NOVEL MECHANISM OF ALUMINUM TOXICITY RELATED TO SUGAR METABOLISM

Y. Yamamoto and T. Sasaki
Institute of Plant Science and Resources, Okayama University, Okayama, Japan
E-mail: yoko@rib.okayama-u.ac.jp

Abstract
Aluminum ion is a major factor to limit crop productivity in acidic soils. Aluminum ion accumulates on root apices, which immediately leads to the inhibition of cell elongation (namely, the inhibition of root elongation). In addition, the enhancement of reactive oxygen species (ROS) production is commonly observed in roots under aluminum stress. These symptoms are also observed in actively growing cells in culture. The elucidation of the mechanisms of aluminum toxicity is necessary to find out effective strategies to overcome aluminum stress. For this purpose, we investigated the responses to aluminum in cultured cell system as well as root system of tobacco, focusing on sugar metabolism. We found that aluminum ion immediately inhibits sugar uptake, which seems to be a mechanism whereby aluminum ion inhibits water uptake (namely, the inhibition of cell elongation). The pathway from the inhibition of sugar uptake till ROS production in the cells under aluminum stress should be elucidated in the future.

Key words: Aluminum toxicity, cell elongation, ROS, sugar uptake, water potential

1.0 Introduction

Free aluminum ions are the main factor responsible for inhibiting root growth in acidic soils (Matsumoto, 2000; Kochian *et al*., 2004). The primary site of aluminum accumulation and toxicity is the growing cells at root tip, where aluminum inhibits elongation after a short-term exposure, and causes necrosis after longer exposure. Primary targets of aluminum seem to be within the cell wall and at the plasma membrane. However, the targets and mechanisms whereby aluminum inhibits elongation have not been fully elucidated. Elongation depends on three events, namely, synthesis of cell wall constituents, loosening of the cell wall, and water uptake. Although aluminum might inhibit any or all of them, we focused on the effect of aluminum on water relations in this study.

A system for studying aluminum responses at cellular level has been developed using a tobacco cell line SL. The cell line system is useful for the study of mechanisms of cell elongation and division, since they are relatively homogeneous and their growth conditions are easily controlled, and each cell is in contact with aluminum ions in medium. In this system, we observed several toxicity symptoms similar to those of roots, namely, cell elongation inhibition, callose production, ROS production, respiration inhibition, ATP depletion (Yamamoto et al., 2002, 2003). The details of aluminum toxicity mechanisms can be investigated in this simple cell system.

Here, we investigate the mechanism of elongation inhibition by aluminum, focusing on water relations in both cultured tobacco cells and tobacco seedlings. We report that along with blocked elongation, aluminium-treated cells fail to accumulate soluble sugars and have a low cellular osmolality. Aluminium reduces sugar uptake substantially within a few hours. We suggest that the inhibition of sugar uptake by aluminum is a primary event, responsible for lowered osmolality and hence lowered water uptake and the inhibition of elongation.

2.0 Materials and Methods

2.1 Tobacco Cells and Seedlings, Media, Aluminum Treatment

A wild-type line (SL) was derived from Nicotiana tabacum L. cv. Samsun (Nakamura *et al*., 1988). Tobacco cells at the logarithmic phase of growth were suspended in medium containing 3 mM CaCl2, 88 mM sucrose, and 20 mM MES, pH 5.0 adjusted with bis-tris propane (treatment medium) and various concentrations of AlCl3 at a cell density of 10 mg fresh weight per mL, and cultured for up to 18 h on a rotary shaker operated at 100 rpm at 25˚C in the dark (Abdel-Basset et al. 2010). Aluminum-treated cells tended to adhere to glassware; therefore, glassware was coated with Sigmacoat (Sigma-Aldrich, St. Louis, MO. USA). Seeds of tobacco (cv. Samsun) were surface sterilized, and then placed on the nylon net set in the mount which was floated on Ruakura growth medium at pH 6.0 (Snowden and Gardner, 1993) on a cycle of 16-h days (~200 µmol m^{-2} s^{-1} light) and 8-h nights. At about 7-day after germination, root parts were treated with aluminum in Ruakura medium containing 10 mM Mes at pH 5.0 for up to 3 days. If it was necessary, sucrose was added in the treatment medium, and the seedlings were treated in the dark, unless otherwise indicated. For fresh weight measurement, cells in 10-mL aliquots were harvested on filter paper by vacuum filtration and fresh weight was determined (Abdel-Basset *et al*., 2010).

