高等植物のアルミニウム毒性と耐性の生理学的および生化学的研究*

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Physiological and Biochemical Studies on Aluminum Toxicity and Tolerance in Higher Plants

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Ⅰ. General Introduction

Approximately 40% of the world's cultivated lands, and up to 70% of the potentially arable lands, are acidic. In acidic soils, aluminum (Al) toxicity has been identified as a major factor limiting root growth of agriculturally important plants. In addition, interest in the phytotoxicity of Al has increased recently due to its role in plant decline in areas affected by acid precipitation and oxidation of sulfur compounds that are involved in releasing Al from mineral deposits in soils. The initial and most dramatic symptom of Al toxicity is inhibition of root elongation, which can be observed within 1-2 hr after exposure to Al. Although considerable research has been directed to elucidating the mechanisms of Al toxicity, the process(es) of inhibition of root elongation by Al and the mechanisms of Al tolerance remain to be characterized. It is known that the apoplasm serves as a major sink of Al in plants. Furthermore, current literatures suggest that the plasma membrane and cell walls seemingly play a crucial role in exclusion of Al from the root symplasm. However, an Al effect on root cell walls has not been extensively studied. Therefore, in the present studies changes in cell wall components induced by Al as well as Al interactions with the root cell walls were investigated in order to clarify the mode of action of Al in growing root tissues. This study helps us to understand some of the fundamental processes that induce Al toxicity and also confer Al tolerance on plants.

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Ⅱ. Aluminum-induced rapid root inhibition and changes in cell wall components of squash seedlings

Growth of etiolated squash (*Cucurbita maxima* Duch.) roots was significantly inhibited by 1mM $AlCl_3$ as early as 1 hr after treatment. The growth inhibition was confined to the elongating zone (1-6 mm from the tip). Microscopic observation confirmed that there was no swelling or apparent change in the root treated with Al within 6 hr, although notches of the epidermis without swelling were observed in the elongating zone only after 10 to 12 hr. The results clearly indicate that Al rapidly reduced squash root growth by inhibiting cell elongation.

Chemical analysis of cell wall polysaccharides from roots revealed that Al increased pectin, hemicellulose and cellulose contents after 3 hr of treatment. The effect of Al on pectin content was found in the elongating zone including the root tip, whereas the increase in cellulose content was confined only to non-elongating zones. Hemicellulose content was increased in all the regions along the root axis. Analysis of the neutral sugar constituents of pectin indicated that the increases were due mainly to the increases in Gal, Ara and Glc components. 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 of main pectic polysaccharides. Analysis of the sugar composition of hemicellulose showed that Glc, Xyl and Gal are the main components. The increases in the amount of Glc and Xyl are likely attributed to the increase in xyloglucan content by an Al effect. Although changes in the level of polysaccharides appear to be a consequence of growth inhibition, the Al effect on increases in the amount of pectin and hemicellulose was first found in this study. There are two possible explanations for the wall changes: stimulation of the synthesis or inhibition of the degradation.

Firstly, cell wall synthesis has been associated with the mechanism of Al tolerance. 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 and leading to detoxification of Al. Furthermore, removal of root mucilage increased the sensitivity of the root to Al, suggesting that Al was bound to the secreted mucilage. Therefore, the present data suggest that a drastic increase in pectin of squash root walls induced by Al in the root tip could offer protection and played an important role in the Al tolerance mechanism.

Secondly, non-cellulosic polysaccharides of cell walls undergo massive turnover during elongation growth. If Al inhibits the degradation of polysaccharides, such inhibition results in the accumulation of non-cellulosic polysaccharides in the cell walls. Degradation of non-cellulosic polysaccharides is a prerequisite for auxin-induced cell elongation. Therefore, inhibition of such degradation possibly results in inhibition of cell elongation.

