The role of ethylene metabolism in the short-term responses to aluminium by roots of two maize cultivars different in Al-resistance

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Abstract

The possible role of ethylene in the initial signal transduction of Al-induced root growth responses was investigated in two tropical maize (Zea mays) varieties that differ in Al resistance: ATP SR Yellow and HS 701 B. The intensity of Al toxicity effects were evaluated after short (4 and 24 h) exposure to 50 μM Al in complete low ionic strength nutrient solution. Relative root elongation rates (RER) and callose formation in root tips were used as stress indicators. Ethylene production by the root tips and 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase activities were analysed. After 24 h exposure to Al, both less callose production and higher RER indicated that ATP SR Yellow was more Al resistant than HS 701 B. The Al resistance of ATP SR Yellow, however, was not expressed after 4 h exposure to Al, when increased callose and decreased RER were observed. In any of the varieties and after any of the time-treatments an Al-induced increase of ethylene production was found. Our results indicate that the Al-resistance genes were not constitutively expressed in the absence of Al in the medium, but activated upon exposure to Al. An efficient protection against Al was achieved after a lag time of more than 4 h. Enhanced ethylene formation does not seem to play a role either in the Al-induced inhibition of root elongation or in the induction of the resistance mechanism. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Aluminium toxicity is considered one of the most important abiotic stress factors limiting crop production on acid soils in the tropics (Foy, 1984). Many investigations have demonstrated that toxic Al concentrations rapidly affect root elongation and that root tips are the primary site of Al-induced injury (Ryan et al., 1993; Llugany et al., 1995; Sivaguru and Horst, 1998; Vázquez et al., 1999). The mechanisms of Al-induced inhibition of root growth are not clearly established (Barceló et al., 1996). Early experiments have demonstrated that Al inhibits root cell division (Clarkson, 1965). However, the fact that Al can
inhibit root elongation within minutes, in combination with the formerly common view that Al enters slowly into the symplasm, has led to the hypothesis that toxic effects of Al in the apoplast are responsible for the fast inhibition of root growth (Horst, 1995). Recently it was found that Al can cross the plasmamembrane within minutes or, at least, a few hours (Lazov et al., 1996; Rengel and Reid, 1997; Vázquez et al., 1999), so that inhibition of cell division or other toxic effects inside the cells may also be responsible for early root effects. According to Lazov and Holland (1999) the effects of Al on cell extension would be largely reversible, while cell division is irreversibly inhibited by Al.

Besides the inhibition of root elongation, early symptoms of Al-toxicity in roots are the depletion of microtubuli in the distal transition zone of the root apex (Sivaguru et al., 1999), apical swelling, callose deposition (Wisemeier et al., 1987; Llugany et al., 1994; Massot et al., 1999), the formation of barrel-shaped root cells (Gunsé et al., 1997) and the swelling of cell walls in the tip (Vázquez et al., 1999). Similar effects have also been reported for other stress factors and may be related to ethylene (Lynch and Brown, 1997; Morgan and Drew, 1997). Whether or not ethylene plays a role in the early signal transduction of Al-induced root growth inhibition is not established. In order to test the hypothesis of ethylene being involved in root responses to Al in this study we analysed the influence of Al on ethylene production in root tips of maize varieties that differ in root growth responses to Al. Callose production and morin staining were used in order to visualize the varietal differences in Al-sensitivity after short-term (hours) Al exposure.

