Aluminium localization and toxicity symptoms related to root growth inhibition in rice (*Oryza sativa* L.) seedlings

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We correlated root growth inhibition with aluminium (Al^{3+}) localization and toxicity symptoms in rice roots using seedlings of two genotypes (tolerant and sensitive) that were exposed to different $AlCl_3$ concentrations. Al^{3+} localization was evaluated by hematoxylin in primary roots and by morin in cross-sections of the root tips. Neutral invertase enzyme activity and callose (1→3, β-D-glucan) accumulation were observed and compared with Al^{3+} accumulation sites. Root growth was inhibited by Al^{3+} in a concentration-specific manner and proportional to the increase of hematoxylin staining, being more pronounced in the sensitive genotype. Morin staining showed the presence of Al^{3+} deep within the roots of the sensitive genotype, indicating that the metal was able to penetrate beyond the first few cell layers. In the tolerant genotype, Al^{3+} penetration was restricted to the first two cell layers. Ruptures in exodermis and epidermis layers by lateral root protrusions in both genotypes allowed Al^{3+} to enter into the roots. More intense activity of invertase in roots of the tolerant genotype was also observed, which could be related to greater root growth of this cultivar when submitted to Al^{3+} stress. Moreover, Al^{3+}-induced callose accumulation was a late response occurring in the same areas where Al^{3+} was present.

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1. Introduction

Considerable attention has been focused on assessing the impact of aluminium toxicity on cultivated plants, because its stress is often the primary factor limiting crop production in acid soils (Kochian 1995). The use of aluminium-resistant plants is a part of crop management strategy for agricultural production in acid soils, since this factor is particularly common in the world (Larsen *et al*. 1996). At low pH, the release of toxic aluminium soluble forms (particularly Al^{3+}) is enhanced by the dissolution of Al-containing compounds, thus becoming available to interact with plants and other organisms (Samac and Tesfaye 2003).

Different plant species and genotypes show distinct variation in tolerance and/or sensitivity to Al^{3+}, generating a broad spectrum of responses to Al^{3+} exposure (Ezaki *et al*. 2000). Several studies have provided evidence that the root apoplast plays a critical role in the tolerance mechanism, based on efflux of organic acids such as malate and citrate (Ma 2000; Horst *et al*. 2010). These substances are able to form a strong complex with aluminium, thus chelating Al^{3+} and rendering aluminium to a non-phytotoxic state. Ma *et al*. (2002) and Yang *et al*. (2008) reported that root organic acid secretion does not appear to contribute to differential Al^{3+} resistance among rice cultivars, and root cell walls may play an important role in excluding Al^{3+} specifically from the rice root. Another response is callose (1→3, β-D-glucan) formation, which is synthesized by plants in response to biotic and abiotic stress (Verma and Hong 2001). Aluminium ions can elicit callose formation, indicating Al^{3+}-injury to roots (Sivaguru and Horst 1998). Its deposition is considered as a marker for Al^{3+} toxicity (Rincon and Gonzales 1992) and is believed to mediate Al^{3+} toxicity effects (Sivaguru *et al*. 2000). Moreover, the impact caused by Al^{3+} can provoke alterations in morphology (Alvarez *et al*. 2012) and physiology of the roots, modifying the activity of numerous regu-

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latory enzymes such as invertases (Simon *et al*. 1994). For the division, elongation and expansion of root cells, sucrose can be taken up; it must be hydrolysed to glucose and fructose by invertase, an extracellular enzyme secreted into the acidic wall of cells (Koch 2004). Sucrose cleavage can initiate a distinctive profile of sugar signals, which can have profound developmental effects (Koch 2004). Thus, it is probable that the alterations in invertases activity induced by Al^{3+} can be part of a toxicity resistance mechanism to aluminium acting on cell walls.

In spite of the progress made, Al^{3+} exclusion mechanisms of some of the most Al^{3+}-resistant plant species such as rice (*Oryza sativa* L.) are still far from being well understood (Cai *et al*. 2011) and the cause of Al^{3+} toxicity needs much greater investigation (Horst *et al*. 2010). Selection of genotypes that tolerate Al^{3+}, as well as the understanding of this metal toxicity, ought to benefit breeders in rice-growing countries affected by Al^{3+} availability in soils. The identification of the primary anatomical sites and metabolic pathways involved in Al^{3+} toxicity are essential to better understand why some plants are tolerant to this metal while others are sensitive (Delhaize and Ryan 1995), as well as to comprehend its effects on plant survival. In this research, two rice genotypes with different sensitivities to Al^{3+} were used, since related genotypes are valuable study models for analysing mechanisms of toxicity and tolerance (Foy *et al*. 1978). It is probable that different sites of Al^{3+} accumulation in roots are related to the expression of distinct tolerance mechanisms. Thus, to confirm these hypotheses, root growth inhibition, invertase activity and callose accumulation were analysed and related to the sites of Al^{3+} accumulation revealed by hematoxylin and morin dyes.