Aluminum sensitivity of cell lines was determined as post-treatment growth capacity in the absence of aluminum (Abdel-Basset *et al*., 2010). In brief, cells for assay were harvested (in 10-mL aliquots), washed, suspended in 30-mL of nutrient medium, and cultured for 7 days. The increase in fresh weight over that week was called post-treatment growth and expressed as a percent of the control.

2.2 Assessment of Osmolality of Medium and Cell Sap

Osmolality of the solutions and cell sap were determined with a freezing point osmometer; Model 210, Fiske Associates, Norwood, MA, USA (Abdel-Basset *et al*., 2010).

2.3 Measurements of Soluble Sugar Contents, Sugar Composition, and Sucrose Uptake

Soluble sugars in cultured cells and root apices (5 mm) were extracted with 80% ethanol at least two times at room temperature, and the measurement of soluble sugar contents was performed by the anthrone reaction, and was expressed as glucose equivalents (Abdel-Basset *et al.,* 2010). This assay quantifies principally free hexose and the hexose moiety derived from oligosaccharides during heat treatment with anthrone-sulfuric acid reagent.

To determine the rate of sucrose uptake in tobacco cells, [U-^{14}C] sucrose was added to the culture and incubated. Then, aliquots of the culture were harvested on a glass membrane filter by vacuum filtration and washed with the treatment medium. The cells on the filter were dried, and the radioactivity on the filter was determined with a scintillation counter (Abdel-Basset, *et al*., 2010).

3.0 Results and Discussion

3.1 Tobacco Cell System

Over an 18-h treatment period, aluminium inhibited the increase in fresh weight (mainly due to water uptake) almost completely and decreased cellular osmolality and internal soluble sugar content substantially (Abdel-Basset et al., 2010). Soluble sugar comprised mainly glucose (32% of total), fructose (36%) and sucrose (16%) in control, and glucose (34%), fructose (38%) and sucrose (8%) in aluminium-treated cultures. In aluminium-treated cultures, fresh weight, soluble sugar content, and osmolality decreased over the first 6 h and remained constant afterward, contrasting to their 6 continued increases in the untreated cultures. Aluminium did not affect the concentrations of major inorganic ions. The rate of sucrose uptake, measured by radio-tracer, was reduced by approximately 60% within 3 h of treatment (Abdel-Basset et al., 2010). Aluminium also inhibited glucose uptake. On the other hand, aluminium responses such as the evolution of ROS and the loss of growth capability were observed after 6-h exposure to aluminium. Thus the events related to aluminum-induced cell elongation inhibition such as the repression of water uptake and the repression of sucrose uptake occur simultaneously as early events, while the events related to aluminuml-induced cell death such as ROS production and the loss of growth capability, as well as respiration inhibition, ATP depletion and callose production (Yamamoto *et al*., 2002; Yamamoto *et al*., 2003), occur as late events. Further separating the effects of aluminium on elongation and cell survival, sucrose starvation for 18 h inhibited elongation and caused similar changes in cellular osmolality but stimulated the production of neither ROS nor callose and did not cause cell death (Abdel-Basset *et al*., 2010).

Taken together, we propose that the inhibition of sucrose uptake in tobacco cells is a mechanism whereby aluminium inhibits elongation, but does not account for the induction of cell death.

3.2 Tobacco Root System

Root elongation of tobacco seedlings depends on light. In the presence of light, root elongation occurred continuously at least for 4 days (~2.3 mm d^{-1}) (Figure 1A), with an increase in free sugar content at root apex (Figure 1B). However, in the absence of light, root elongation was completely inhibited (Figure 1A) and the free sugar content at root apex was decreased (Figure 1B). These results strongly suggest that root elongation depends on the supply of photoassimilates (sucrose) from shoots. To support this, the exogenous supply of sucrose (or glucose) in medium supported root elongation even in 7 the dark condition (Figure 2). Under the dark condition with exogenous sucrose (50 mM), however, the addition of aluminum in medium immediately prevented the increase in free sugar content at root apex and root elongation. Net increases in free sugar content in root apex (µg $apex^{-1}$) were 60 (at 2 h), 960 (6 h) and 1279 (24 h) in the absence of aluminum, while 0 (2 h), 68 (6 h) and 711 (24 h) in the presence of 200 µMAl. After 24 h treatment with 200 µM Al, root elongation level was 40% of control. These results strongly suggest that the inhibition of sucrose uptake by aluminum simultaneously inhibits cell elongation.

