Aluminum alters the physiological characteristics of plasma membrane in agriculturally important crops

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Abstract

Aluminum (Al) in acid soils, which comprise approximately 30-40% of worldwide arable land, has been recognized as a serious global problem with regard to crop production. Phytotoxic species (Al\(^{3+}\)) at low pH has been shown to exert a profound inhibitory effect on root elongation. Here we have analyzed the physiological and biochemical responses of plasma membrane (PM) to Al stress in important agricultural crops. The close correlation between Al-induced growth inhibition, Al accumulation and calllose formation in root apices was found. The changes in surface pH mediated by altered dynamics of H\(^+\) efflux and influx across the root tip PM play an important role in root growth as affected by Al. Also, a zone-specific depolarization of PM surface potential coupled with inhibition of H\(^+\)-ATPase activity. Interestingly the hydraulic conductivity in Al-sensitive cultivar was markedly reduced only in root tip cells but the turgor pressure was not affected. These effects may indicate a direct Al interaction with H\(^+\)-fluxes and water transport of the PM in root apices.

Media summary

Al-induced exudation of organic acids, as a basis for the mechanism of Al tolerance, is accompanied with changes in PM surface potential, the activation of H\(^+\)-ATPase and water transport.

Key Words

Aluminum stress, root growth, plasma membrane, H\(^+\)-ATPase, tolerance, water transport

Introduction

Al is one of the most abundant minerals in the soil accounting for approximately 7 %. Acidic soils occupy about 40% of agricultural land around the world and the phytotoxic species of Al\(^{3+}\) is solubilized to the levels that inhibit root growth resulting in crop losses (Matsumoto et al. 2001). The root apex, which is the primary target sites for Al, plays a central role in the expression of Al-tolerance and toxicity (Ryan et al. 1993). Exclusion of Al by chelation with organic acid anions, such as citrate (Miyasaka et al. 1991), malate (Delhaize et al. 1993) and oxalate (Ma et al. 1997), excreted from root apices, has been suggested as an Al tolerance mechanism. While the apoplastic and symplastic target sites of Al in plant cells are under debate, several studies have focused on the plasma membrane (PM) as having a key function (Kinraide et al. 1998). PM properties such as surface negativity or zeta potential (Ahn et al. 2001, 2004; Ahn and Matsumoto 2006) have been reported to be altered by Al, and may be important as barriers to the passive movement of Al into root cells. The present study mainly focuses on plant responses to Al stress at the membrane level in root apices of several economically important crops, which would be useful to understand Al toxicity and tolerance mechanism.

Methods

Uniform seedlings of squash, wheat and soybean were grown in a controlled-environment chamber with a 14/10 h day/night cycle. The primary root length was measured with a ruler after the
commencement of Al treatment. The Al contents were determined by spectrophotometer after dilution. PM vesicles were prepared at 4°C and H⁺-pumping and H⁺-ATPase activities were determined. To visualize alkalization along single intact roots in the presence or absence of Al a modified agarose plate technique was used. The electrophoresed PM proteins were electroblotted onto PVDF membrane and the H⁺-ATPase was detected using standard western blot protocol with an antibody raised against maize H⁺-ATPase. Immunolocalization of H⁺-ATPase was performed essentially as described by Sivaguru et al. (1999). The zeta potential of PM vesicles isolated from root was calculated from the mobility in free-flow electrophoresis. A cell pressure probe was used to measure the hydraulic conductivity of root cortical cells as described by Zimmermann et al. (2000).

Results

Al-induced growth inhibition is associated with impaired H⁺-flux across PM in roots
The presence of Al, determined by highly sensitive growth-measuring method, preferentially inhibited the central elongation zone, which is located 2-4 mm from the tip. There exists close correlation between Al-induced growth inhibition, Al accumulation and callose formation in root apices of squash. The inhibition of H⁺-pumping rate in the highly purified PM vesicles obtained from the Al-treated apical root portions (1 cm) coincided with the inhibition of root growth under Al stress. Using pH indicator (Bromocresol Purple), surface pH of the control root apices was strongly alkalized from the starting pH 4.5 in a time-dependent manner. In contrast, the surface pH changed only slightly in the Al-treated root apices. The changes in surface pH mediated by altered dynamics of H⁺ efflux and influx across the root tip PM play an important role in root growth upon exposure to Al.

Aluminum inhibits H⁺-ATPase activity and alters PM surface potentials permanently in roots
The spatial pattern of Al-induced root growth inhibition is closely associated with inhibition of H⁺-ATPase activity coupled with decreased surface negativity of plasma membrane (PM) vesicles isolated from apical 5 mm root segments of squash plants. The highest positive shifts (depolarization) in zeta potential of the isolated PM vesicles from 0-5 mm regions of Al treated roots were corresponded to pronounced inhibition of H⁺-ATPase activity. The depolarization of PM vesicles isolated from Al treated roots in response to added Al in vitro was less than that of control roots, suggesting, particularly in the first 5 mm root apex, a tight Al binding to PM target sites or irreversible alteration of PM properties upon Al treatment to intact plants. Immunolocalization of H⁺-ATPase revealed decreases in tissue-specific H⁺-ATPase in the epidermal and cortical cells (2-3 mm from tip) following Al treatments. These results provide the first circumstantial evidence for a zone-specific depolarization of PM surface potential coupled with inhibition of H⁺-ATPase activity. These effects may indicate a direct Al interaction with H⁺-ATPase from the cytoplasmic side of the PM.

PM surface potential and H⁺-ATPase activity in near-isogenic wheat lines differing in tolerance to aluminum
This study aims to clarify the effect of Al on the surface potential (zeta potential) and PM H⁺-ATPase activity, as linked to exudation of organic acid anions, using near-isogenic Al-tolerant (ET8) and Al-sensitive (ES8) wheat lines. After 4-h in vivo treatment with Al (2.6 µM), H⁺-ATPase activity and H⁺ transport rate were decreased and zeta potential was depolarized in PM vesicles from root tips (10 mm) of Al-sensitive ES8 but not of Al-tolerant ET8. In vivo treatment with 50 µM malate alleviated deleterious Al effects on H⁺-ATPase activity and the zeta potential in PM vesicles isolated from root tips of ES8. In PM vesicles from root tips, in vitro treatment with 10 µM Al had a greater inhibitory effect on H⁺-ATPase activity of ES8 than that of ET8 but not of the region distal to the root tip. Al-induced exudation of malate, as a basis for the mechanism of Al tolerance, is accompanied by changes in PM surface potential and the activation of H⁺-ATPase.

Differential response of water transport activity in Al-sensitive and –tolerant soybean roots
In both cultivars, turgor pressure (about 0.1-0.2Mpa) was not altered by the presence of Al for 24 h. However, hydraulic conductivity was more reduced in sensitive cultivar than tolerant one. It is suggested that the gating of aquaporins is involved in such response.
Conclusion

The present results imply the important role of plasma membrane (PM) in terms of tolerant mechanism to Al stress. When plants are under Al stress, followings are considered to be important observations made in the present study:

1) The inhibition of root growth was dependent on Al concentration and the duration of exposure.

2) The damage occurred preferentially in the root tip with high Al accumulation and callose formation.

3) Al altered dynamics of H⁺ efflux and influx (changes in surface pH) across the root tip PM.

4) Al inhibited the H⁺-ATPase activity by permanently altering PM surface potentials.

5) Al induced PM surface potential and H⁺-ATPase activity in near-isogenic wheat lines.
differing in tolerance to Al.
6) Al also reduced the hydraulic conductivity in root tip cells.

References