2. Materials and methods

2.1 *Plant materials and growth conditions*

Two rice (*Oryza sativa* L) cultivars, Caiapó (Al^{3+}-resistant) and IAC-1289 (Al^{3+}-sensitive), were selected for this study. Prior to germination, all seeds were surface sterilized in 5% sodium hypochlorite for 20 min, rinsed thoroughly with water and allowed to imbibe in water overnight in the dark at 25±2°C. Deionized water was used throughout the experiments.

After imbibition, two pieces of filter paper were placed on each of 12 Petri dishes, 6 per cultivar, with 25 seeds sown on each dish and moistened with 5 mL of deionized water. All Petri dishes were covered by lids and placed for 24 h in a germination chamber at 30°C, under 12 h photoperiod. Ten healthy and uniform seedlings from each cultivar were then individually transferred to 70 mL glass test tubes containing deionized water. Styrofoam rings were used in order to hold them in place. The tubes were randomly placed in a growth room to ensure normal growing conditions. Artificial light was supplied at the intensity of 200 μmol $m^{-2}s^{-1}$ photon flux density at plant base level during the 16 h day, and the temperature was kept at 25±5°C. On the sixth day after germination, both cultivars were treated for 48 h with solutions of 200 μM $CaCl_2$ and $AlCl_3$ adjusted to pH at 4.0± 0.02 by addition of 0.1 M HCl or 0.1 M NaOH.

For better characterization of root growth response, the seedlings were exposed to a broad range of Al^{3+} concentrations (0, 20, 40, 60, 80, 100, 120, 140 and 160 μM of $AlCl_3$) in an experimental design of the 2 cultivars × 10 repetitions × 9 treatments.

2.2 *Root growth and aluminium localization*

Relative root elongation rate (RRE) during Al^{3+} treatment was determined by measuring the length of the whole root before and after 48 h of exposure to Al^{3+}. Root length was measured from the root–shoot junction to the tip of the primary root using a ruler (Tice *et al*. 1992). RRE data was submitted to ANOVA analysis. Means were compared by Tukey test at the $P \leq 0.05$ level of confidence (free software BioEstat 5.0).

In a parallel experiment to assess Al^{3+} accumulation and localization, 4 seedlings of each cultivar was exposed to 0, 40, 80 and 160 μM of Al^{3+} for the accumulation assay, and to 0 and 80 μM Al^{3+} for the localization assay, were washed with deionized water to remove unabsorbed Al^{3+} ions and stained as described below. Two independent experiments comprising three replicate sets of samples were analysed. Whole intact roots were stained with hematoxylin for 30 min, washed further in water and then photographed 20 min later when colour development had reached its maximum. The dye solution of hematoxylin was prepared 24 h before the assay by adding 2 g of hematoxylin, 0.2 g of KIO_3 and 0.1 mL of 0.1 M NaOH into 1 L of deionized water (Tice *et al*. 1992). For morin staining, roots were washed with NH_4OAc buffer (pH 5.0±0.01) for 10 min before being stained with morin for 30 min (Eggert 1970). The dye solution was prepared just before the procedure by adding 0.032 g of morin into 1 L of the buffer. After the staining procedure, the roots were washed again in the same buffer until the dye no longer eluted from the roots. Root segments bearing lateral roots were excised and transferred to a glass slide for observation using a microscope coupled with fluorescence. Free-hand sections were made with sharp razor blades, after the roots had already being stained with morin, in order to avoid artifacts related to tissue redistribution of Al^{3+}. The collected root cross-sections were rinsed in several changes of deionized water. The slides were prepared by mounting the sections in water and immediately examined and photographed.

2.3 *Invertase activity assay*

To detect invertase activity, 6 seedlings of each cultivar were exposed to Al^{3+} concentrations (0, 80, 160 µM) and root segments were incubated in a neutral reaction medium buffer containing 0.38 mM sodium phosphate (pH 7.5), 0.024% tetrazolium blue, 0.014% phenazin metasulphate, 30 U of glucose oxidase and 30 mM of sucrose at room temperature for 3 h (Doehlert and Felker 1987; Zrenner *et al*. 1995). For the control, an incubation medium without sucrose was used. A second control was performed without glucose oxidase. The samples were photographed on an Olympus light microscope.