4.0 Conclusion

In plant cells, the increase in osmolality in vacuole is fundamentally necessary for water uptake, and the increase in osmolality will be performed by increases in inorganic ion concentrations and/or free sugars. Based on the responses to aluminium commonly observed in cell culture and root systems of tobacco, we propose that the accumulation of aluminium on actively growing cells at root apex or in culture inhibits the accumulation of free sugar in vacuole via the uptake inhibition of sucrose and/or glucose, which could be a mechanism whereby aluminium inhibits cell elongation, but does not directly account for the induction of cell death (Figure 3).

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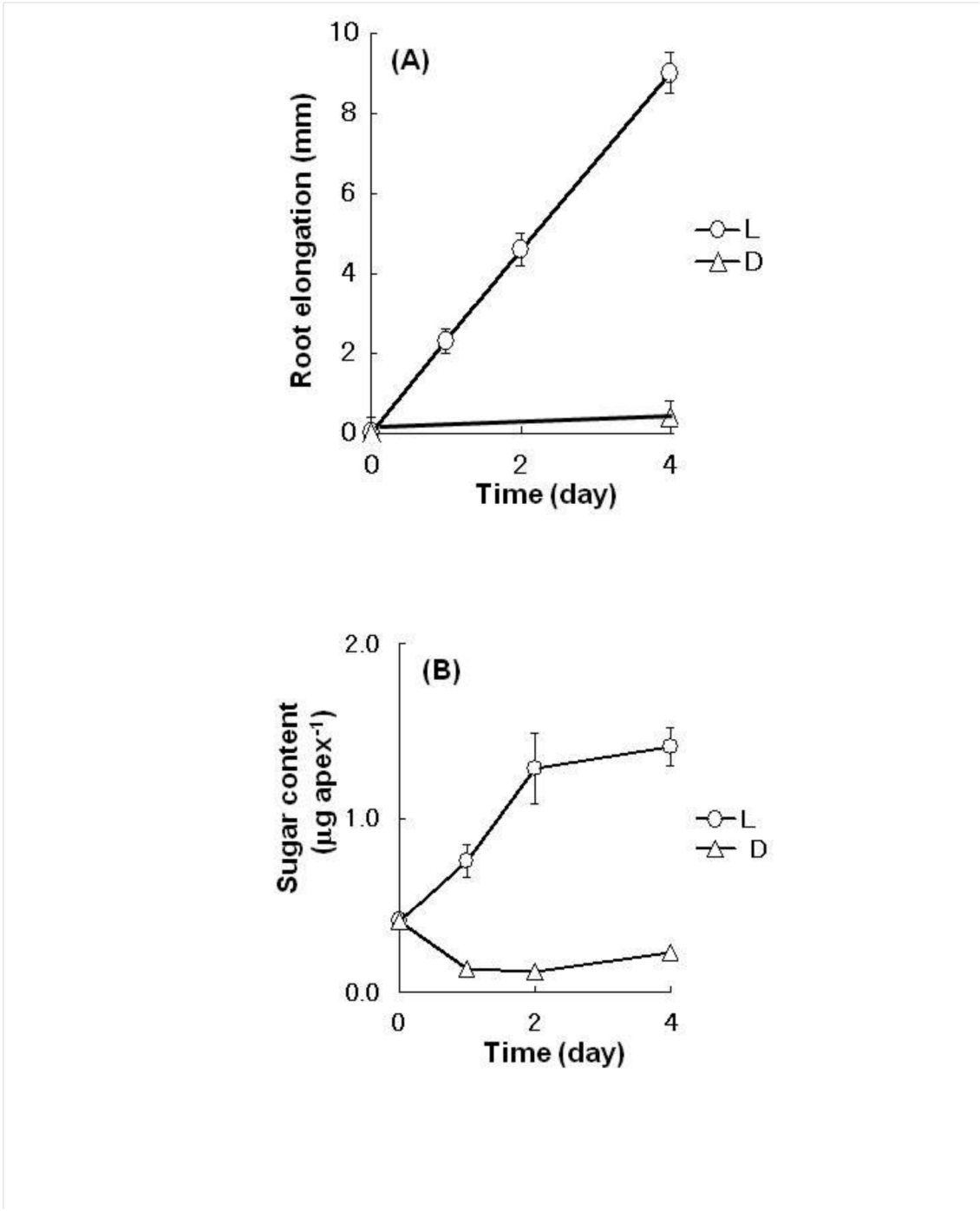

Figure 1: Root elongation of tobacco seedlings depends on the light

Tobacco seedlings were hydroponically cultured in growth medium in the light (O) or dark (△) condition for up to 4 days. At times, root length (A) and free sugar content at root apices (B) were determined.

