zones. Hemicellulose content increased in all of the regions along the root axis. The increase in the pectin fraction was due to the increases in uronic acids, galactose, and arabinose constituents, whereas hemicellulose content changed due to increases in glucose, xylose, galactose, and arabinose. The results clearly indicate that aluminum rapidly reduced squash root growth by inhibiting cell elongation and altering metabolism of cell-wall polysaccharides in the nonelongating zone as well as in the elongating zone.

Al is the most abundant metal in the earth's crust. Most of the Al is incorporated into aluminosilicate soil minerals; very small quantities appear in soluble forms capable of influencing biological systems (Driscoll and Schecher, 1988; May and Nordstrom, 1991). However, solubilization of Al-containing minerals is enhanced in acidic environments (Bolan et al., 1991). Solubilized Al ions in acid soils are known to be toxic to the growth of agriculturally important plants (Foy et al., 1978). In addition, the physiological mechanisms of its toxicity have been explained by the inhibition of cell division (Clarkson, 1965; Morimura et al., 1978), cell elongation (Wallace and Anderson, 1984), or mineral uptake (Clarkson, 1967; Foy et al., 1978).

Al was absorbed in large amounts in the tip of growing roots of many plants (Matsumoto et al., 1977; Wagatsuma, 1983), but the toxic effects such as cell destruction were not restricted to the meristematic region (Huck, 1972; Wagatsuma et al., 1987). Therefore, Al probably causes effects other than the inhibition of cell division. Al-induced mineral deficiencies could not explain the rapid toxic effects of Al on the growth of plants (Taylor, 1988). Thus, rapid inhibition of root growth by Al is likely caused by the inhibition of cell elongation, which is closely associated with metabolism of cell-wall polysaccharides (Hayashi, 1989; Sakurai, 1991). Furthermore, if the apoplasm has an important role in the tolerance of Al toxicity (Allan et al., 1990; Taylor, 1991), the role of the cell walls of growing roots needs to be determined. However, an Al effect on root cell walls has not been extensively studied. In the present experiments, we treated the intact roots of squash seedlings with Al for short periods and then analyzed the cell-wall components to clarify the mode of action of the direct effect of Al on the root cell walls.

MATERIALS AND METHODS

Plant Materials

Seeds of squash (*Cucurbita maxima* Duch. cv Houkou-Aokawa-amaguri; Takayama Seed Co., Kyoto, Japan) were soaked for 15 h in tap water and germinated in the dark for 48 h on two layers of moistened filter paper (No. 514 A; Toyo Roshi Co., Tokyo, Japan). Then the germinated seeds with uniform root lengths of approximately 2 cm were cultivated hydroponically for 24 h in the dark at 25 ± 0.5°C in one-fifth-strength Hoagland nutrient solution. The seeds were placed on a stainless steel mesh (5 × 5 mm), which was held 5 mm above the solution level.

Growth Experiments

Measurement of Root Elongation

After 24 h, each axial root from 16 seedlings was marked with India ink at 1-mm intervals from the tip. Then one-half of the marked roots were transferred to one-fifth-strength Hoagland solution from which phosphate was omitted and adjusted to pH 4.5 with 0.01 M HCl. The other one-half of the marked roots were transferred to the incomplete Hoagland solution containing 1 mM $AlCl_3 \cdot 6H_2O$ (Katayama Chemical Co., Osaka, Japan) adjusted to a final pH of 4.5 using 0.01 M NaOH. The length of each marked root segment was measured 6 h after the treatment by using a binocular microscope. In another experiment, the whole root lengths from 10 seedlings were measured with a ruler after 0, 1, 3, and 6 h of treatment.

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* Corresponding author; fax 81-824-24-0758.

Abbreviations: NS, neutral sugar; Rha, rhamnose; UA, uronic acid; WS, water-soluble fraction.

