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Does root exudation of phenolics play a role in aluminium resistance in maize (*Zea mays* L.)?

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Abstract

Aluminium (Al) toxicity is widely considered to be the most important growth-limiting factor for plants in strongly acid mineral soils (pH < 5.0). Here the root-growth response of an Al-resistant maize variety (*Zea mays* L. var Sikuani) was studied at 0, 20 and 50 µM Al. Root elongation was followed over the first 48 hours of Al treatment, and during the initial 10 hours elongation was determined on an hourly basis. In addition, we investigated the role of root exudation of phenolics as a potential mechanism of Al detoxification and resistance. Aluminium resistance was only observed after exposure to 50 µM Al, and not after exposure to 20 µM, suggesting that there is a threshold Al concentration before the mechanisms of Al resistance are activated. Aluminium triggered release of catechol and of the flavonoid-type phenolics: catechin and quercetin. The exudation of phenolics was enhanced with increasing Al concentration: total exuded phenolics were about 10-fold higher at 50 µM than at 20 µM Al. The flavonoid-type phenolics, to date unconsidered, appear to play a role in the mechanism(s) of Al resistance.

Introduction

From recent evidence it appears that organic acids, which are able to form chelates with Al, play an important role in the detoxification of Al both externally and internally (Ma, 2000). If the formation of such Al-organic chelates has an important role in the mechanisms of Al resistance it follows that other oxygen donor compounds with strong Al-binding affinity could play an equally vital role in Al resistance. Several types of phenolics, such as the flavonoid-type phenols, also show strong Al-chelating ability and their exudation by root tips could potentially detoxify Al. The present work tests this hypothesis by examining the role of flavenoid-type phenolics in Al resistance in maize

Materials and methods

Plant material and growth conditions.

Seedlings of maize (*Zea mays* L. cv Sikuani) were grown in cell culture flasks (700 ml capacity) in continuously aerated solutions at pH 4.3. The composition of the nutrient solution was as follows (in µM): 500 $Ca(NO_3)_2$, 395 K_2SO_4, 5 KH_2PO, 100 $MgSO_4$, 200 NH_4NO_3, 0.06 $(NH_4)_6Mo_7O_{24}$, 5 $MnSO_4$, 0.38 $ZnSO_4$, 0.16 $CuSO_4$, 16 H_3BO_3, and 10 FeEDTA. Aluminium was added in the form of $AlCl_3.6H_2O$ at nominal concentrations of 0, 20 and 50 µM.

Cell culture flasks containing plant roots were photographed using OPTIMAS v6.0 at time 0 (before Al treatment), and then after 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 24 h, and 48 h of Al treatment. Root elongation rate (RER, cm $root^{-1}$ h^{-1}) was measured by overlaying the photographs in Photoshop v5.1.

Collection of root exudates

Seeds of the cv Sikuani were surface sterilised with 5% NaOCl and germinated in autoclaved glass Petri dishes on filter paper moistened with sterile 1 mM $CaSO_4$. Seedlings were then transplanted into sterile containers with two compartments containing sterilised growth solution (as above). The root tips of two primary roots were secured through two holes in the wall of the inner compartment. Seedlings were treated with 0, 20 or 50 µM Al for 24 hours.

Analysis of phenolic compounds

Extraction of soluble phenolics was performed according to Solecka *et al.* (1999). The phenolic fractions were identified by an HPLC procedure by means of a C18 Nucleosil column and two solvents: the solvent A was composed of 2 % acetic acid (v/v, in water) and the solvent B of acetonitrile-water-acetic acid (8:2:0.2, v/v/v). Phenolic compounds were monitored and analysed with a diode-array detector and compared with external standards.

Results

Root elongation

Aluminium at 20 µM significantly reduced root elongation ($p \leq 0.001$): at harvest, RER of Al-treated plants was 46 % that of control plants. Aluminium-induced inhibition of root growth started after 7 hours of exposure (Fig. 1). In contrast, after exposure to 50 µM the Sikuani variety showed Al resistance. Aluminium significantly reduced root elongation almost immediately (within 30 minutes of exposure Fig. 1), however, after 4 hours of exposure, RER recovered and there were no further significant differences between the RER of control plants and those of Al-treated plants.

Exudation of phenolics

Four main phenolics were detectable: catechin, catechol, curcumin and quercetin. Secretion of catechin or quercetin by seedlings grown in control nutrient solutions was either not detected or detected at low concentrations (about 2.5 nmol h^{-1} tip^{-1}). Increasing Al concentration from 20 to 50 µM stimulated root tip exudation (Fig. 2).