2. Materials and methods

2.1. Plant material and growth conditions

Maize (Zea mays L.) grains of the Al resistant variety ATP SR Yellow (C. The, Yaounde, Cameroun) and the Al sensitive variety HS 701 B (EMBRAPA, Siete Lagoas, Brazil) were germinated between layers of filter paper moistened with 0.4 mM CaCl₂ for 7 days. Uniform seedlings with grain were transferred to beakers (volume 30 L, 30 plants per beaker) containing continuously aerated nutrient solution of the following composition in μM: 200 CaSO₄, 100 MgSO₄, 400 KNO₃, 300 NH₄NO₃, 15 MnSO₄, 0.38 ZnSO₄, 0.16 CuSO₄·5 H₂O, 10 Fe-EDTA, 5 NaH₂PO₄, 16 H₂BO₃, 0.06 (NH₄)₆Mo₇O₂₄·4H₂O, pH 4.5 and supplemented or not (control) with 50 μM Al in the form of AlCl₃. The sum of monomeric Al species in the solution with a nominal total Al concentration of 50 was 32 μM, according to the short-term colorimetric method of Kerven et al. (1989). The plants were grown in a growth chamber under the following conditions: 16 h light/8 h darkness; PPFD 330 μM m⁻² s⁻¹; day/night temperature 24/18°C and RH 50/80%.

2.2. Root elongation

The length of the longest root per plant, the primary root, was measured with a ruler before and after the 4- and 24-h treatments and the root elongation rates were calculated as the difference between initial and final length divided by the exposure time.

2.3. Visualization of callose and Al in root tips

For the visualization of callose, the tips (5 mm) of roots were cut with a razor blade and immediately fixed in 70% ethanol (v/v) containing 5% formaldehyde and 5% propionic acid. The fixed tips were stained with 0.05% anilin blue (BDH 340034C) in 0.15 M K-phosphate buffer (pH 9.0). The tips were gently squashed between a microscope slide and its cover. The preparations were observed with a fluorescence microscope (Nikon Optiphot 2, Hg lamp, filter UV-1A, excitation filter 365/10 nm, dicroic mirror 400 nm, barrier filter 400 nm). Fluorescence from the epidermal cells and from the underlying outer cortical cells could be observed. Images were digitalized with a CCD camera (Supreme color CV-950) and a video card (IMAGRAPH Precision/Chroma-P, Imagraph Corp., MA).

Aluminium in the root tips was visualized by morin staining according to Tice et al. (1992).
Observations were made with a fluorescence microscope (Nikon Optiphot 2, Hg lamp, filter BV-2A, excitation filter 400–440 nm, dichroic mirror 455 nm, barrier filter 470 nm). The images were captured as described above.

2.4. Ethylene, ACC synthase and ACC oxidase

For ethylene analysis, 12–16 root tips (5 mm) per treatment and time sample were placed in a glass vial (3 ml capacity) containing 1 ml of the treatment solution. The vials were closed with a septum cap, and kept in darkness at 20°C. After 4 or 24 h gas, from the vial headspace was extracted and injected in a gas chromatograph (Hewlett Packard 5890 Series II) equipped with a 2 m 1/8” 60/80 packed alumina column (Supelco Inc., Bellefonte). Analysis were conducted under the following conditions: carrier gas, He (30 ml min⁻¹), oven temperature 105°C, injector temperature 110°C, FID, air/H₂ (300/30 ml min⁻¹), FID temperature, 250°C.

For the determination of ACC synthase and ACC oxidase activities, 5 mm root tips were frozen in liquid N₂ and homogenized with K-phosphate buffer (Riov and Yang, 1982). ACC synthase activity was determined according to Lizada and Yang (1979) and ACC oxidase activity was analysed following the method of Fernández-Maculet and Yang (1992).

2.5. Statistical analysis

All measurements were performed after 4 and 24 h exposure on at least four replicates per treatment and time sample. The data were subjected to a two-way analysis of variance (ANOVA) using \( P < 0.05 \) as critical level of significance.

3. Results

3.1. Aluminium effects on root elongation and callose formation in root tips

Clear differences in the growth responses to Al of roots from both maize varieties were observed after the 4 and 24 h Al treatments (Fig. 1). The root elongation rate of plants from the Al-sensitive variety HS 701 B was unaffected by the 4 h exposure to Al, but after 24 h the root elongation rate was severely inhibited, reaching only 15.6% of the control value. Roots from the Al-resistant variety ATP SR Yellow exhibited a faster root growth response. In this variety root elongation was significantly reduced after only 4 h exposure to Al (Fig. 1). However, after 24 h no differences in root elongation rates between control and Al-treated plants were observed in this variety.