Al Treatment for the Cell-Wall Analysis

Seedlings that were 3.6 d old were transferred to one-fifth-strength Hoagland solution (without phosphate) with or without 1 mM $AlCl_3$ (pH 4.5). After 0, 1, 3, and 6 h of treatment, 25 axial roots (15 mm long) were excised. In some experiments, groups of roots were harvested and then cut with a multibladed cutter into seven consecutive sections (2.2 mm in length) beginning at the tip. All manipulations of the growth experiments were conducted under a dim green light at 24 ± 1°C.

Cell-Wall Fractionation

Cell walls were fractionated by the method of Sakurai et al. (1987a). A group of 25 roots or 85 root segments was fixed in boiling methanol for 10 min. The methanol extract was designated as the methanol-soluble fraction. Rehydrated roots or root segments were homogenized in deionized water with a glass homogenizer (1.6 × 14.4 cm; Iwaki Glass Co., Tokyo, Japan). The homogenate was centrifuged for 10 min at 1000*g*, and then the residue was washed with deionized water, acetone, and a methanol:chloroform mixture (1:1, v/v) and air dried. The WS was collected after denaturation of proteins by adding TCA (10%, v/v). The dried wall material was treated for 2 h with 2 units/mL porcine pancreatic α-amylase (type I-A, Sigma) in Na-acetate buffer (pH 6.5, 50 mM) to remove starch and then for 18 h with 200 mg/mL pronase (Actinase; Kaken Kagaku Co., Tokyo, Japan) in Na-phosphate buffer (pH 6.5, 50 mM) containing 5% ethanol to remove proteins.

Next, the pectic substance was extracted from the cell walls by three treatments with 50 mM EDTA at 95°C for 15 min. Then the hemicellulose was extracted for 18 h with 17.5% NaOH containing 0.02% $NaBH_4$. The alkali-insoluble fraction was designated as the cellulose. The hemicellulosic fraction was neutralized with glacial acetic acid. The pectic and neutralized hemicellulosic fractions were dialyzed against deionized water for 20 h.

The total sugar content of each fraction was determined by the phenol-sulfuric acid method (Dubois et al., 1956), and the UA contents were determined by the Blumenkrantz and Asboe-Hansen (1973) procedure. The NS composition of the pectin and hemicellulose fractions was determined by GLC according to the method of Albersheim et al. (1967). All experiments were repeated at least three times. Data from one experiment with triplicated samples are given.

RESULTS

Effect of Al on the Growth of Squash Roots

Growth of etiolated squash roots was inhibited by Al after 1 h of treatment (Fig. 1). The growth inhibition was confined to the region 1 to 6 mm from the root tip (Fig. 2). Microscopic observation revealed that the meristematic region of squash roots was restricted to the distal 1-mm region. There was no swelling or apparent change in the root treated with Al within 6 h, although notches of the epidermis without swelling were observed in the elongation zone after only 10 to 12 h.

Analysis of the Cell-Wall Compositions

The immediate inhibition of root growth by Al suggested a direct effect of Al on cell elongation. Cell walls were prepared from the 15-mm-long axial root tissues treated with or without 1 mM Al at pH 4.5, and wall components were analyzed 0, 1, 3, and 6 h after the treatment (Fig. 3). In the absence of Al, the cell-wall fractions did not change during the 6-h period. The roots did not show any significant change in sugar content of the cell-wall fractions 1 h after Al treatment. After 3 h, Al increased the NS content of the WS and the pectin, hemicellulose, and cellulose fractions. The UA content of pectin was increased by exposure to Al for 6 h. Al slightly inhibited the increase of NS content in methanol-soluble fraction. Al did not have significant effects on the UA content of the WS or the hemicellulose fraction during the 6-h period.