2.4 *Callose accumulation assay*

Accumulation of Al^{3+}-induced callose was appraised by an aniline blue staining procedure after 6 seedlings of each cultivar were exposed to 0, 80 and 160 µM of Al^{3+} for 24 and 60 h. Whole roots were fixed in 10% formaldehyde, 5% glacial acetic acid and 10% ethanol (FAA) for 1 h to avoid wound callose formation. After this period the first centimeter of the root tip was excised and washed thoroughly in deionized water. The 1 cm segments were stained for 10 min in a solution of 0.1% water soluble aniline blue in 50 mM Glycine-NaOH buffer at pH 9.5 (Kauss 1992). The segments were mounted in the staining solution, gently squashed on a glass slide and observed with a fluorescence microscope. For the localization of callose in roots, the tissues were exposed only to 0 and 80 µM of Al^{3+} for 48 h. After this period, the roots were fixed and encased in wax and the cross-sections were made in a microtome. The prepared slides were stained with the aniline blue solution and observed using laser confocal scanning microscopy (ZEISS, LSM 510 – Germany).

3. Results

3.1 *Root growth inhibition*

Dose-dependent curves of root elongation inhibition are shown in figure 1. The RRE values are shown as the means of the six samples obtained from two independent experiments. Gravity-induced bending did not interfere with root elongation measurements because the roots were positioned vertically. The results indicate that the cultivars varied in their response to increasing concentrations of Al^{3+}. IAC cultivar plants had a greater inhibition rate than Caiapó. An 80% reduction in root growth of IAC occurred with 4 times lower concentrations of external Al^{3+} supply than those required to induce the same effect in Caiapó plants. At Al^{3+} concentrations of 40, 80 and 120 µM, differences between cultivars were significant ($P \leq 0.05$). It might also be noted

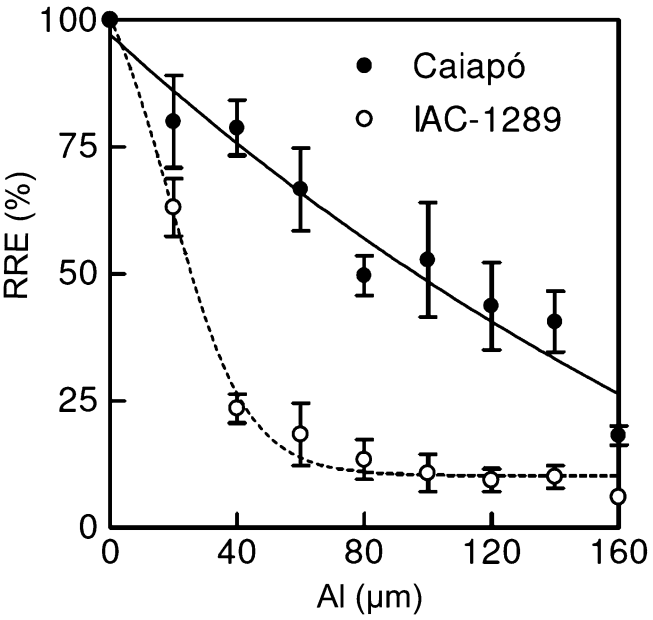

Figure 1. Relative root elongation (RRE) of rice seedlings of the genotypes Caiapó and IAC-1289, after 48 h at different Al^{3+} concentrations. Root length is expressed as a percentage of the control group (means±SE, n=6).

that at 160 µM Al^{3+}, there was no significant difference in RRE values for either of the tested cultivars.

Control plants showed white and healthy roots throughout the experiment. After 48 h, the Al^{3+}-treated roots presented an occasional occurrence of brown coloured spots as well as a swollen appearance. IAC plants exposed to 80 and 160 µM and Caiapó exposed to 160 µM of Al^{3+} had root cap cells that were peeled off and had visible root damage (figure 2).