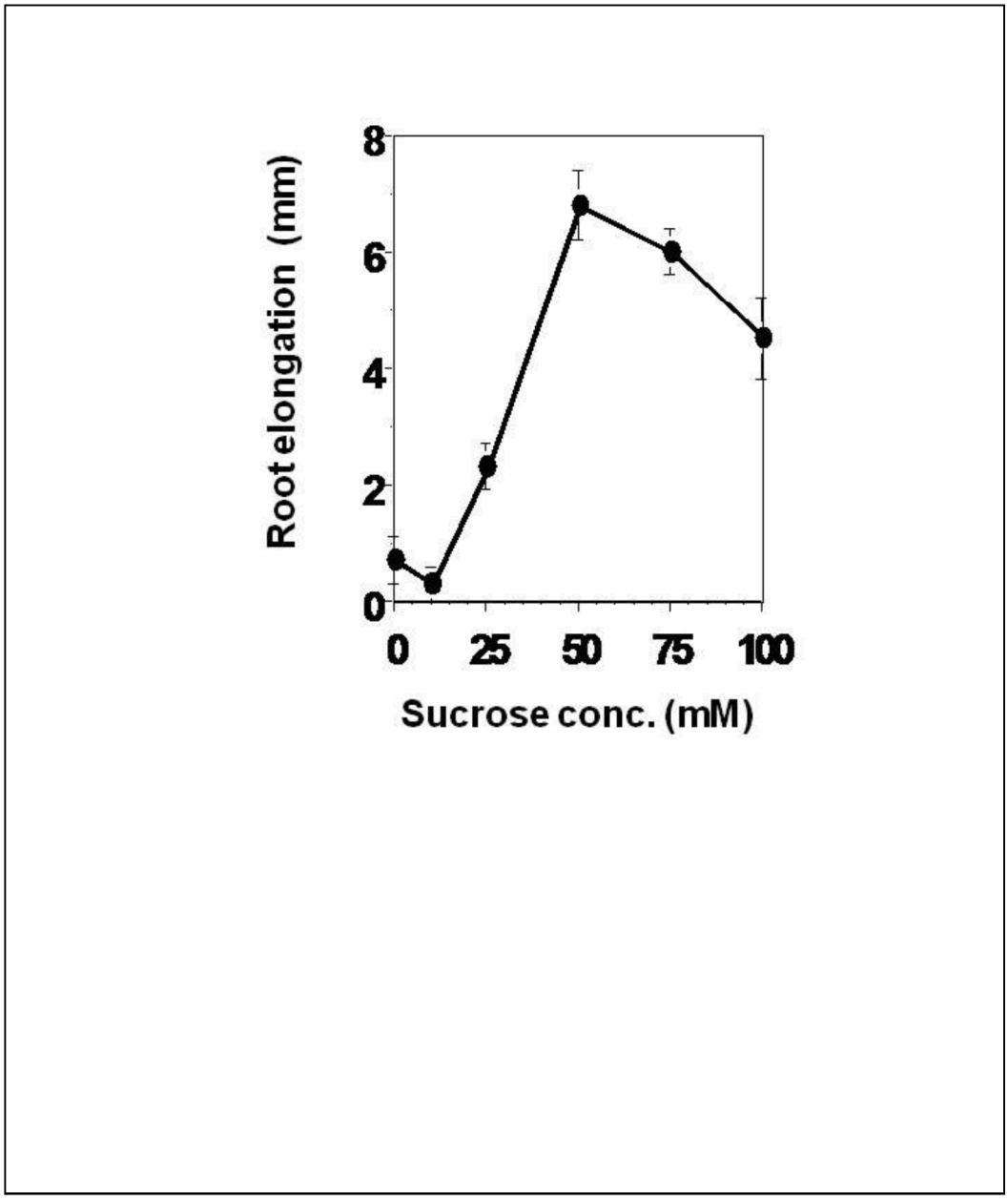

Figure 2: Exogenous supply of sucrose supports root elongation of tobacco seedlings in the dark

Tobacco seedlings were hydroponically cultured in growth medium containing various concentrations of sucrose, in the dark for up to 3 days. Root length was measured at a start and after 3 days, and root elongation during the culture was determined.