Aluminium-induced callose deposition was observed especially in cortex cells of 5 mm root tips (Fig. 2). In maize variety HS 701 B intensive fluorescence indicating callose formation, was observed after 4 and 24 h exposure to Al (Fig. 2B, C). In contrast, root tips from variety ATP SR Yellow exhibited much callose after 4 h, but not after 24 h exposure to Al (Fig. 2E, F).

3.2. Morin-stainable Al in root tips

Root tips from both maize varieties significantly differed in Al accumulation as shown by fluorescence microscopy observations on root tips stained with the Al specific morin reagent (Fig. 3). In variety HS 701 B, Al accumulation in tips

![Fig. 1. Relative elongation rates of roots (percentage of control without Al) from Al-resistant variety ATP SR Yellow and Al-sensitive variety HS 701 B exposed to 50 µM Al for 4 or 24 h.](image-url)
Fig. 2. Fluorescence microscopy images of the surface of primary roots (5 mm from tip) of the Al-sensitive variety HS 701 B (A–C) and Al-resistant variety ATP SR Yellow (D–F) exposed to control (A, D) or 50 μM Al treatments for 4 (B, E) or 24 h (C, F). Tips were stained with anilin blue. Callose yields green fluorescence.
Fig. 3. Fluorescence microscopy images of tips of primary roots stained with morin for Al detection. A, B, and C show tips from Al-sensitive HS 701 B exposed to control (A) or 50 μM Al for 4 (B) or 24 h (C). D–F show tips from the corresponding treatments of Al-resistant ATP SR Yellow.
increased with the exposure time (Fig. 3B, C), and after 24 h the entire 5 mm tips exhibited the bright green fluorescence in an uniform way. The more mature zone of the tips from variety ATP SR Yellow exhibited more intense morin staining after 4 h exposure to Al (Fig. 3E) than that of variety HS 701 B (Fig. 3B), while the apical part of the tip was less stained in ATP SR Yellow. After 24 h, clearly less morin stainable Al accumulated in the cap and the meristematic zone in the Al resistant ATP SR Yellow (Fig. 3F) than in Al sensitive HS 701 B (Fig. 3C).

3.3. ACC synthase and ACC oxidase activities, and ethylene in root tips

Under control conditions, Al-resistant variety ATP SR Yellow exhibited lower ACC synthase activity (Fig. 4C) than the Al sensitive HS 701 B (Fig. 4D), while the contrary was observed for ACC oxidase activity (Fig. 4E, F). No varietal differences in ethylene production of root tips under control conditions were found (Fig. 4A, B). A 4 h-exposure to Al increased ACC synthase activity (Fig. 4C) and decreased ACC oxidase (Fig. 4E) activity in the Al-resistant ATP SR Yellow, while in the Al sensitive HS 701 B no statistically significant effects of Al on these enzymes were observed (Fig. 4D, F). After longer (24 h) Al treatment, less ACC synthase activity was observed in both maize varieties (Fig. 4C, D). In ATP SR Yellow, ACC oxidase activity was also decreased by the 24-h Al exposure (Fig. 4E), while no statistically significant effects were observed in variety HS 701 B (Fig. 4F). Within the short time frame of this experiment no influence of Al on ethylene production in the root tips was observed (Fig. 4A, B).