3.2 *Aluminium localization by hematoxylin and morin dyes*

Al^{3+} localization and accumulation was assessed by two Al^{3+} indicator stains, morin and hematoxylin. Both Al^{3+} indicators showed homologous results and allowed the visual detection of Al^{3+} localization in root tissue. When whole roots were analysed, as shown in figure 2, higher concentrations of Al^{3+} in the root tissues caused more intense staining in IAC than in Caiapó plants, this response being more pronounced at the root tip. Control roots absorbed the hematoxylin stain, but there was no reaction with Al^{3+}, and therefore they appeared dull red. The same result was seen for Caiapó at 40 µM. In addition to the absence of Al^{3+} accumulation in the control and in Caiapó roots exposed to 40 µM Al^{3+}, the transparent appearance of the root caps could also be noted. Al^{3+} uptake at 40 µM occurred only in IAC at the root tip but not at the root cap; this region also

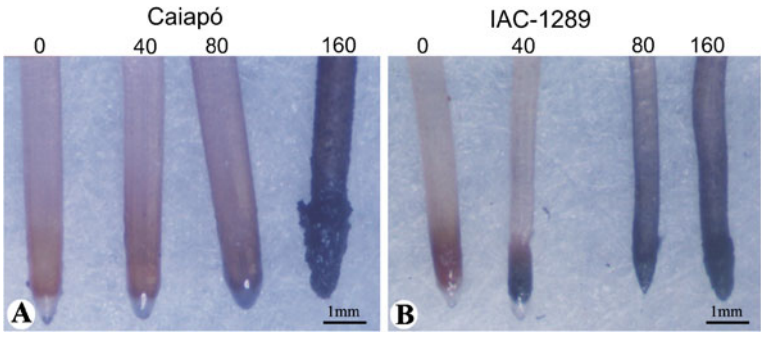

Figure 2. Apex of primary roots from rice seedlings of the genotypes (**A**) Caiapó and (**B**) IAC-1289, exposed for 48 h to 0, 40, 80 and 160 μM Al^{3+}, stained with hematoxylin 2%.

kept its transparent appearance. At 80 μM, the roots of both cultivars showed blue staining, including the root cap, but it was more intensely pronounced in IAC, demonstrating that in this cultivar, Al^{3+} accumulation was greater than in Caiapó at the same Al^{3+} concentration. The roots of IAC and Caiapó exposed to 160 μM of Al^{3+} were strongly dyed, showing high amounts of Al^{3+} in tissues (figure 2).

Caiapó root cross-sections showed considerably reduced morin staining when compared with IAC. But the difference was more substantial at the location of the fluorescent signals, which were stronger at the periphery for Caiapó and in the stele of IAC, as seen in figure 3. In Caiapó, Al^{3+} was specifically located at the outer cells and its entrance was blocked by the exodermis (figure 3B and D).

When the root segments bearing lateral roots were analysed, no difference could be observed between the cultivars, although there was a strong signal at the base of the lateral root (figure 3E and F), demonstrating a possible site for aluminium penetration.

3.3 *Invertase activity assay*

Even after 48 h of exposure to 80 and 160 μM of Al^{3+}, Caiapó roots presented great activity and expression of invertase (figure 4). This activity was more accentuated in the apex, where cellular divisions are pronounced in more basal portions of roots. However, in IAC roots the invertase activity was less when compared to Caiapó. After exposure to 160 μM of Al^{3+}, roots of IAC showed great invertase activity in root apexes and in lateral root protuberances (figure 4A). The activity and expression of invertase was more intense in Caiapó than IAC roots.

3.4 *Callose accumulation assay*

Under the fluorescence microscope, callose appeared as bright green-yellowish dots and the primary natural fluorescence of root tissues showed a vivid blue color (figure 5). The root samples of both cultivars at 12 and 24 h of Al^{3+} exposure did not present any different response when compared to the control plants. In all treatments, an occasional and faint fluorescent signal could be observed in odd positions of the 1 cm root tips. The lack of signal in some samples and the absence of image repetition shows that in spite of all the care taken to handle the roots, wound callose was formed in some cases. After 60 h of exposure to 80 μM of Al^{3+}, a homogeneous callose deposition pattern was observed in all samples at the apex of IAC and Caiapó roots. However, localization results demonstrated that the visual attempt to determine callose accumulation was not completely accurate. Using Confocal microscopy assay, callose deposition was observed in both cultivars (figure 6). In IAC, the Al^{3+}-induced callose was present randomly in the epidermis, cortex and stele. Contrastingly, a ring of callose deposition around the cortex and surrounding the exodermis cells could be observed in Caiapó in response to external Al^{3+} supplies (figure 6A and C).

4. Discussion

The visual toxic symptoms of Al^{3+}-treated roots were very similar to those reported earlier (Lazof *et al*. 1996; Alvarez *et al*. 2012). The brown spots could be due to oxidative stress or to the accumulation of phenolic compounds (Nagy *et al*. 2004). Root tip bending and peeling of cells are suggested by Yamamoto *et al*. (2001) to be the result of inhibition of peripheral cell elongation.

