Physiological Assessment of the Actual Role of Aluminum Exclusion Conferred by Citrate Release as an Aluminum Tolerance Mechanism in Maize

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INTRODUCTION

The exudation of organic acids in the rhizosphere is one of the mechanisms for Al tolerance best supported by scientific evidence in several plant species. Several experiments conducted at Embrapa Maize and Sorghum have showed the presence of these compounds in the nutrient solution in which the roots have been immersed, but this strategy did not allow us to locate the exudation zone to the root apex or elsewhere along the root. However, the root apex is considered to be the main site for the toxic effect of aluminum. Thus, knowledge of the spatial aspects of organic acid exudation along the roots is necessary to verify the effectiveness of this mechanism for Al tolerance in maize. The objective of this study was to identify and quantify organic acid exudation in response to Al along the roots of three maize lines contrasting for tolerance to Al toxicity.

MATERIAL AND METHODS

Three maize lines with different levels of aluminum tolerance were used: Cateto – tolerant, L3 – intermediate tolerance and L53 – susceptible, in two experiments in which the effects of time of exposure to Al and Al concentration on the exudation of organic acids from the root apex were evaluated. Seedlings were subject to 40 µM Al^{+3} activity for 0, 12, 24 and 48 hours, or to 48 hour-long treatments at 0, 20, 40 and 80 µM of Al^{+3} activity. The seedlings of each treatment were transferred to trays containing germination paper moistened with a 500 µM $CaCl_2$ basal solution and Al activities corresponding to the 4 treatments. Four-milimiter Whatman chromatography paper discs were placed in contact with the root apex of the seedlings for collection of organic acids. The remainder of the roots was covered with germination paper. Four replications of 12 seedlings were used for each treatment.

After two hours of contact with the roots, the chromatography paper discs were transferred to plastic tubes containing 1 ml of mili Q water, agitated with a vortex and filtrated. Organic acids were analyzed using a Shimadzu LC-10 model HPLC with a GROM-SIL 250 x 4 mm column, C-18, a mobile phase of 18mM KH_2PO_4 and a flow of 0,4 ml per minute. The organic acids were identified and quantified at a wavelength λ = 215nm and compared with an external standard containing malic, citric, succinic, cis and trans aconictic acid. Selected samples were analyzed for verification of possible contamination caused by microorganisms.

A second experiment was conducted to evaluate the sites of organic acid release along the root - the root tip, 15mm, and 50 mm from the root tip - after 48 hours of exposure to 40μM Al activity. The methodologies for exudate collection and determination were the same as described for the preceding experiment.

A third experiment was conduced to evaluate the aluminum concentration in root tips after 6, 12, 24 and 48 h of 40μM Al activity. Aluminum concentration was quantified by a colorimetric assay.

RESULTS AND DISCUSSION

Citrate was the main organic acid released from maize roots. Citric acid exudation increased with the exposure time and with increasing Al activities in the nutrient solution for the tolerant lines Cateto and L3 (Figures 1 and 2). The sensitive line L53 released smaller amounts of citrate in comparison to the tolerant lines, and did not show an increase in the exudation rate with time of exposure to Al. The sensitive line showed a similar behavior to the tolerant lines, but with a significantly smaller increase in the rate of exudation of citrate in response to increasing Al activities. The citric acid release was higher for the Al tolerant lines Cateto and L3 at the root tip than at 15 or 50 mm from the root tip after 48 hour in presence of 40 μM of Al activity (Figure 4). The enhancement of citric acid exudation in response to increasing Al concentrations and the greater exudation at the root apex were thus consistent with the role of organic acid exudation as an aluminum tolerance mechanism in maize. On the other hand, the higher exudation observed in L3 in relation to Cateto did not provide comparatively greater tolerance to Al. This can be verified by the greater reduction of seminal root growth of L3 after two days of exposure to 40 μM Al activity. (Figure 3). Even so, the higher exudation rate of L3 relative to L53 and Cateto did provide greater Al exclusion, as L3 showed smaller Al accumulation after 48 hours of exposure to Al (Figure 5). Our results suggest that other factors should be contributing to the high level of Al tolerance observed in Cateto besides an exclusion mechanism based on citrate exudation.