Effect of Al on the Cell-Wall Polysaccharides Fractionated from the Different Region of Squash Roots

Figure 4 shows the effect of Al on the wall polysaccharides along the root axis after 6 h of treatment. In the absence of Al, sugar contents of none of the cell-wall fractions of root segments excised from different regions changed after 6 h, except that the sugar contents of the control segment excised 2.2 to 4.4 mm from the tip were slightly lower than those of the initial segment. Exposure to Al increased the content of NSs of pectin in the distal part (0–8.8 mm from the tip), increased the cellulose content in the proximal end (6.6–15.4

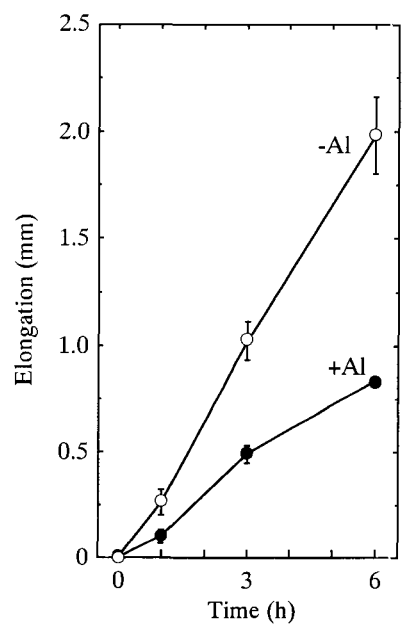

Figure 1. Time-course effect of Al on the growth of squash roots. Seedlings (63 h old) were grown for 1 d in the dark at 25 ± 0.5°C in one-fifth-strength normal Hoagland solution and then treated with (●) or without (○) 1 mM $AlCl_3$ in one-fifth-strength modified Hoagland solution without phosphate. The solution pH was adjusted to 4.5 using 0.01 M HCl or NaOH. The increment of root length was measured by ruler. Bars indicate SE (n = 10).

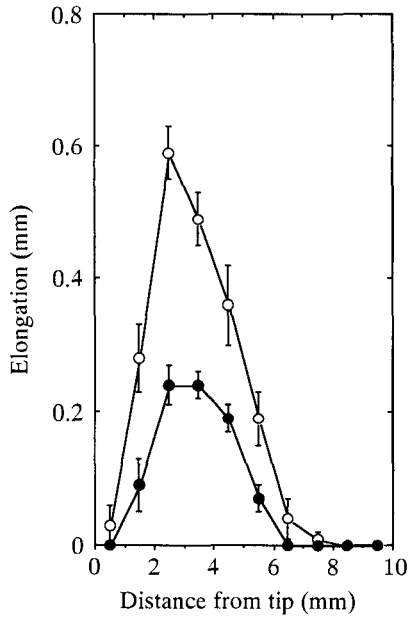

Figure 2. Inhibitory effect of Al on different regions of squash roots. Axial roots of 3.6-d-old seedlings were marked with India ink at 1-mm intervals from the tip and then treated with (●) or without (○) 1 mM $AlCl_3$ in one-fifth-strength modified Hoagland solution without phosphate at pH 4.5 in the dark at 25 ± 0.5°C. After 6 h of treatment, the length of each marked root region was measured using a binocular microscope. Bars indicate SE (n = 8).

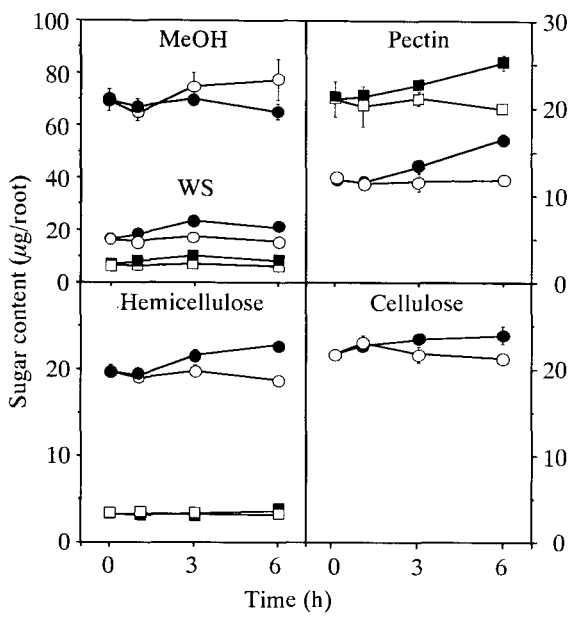

Figure 3. Kinetic changes in the sugar contents of WS and methanol (MeOH) and cell-wall fractions of squash root tissues produced by Al treatment. After treatment with (■, ●) or without (□, ○) 1 mM $AlCl_3$ at pH 4.5 in the dark, groups of 25 distal root segments (15 mm in length) were harvested for the cell-wall analysis. MeOH, Methanol soluble; NS (●, ○); UA (■, □). Bars indicate SE (n = 3).