4. Discussion

The Al-resistance of maize variety ATP SR Yellow also exhibited higher Al resistance than HS 701 B as shown by relative root elongation rates (Fig. 1) and callose formation (Fig. 2) after 24 h exposure to Al. Nonetheless, variety ATP SR Yellow reacted quite Al-sensitive after a shorter exposure time (4 h). This observation is in line with previous investigations on Al–resistance in maize that also report a change in the effects of Al on both relative root elongation rates and cell wall damage during the initial 24 h exposure (Llugany et al., 1995; Vázquez et al., 1999). Moreover, in the present experiment the use of callose formation, a very susceptible marker for Al-sensitivity in maize (Horst et al., 1997), also demonstrates this change in the response to Al of the Al-resistant maize variety between 4 and 24 h exposure (Fig. 2E, F). Our data thus show that there is a lag time of more than 4 h before the root tips are efficiently protected against the toxicity of Al. The phenotypic plasticity of the resistant variety suggests that in maize the genes responsible for Al-resistance are not constitutively expressed in the absence of Al but activated upon exposure to toxic Al levels. According to current knowledge, Al-resistance in maize can be brought about by the excretion of organic acids, mainly citrate, by the root tips (Pellet et al., 1995; Jorge and Arruda, 1997). The accumulation of organic acids in the apoplastic space of root tips and at the root tip surface would decrease Al toxicity by the formation of less toxic Al-organic acid complexes at these sites. The decrease of intensity of morin fluorescence in the apex of root tips of ATP SR Yellow after 24 h exposure to Al (Fig. 2F) in comparison to the 4 h treatment (Fig. 2E) supports the view of a change in Al speciation in the tips of Al-resistant ATP SR Yellow after a lag time of more than 4 h. This change in Al speciation would be responsible for the recovery of the relative root elongation rate and the decrease of callose formation in root tips of the Al-resistant variety ATP SR Yellow between 4 and 24 h exposure. Accordingly, in the Al-sensitive variety HS 701 B both morin-stainable Al and Al-toxicity symptoms, in the form of callose depositions and inhibition of relative root elongation rates, increased with the time of exposure to Al-toxicity stress, because plants from this variety were unable to detoxify the Al.
In spite of the fact that maize plants showed severe root growth inhibition and stress-induced callose formation, ethylene production was either unaffected or decreased by Al toxicity. Several authors have found that ion toxicity stress can promote ethylene production in plants. Stress
ethylene formation has been found in plants exposed to toxic concentrations of Cd (Fuhrer, 1982), Cu or Zn (Gora and Clijsters, 1989). In contrast, other metal ions at toxic concentrations, such as Cr, Co and Ni inhibit rather than promote ethylene production (Yu and Yang, 1979; Poschenrieder et al., 1993). The effect of metal toxicity on ethylene production seems to depend on the type of metal ion and its concentration (Abeles et al., 1992). However, no clear relationship between the effect of metal ions on ethylene production and their binding preferences can be established. The aquo Al(III) ion is considered as a class A metal ion with high affinity for phosphate and other ligands with oxygen as metal-binding donor atom (Martell and Motekaitis, 1989); also Cr(III) preferentially forms complexes with N and O donor atoms as ligands and has little affinity for sulphide (Barceló and Poschenrieder, 1997). In contrast, metal ions that are known to enhance ethylene production in plants such as Cu²⁺, Cd²⁺ and Zn²⁺ show substantial Class B characteristics and preference for binding to nitrogen and sulphur centres (Woolhouse, 1983). However, Co²⁺ and Ni²⁺, metal ions that tend to decrease ethylene production, also have considerable Class B characteristics. Moreover, an interpretation of the differential effects of ion toxicity on ethylene production based on the binding preferences is hampered by the doubts about the ionic species that really is responsible for the primary toxicity effect in the plant tissues. This holds especially true for Al.

Metal toxicity-induced increase of ethylene production can be caused by either or both enhancement of lipid peroxidation and increased ACC synthase activity (Abeles et al., 1992). In this experiment, an Al-induced enhancement of ACC-synthase activity was only observed in the 4 h Al-treatment of the Al-resistant variety ATP SR Yellow. This increase, however, was accompanied by a decrease of ACC oxidase, i.e. the capacity to transform ACC into ethylene, and no promotion of ethylene evolution was detected.

Increased peroxidation of membrane lipids in root tips of soyabean has been observed only after long-term exposure to Al (Cakmak and Horst, 1991). Our observation, that short exposures to Al do not enhance ethylene production in root tips, is also in line with the view that lipid peroxidation is not an early event in the Al toxicity syndrome. In the view of our results enhanced ethylene formation does not seem to play a role either in the Al-induced inhibition of root elongation or in the induction of the resistance mechanism.

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