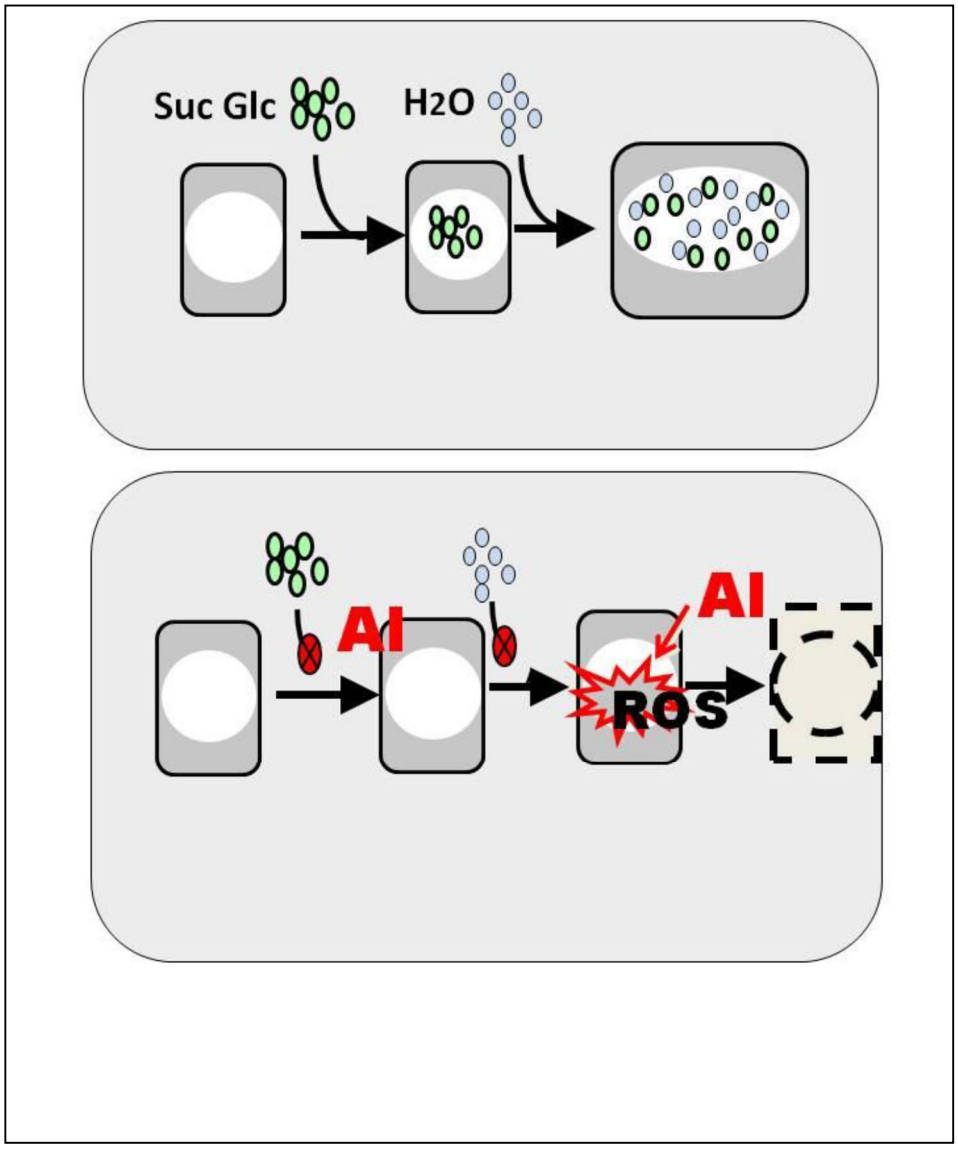

Figure 3: Model for a mechanism of aluminum-induced cell elongation inhibition in tobacco cells

Without aluminum (Top), the uptake of sugars increases osmolality, which could be a motive force of water uptake into vacuole, hence cell elongation. With aluminum (bottom), the uptake of sugar is inhibited, which prevents water uptake, hence cell elongation. The aluminum-induced root elongation inhibition seems to be explained by this mechanism. Other events caused by aluminum, such as callose production, respiration inhibition, ROS production, ATP depletion, start in relatively late phase and seem to be related to cell death.