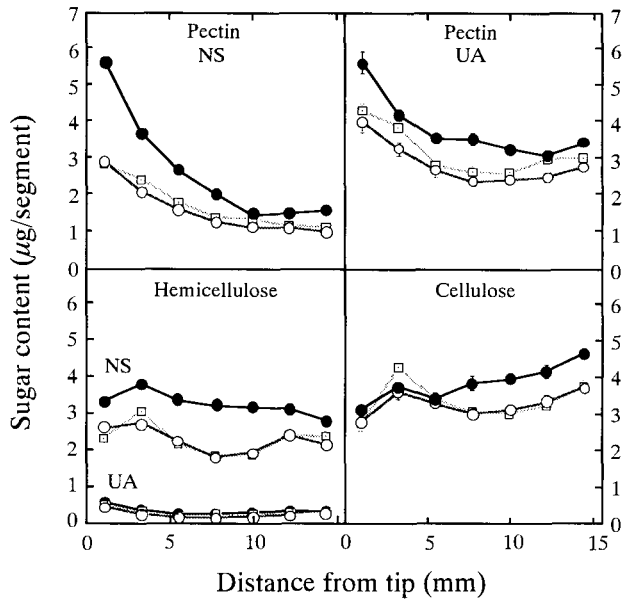

Figure 4. Changes in the sugar contents of cell-wall fractions from different regions of squash root produced by Al treatment. Intact roots were treated with (●) or without (○) 1 mM $AlCl_3$ at pH 4.5 for 6 h in the dark and then cut into seven consecutive segments (2.2 mm in length) from the tip. ⊡, Initial root. Bars indicate SE (n = 3).

mm from the tip), and uniformly increased the amount of UAs of pectin and NSs of hemicellulose along the axis.

Effect of Al on the Sugar Compositions of Pectin and Hemicellulose

To specify particular neutral components attributed to the increases in the amounts of pectin and hemicelluloses, the NS constituents of cell walls along the root axis treated with or without Al for 6 h were analyzed by GLC. The major components of the NS of pectin were Gal and Ara, which was markedly increased by Al, especially in the root tip (Fig. 5). Al also increased Glc content in the distal end (0–4.4 mm from the tip). Higher Gal and Ara contents in pectin from the distal part of the Al-treated roots contributed to the higher NS contents in the pectin.

The predominant sugar components of hemicellulose fraction were Glc, Xyl, and Gal (Fig. 6). The Glc content was higher in the distal than in the proximal part, whereas the Gal content showed an opposite trend in the initial or control root. Al treatment uniformly increased the Glc content along the axis. It increased Xyl and Ara contents in the middle and proximal parts, and the Gal content was increased in the middle and distal parts.

DISCUSSION

Roots were the main site of Al injury in squash seedlings. Inhibition of the root growth could be detected 1 h after Al treatment (Fig. 1). Such rapid inhibition by Al was reported