Ⅲ. Interactions of Al with cell wall components extracted from the root tips of squash seedlings

The fact that the Al effect on the amount of wall fractions was not found during the 1st hour of treatment, clearly indicates that the early stage of growth inhibition was not

explained merely by the amounts of cell wall fractions or wall components. However, the changes in the cell wall components induced by Al found in later stage are likely involved in the recognition as well as in the subsequent defense response of plants towards pathogens or environmental stress factors. If the cell wall polysaccharides serve as an Al detoxification, Al binding to or interaction with the polysaccharides should first take place within the cell walls and/or at the plasma membrane surfaces.

The Al-binding capacity was tested in vitro as follows; To the purified pectin or hemicellulose (1mg /l solution), 27 μg of Al was added. The mixture was allowed to stand for 1 hr, then eluted through a PD-10 column (Sephadex G-25). Added Al was co-eluted with pectin or hemicellulose both at pH 5.5 (Na-acetate buffer) and pH 6.5 (MES/KOH buffer), indicating that Al was bound to pectin and hemicellulose. In order to confirm the Al-binding capacity of cell wall polysaccharides, a precipitation experiment was conducted. Increases in the amounts of added Al caused precipitation of total sugar content of water-soluble and pectic fractions at pH 6.5, but not at pH 5.5. Added Al caused precipitation of only about 25% of the total sugar content of hemicellulose at pH 6.5. Furthermore, Al co-precipitated with water-soluble or pectic polysaccharides in the molar ratio of 1:1 (polysaccharides : Al), whereas with hemicellulose the ratio was 3:1, suggesting that the Al-binding capacity of water-soluble and pectic polysaccharides was higher than that of hemicellulose.

It is well known that different pHs induce different Al ions in solution. At pH 5.5, most Al exists as $Al(OH)_2^+$ ion, and at pH 6.5, $Al(OH)_4^-$ ion is predominant. The differences in Al ions, such as the net charges and the hydroxyl groups, might be attributed to different Al-bindings within the cell walls. Cis-diol residues of terminal neutral sugars and free carboxyl groups of acidic polysaccharides appear to be good candidates for Al binding or chelation. Thus, Al probably possesses a monovalent binding site at pH 5.5 and a divalent binding site at pH 6.5. The divalent binding of Al might cause a cross-linking between two polysaccharide molecules, resulting in precipitation of Al-polysaccharide complexes in the solution. The monovalent binding of Al at pH 5.5 could not cause precipitation, but could still induce toxic effect, because it reduces the activities of wall degrading enzymes responsible for cell elongation by interfering their access to the substrates.

Since the treatment of squash roots with Al induced increases in Gal and Ara residues of pectin, arabinogalactan (AG) probably involved in Al binding. Larchwood AG (type I or 1,4-linked AG) and gum arabic (type II or 1,3 & 1,6-linked AG) were used in the Al-binding test. The results indicated that Al was specifically bound to AG type II with β -1,3-Gal*p* backbone.

Analysis of Al content of cell wall fractions extracted from the root tips showed that, even in the absence of Al in the culture medium, Al did normally exist in the root tissues mainly in the water-soluble and cellulose fractions. Treatment of roots with Al induced further accumulation of Al in the water-soluble, pectic and cellulose fractions. The results suggest that the roots accelerate the synthesis of cell wall polysaccharides in response to Al for the accommodation to Al toxicity.

Ⅳ. General Discussion

In conclusion, the present studies clearly revealed that Al rapidly inhibits the root growth primarily by inhibiting the cell elongation. Chemical analysis of cell walls from roots indicated that Al increased the amount of acidic and neutral polysaccharides in the actively growing regions and the amount of cellulose in the non-growing regions. Al-binding test and precipitation experiment showed that Al was bound to the cell wall polysaccharides by at least two binding modes: monovalent binding at pH 5.5 and divalent binding at pH 6.5.

The results suggest that acidic and neutral polysaccharides from root cell walls play an important role in Al binding or chelation. Improvement of wall polysaccharide biosynthesis, especially acidic polysaccharides with AG side chains by plant breeding or biotechnology may render Al resistance to the useful crops.