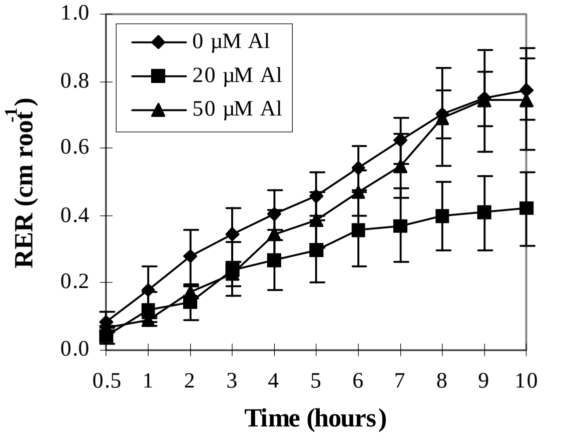

Figure 1. Root elongation rate (RER, ±SE) of cv Sikuani grown in nutrient solution at 0, 20 or 50 µM Al over the first 10 hours of Al exposure.

Discussion

In agreement with our earlier work, the Al-resistant cv Sikuani, when exposed to 50 µM Al, showed a lag time (of at least 3 hours) before Al resistance was observed, after which RER did not differ from that of the controls (Llugany *et al.* 1995, Gunsé *et al.* 2000). This result suggests that in these maize varieties the Al resistance mechanisms require induction upon Al exposure. The observation that Sikuani suffered inhibition of RER by 20 µM Al but not when exposed to 50 µM indicates

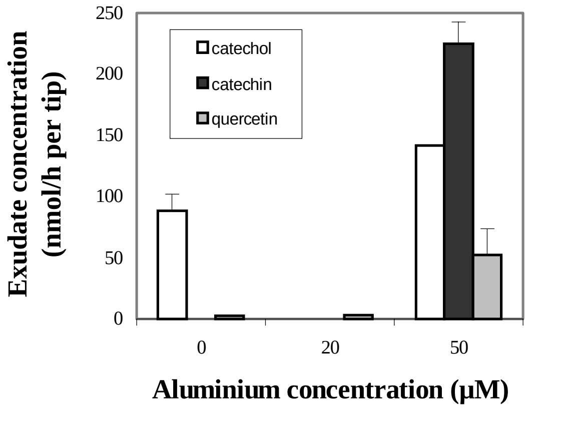

Figure 2. Root exudation of phenolics (nmol h^{-1} tip^{-1}, ±SE) from roots of Sikuani after exposure to 0, 20 or 50 µM Al for 24 hours.

that a minimum Al concentration (or threshold) may be required before the mechanisms of Al resistance are activated.

Al resistance in the maize variety Sikuani of the present study, as in other Al-resistant maize varieties such as C 525 M (Vázquez *et al.*, 1999) or ATP SR Yellow (Gunsé *et al.*, 2000), must be related to some kind of apoplastic detoxification of Al since phenotypic expression of resistance in the form of a recovery of RER coincided with a decrease in stainability of apoplastic Al by either morin (unpublished data) or hematoxylin (Gunsé *et al.*, 2000). According to our results Al-induced enhancement of exudation of phenolics may play a role in the detoxification of Al in the root tip apoplast. The most notable phenolics detected at substantial concentrations in root exudates were catechol and the flavonoid-type phenolics (catechin and quercetin). Curcumin was also detected, and was found at significantly higher concentrations in +Al groups than in controls, but its structural configuration suggests they are unlikely to be potential Al chelators. High concentrations of flavonoid-type phenolics were found in Sikuani at 50 µM Al, where root growth was not inhibited. In contrast, exposure to 20 µM Al did not enhance exudation of flavonoid-type phenolics and inhibited RER.

There is convincing experimental evidence demonstrating that certain flavonoid-type phenolics readily form complexes with Al in the root apoplast. Morin is a well-documented example. This, fluorescent reagent, has been used by many authors for visualising Al in the root apoplast (Gunsé *et al.*, 2000). Morin, the 2',3,4',5,7-pentahydroxyflavone, has high structural similarity to catechin (2-[3,4-dihydroxyphenyl]-3,4-dihydro-1[2H]-benzopyran-3,5,7-triol). The critical stability constant for the catechin-Al complex (3:1 type, log K = 40,92) is not only much higher than that for Al complexes with organic acids but also much higher than the log K for the proton-catechin complexes (Smith and Martell, 1989). Since, in our investigations, both Al-induced enhancement of quercetin and catechin exudation coincided with the maintenance or recovery of the root elongation rates in the Al-resistant cv Sikuani, and the exudation rates of these flavonoid–type phenolics were substantially higher than that of organic acids (data not shown), we propose that these flavonoid-type phenolics may play an important role in the apoplastic detoxification of Al in maize root tips.

References

Gunsé B, Poschenrieder C and Barceló J 2000 Env. Exp. Bot. 43, 73-81.

Llugany M, Poschenrieder C and Barceló J 1995 Physiol. Plant. 93, 265-271.

Ma JF 2000 Plant Cell Physiol. 41, 383-390.

Smith RM and Martell AE 1989 Critical Stability Constants. Plenum Publishers, New york.

Solecka D, Boudet AM and Kacperska A 1999 Plant Physiol. Biochem. 37, 491-496.

Vázquez MD, Poschenrieder C, Corrales I and Barceló J 1999 Plant Physiol. 119, 435-444.