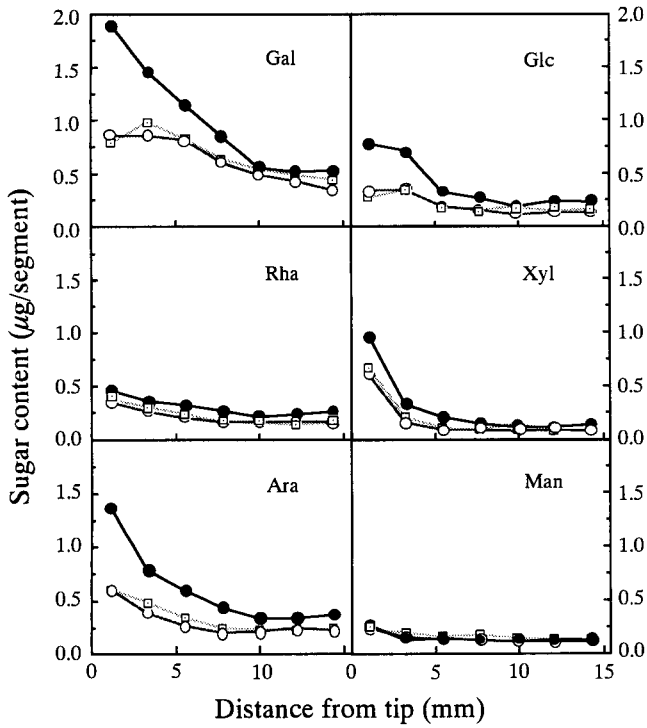

Figure 5. Compositions of the NSs of pectin extracted from different regions of squash root after 6 h of Al treatment. Air-dried pectin extracted from different regions of the roots was hydrolyzed for 1 h with 2 N TFA and subjected to the determination of NS compositions by GLC. ⊡, Initial root; O, without Al; ●, with Al. SE values shown by bars are within the size of the symbols.

in wheat (Wallace and Anderson, 1984; Ryan et al., 1992). Horst et al. (1983) reported that the cell division rate of cowpea roots was drastically reduced after only 5 h of Al treatment. In squash roots, the inhibitory effect of Al was found mainly in the elongation region, indicating that the rapid inhibition is primarily caused by a decline in cell elongation (Fig. 2). It has been demonstrated that chemical components of the cell wall and the mechanical properties of the cell wall are dynamically changed during cell elongation (Sakurai, 1991). Measurements of cell-wall polysaccharides, however, showed that the inhibition of root growth by Al preceded the changes in the level of polysaccharides, suggesting that the wall changes are the consequence of the growth inhibition. Whether the inhibitory effect of the root elongation by Al is related to chemical changes in the cell-wall components or not needs further investigation.

Analyses of squash root walls (Fig. 3) revealed that Al-induced changes in the wall components were significant after 3 h and even more apparent after 6 h of Al exposure. Al increased the sugar contents of all wall fractions in the whole roots except the methanol-soluble fraction. Note that the Al effect on the amount of wall fractions was not found during the 1st h of treatment. This clearly indicates that the early stage of growth inhibition was not explained merely by the amounts of cell-wall fractions or wall components. However, there is the possibility that a shift of mol wt of wall polysaccharides takes place in Al-treated root within 1 h. A decrease in mol wt of wall polysaccharides was reported to be associated with the promotion of cell elongation (Sakurai, 1991). Al effects on molecular shifting of wall polysaccharides are currently being investigated.

An Al effect on increases in the amount of pectin and hemicellulose was first found in this experiment, and there are two possible explanations: stimulation of synthesis or inhibition of degradation. Noncellulosic polysaccharides of the cell walls undergo massive turnover during elongation growth (Labavitch, 1981). If Al inhibits the degradation process of the polysaccharides, it results in the accumulation of noncellulosic polysaccharides in the cell walls. Degradation of noncellulosic polysaccharides is a prerequisite for auxin-induced cell elongation (Masuda, 1990; Sakurai, 1991). Therefore, inhibition of the degradation possibly results in inhibition of cell elongation.

The increase in cellulose content in the nonelongating zone by the Al ion was also first found in this experiment, suggesting that it is involved in the syntheses of secondary walls. It is uncertain whether or not the increase is directly caused by Al treatment, since Matsumoto et al. (1977) and Wagatsuma et al. (1987) reported that localization of Al was primarily higher in the root tip and lower in the proximal part, where the cellulose content was increased.

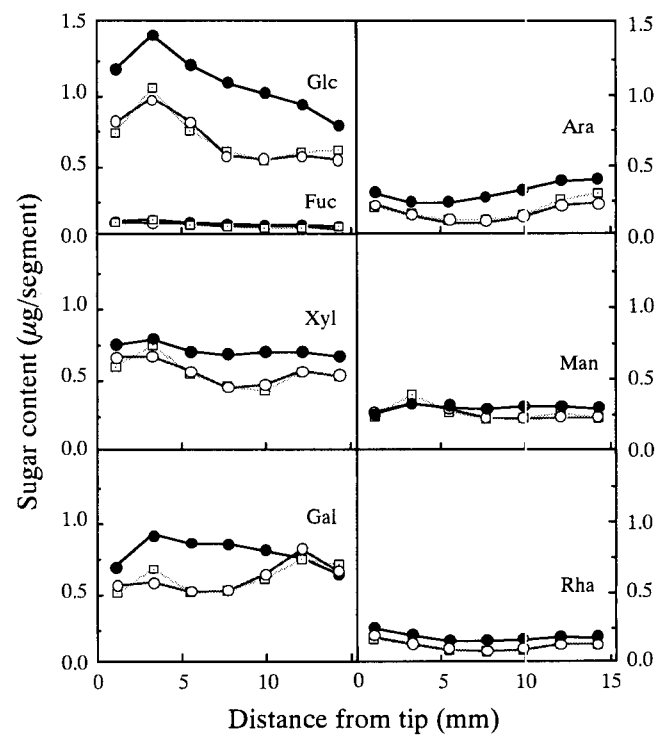

Figure 6. Compositions of the NSs of hemicellulose extracted from different regions of squash root after 6 h of Al treatment. Air-dried hemicellulose extracted from different regions of the roots was hydrolyzed for 1 h with 2 N TFA and subjected to the determination of NS compositions by GLC. ⊡, Initial root; O, without Al; ●, with Al. SE values shown by bars are within the size of the symbols.

Al increased the sugar content of pectin in the distal end (Fig. 4). Analysis of the NS constituents revealed that the increases were due mainly to the increases in Gal, Ara, and Glc components (Fig. 5). Since 6 h of Al treatment did not increase the amount of Rha as much as that of Gal or Ara, the increments of Gal and Ara might result from the extension of arabinogalactan side chains or an increase in the number of side chains of rhamnogalacturonans. Rhamnogalacturonan was considered to have arabinogalactan side chains in squash hypocotyls (Sakurai et al., 1987b). The increase in Glc is not readily explained. Although it might be derived from contaminant starch, amounts of sugar released by α-amylase treatment were similar in experiments with and without Al treatment. Therefore, the increase in Glc may be due to the water-soluble callose (Wissemeier et al., 1987) or xyloglucan fragments. The Gal content in pectin was high in the tip and low in the proximal part, both in the absence or presence of Al. The result is in contrast to that in pea roots reported by Tanimoto (1988).

Analyses of the sugar composition of hemicelluloses (Fig. 6) show that Glc, Xyl, and Gal are the main components. Glc, Xyl, Fuc, and a part of Gal may be the xyloglucan components. The ratios of Glc and Xyl to Fuc content in the elongation zones were 10 and 8, respectively, and similar to that of common xyloglucan structure (Hayashi, 1989). The increases in the amount of Glc and Xyl are likely attributed to the increase in xyloglucan content by an Al effect.

Cell-wall synthesis has been associated with the mechanism of Al tolerance (Taylor, 1991). In particular, the pectic substances were believed to bind or chelate Al^{3+} ions in their free carboxyl groups, resulting in cross-linking of pectin molecules (Klimashevskii and Dedov, 1975) and leading to detoxification of Al. Furthermore, removal of root mucilage increased sensitivity of root to Al (Horst et al., 1982), suggesting that Al was bound to the secreted mucilage. Therefore, a drastic increase in pectin of squash root walls induced by Al in the the root tip (0- to 2.2-mm segment) could offer protection and have an important role in the tolerance mechanisms. Squash was characterized as an Al-tolerant plant (Aimi and Murakami, 1964).

In conclusion, the present results clearly revealed that Al rapidly inhibits the root growth and primarily causes a decline in the cell elongation. Chemical analysis of cell walls from roots indicated that Al increased the amount of acidic and neutral polysaccharides in an actively growing region and the amount of cellulose in a nongrowing region.

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