Species susceptible to aluminium toxicity are most likely to be vulnerable to this element during seedling development, since there is a shift at the source of nutrient supply from seed reserves to active uptake from the external medium. It has been shown that the distal part of the transition zone is the more Al^{3+}-sensitive site of the

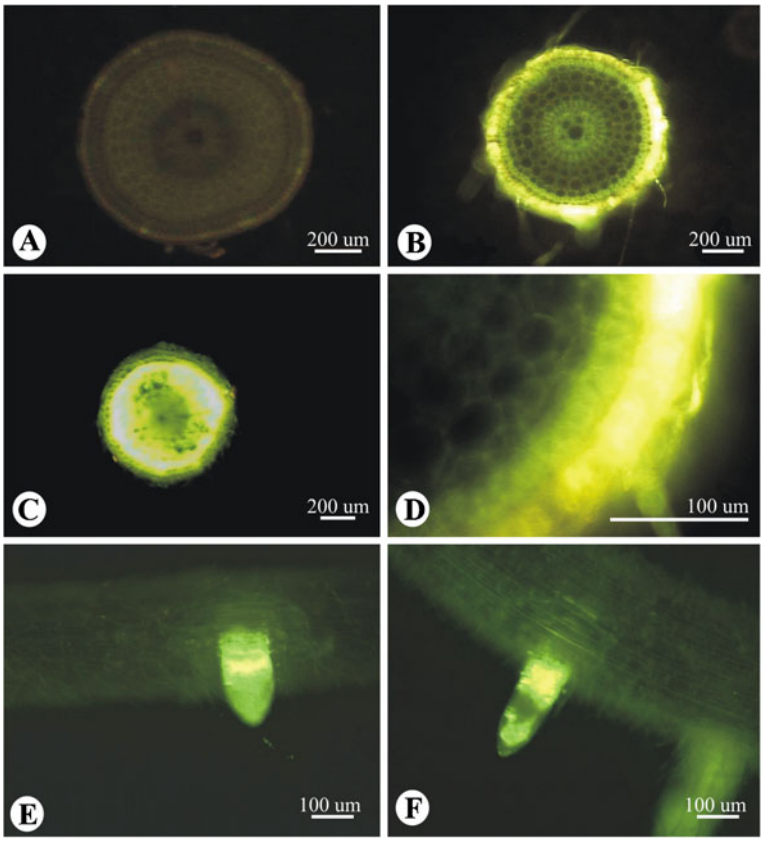

Figure 3. Aluminium localization in cross-sections of rice root tips stained with morin 100 μM after exposure to 80 μM Al^{3+} for 48 h. (**A**) Caiapó control, (**B**) Caiapó+Al^{3+}, (**C**) IAC+Al^{3+}, (**D**) Caiapó+Al^{3+}, (**E**) Caiapó and (**F**) IAC lateral roots from a mature segment of primary roots of rice seedlings.

root apex (Sivaguru and Horst 1998). For this reason, root length is the most accurate parameter for assessing tolerance and sensitivity to Al^{3+} toxicity. Root growth of both rice cultivars was inhibited by Al^{3+} in a concentration-dependent manner, being distinctly more pronounced in IAC than in Caiapó. Previous work has shown the IAC cultivar to be relatively sensitive to Al^{3+} (Marciano *et al*. 2010). Root growth inhibition has become a widely accepted measure of Al^{3+} toxicity in plants, although this symptom *per se* does not offer enough information about the causes of stress that lead to changes in root elongation. The comparison of Al^{3+} accumulation, defined by hematoxylin staining with RRE data, shows that root inhibition is directly related to Al^{3+} accumulation on root tips. In the hematoxylin staining procedure, Al^{3+} acts as a mordant binding to oxidized hematoxylin (hematein) with formation of purple-bluish coloured complexes (Baker 1962). Morin is a fluorescent dye with high sensitivity to Al^{3+} (Eggert 1970) and has been used as a means to detect the presence of Al^{3+} in plant tissues after short-term exposure to this metal (Tice *et al*. 1992; Larsen *et al*. 1996). Despite being highly sensitive for detecting low concentrations of Al^{3+}, neither morin nor hematoxylin staining should be used to study the distribution of Al^{3+} between cell compartments (Eticha *et al*. 2005). However, these staining procedures are relatively simple and fast tools to examine Al^{3+} localization and accumulation in root tissues.

The concentration of Al^{3+} (80 μM) chosen for the localization study was lower than that which caused the maximum inhibitory effect on root growth, with more pronounced effects in subsequent toxicity symptoms such as apex swelling and lesion development. However, at this Al^{3+} concentration it is possible to discriminate the Al^{3+} effects in IAC and Caiapó genotypes. Different patterns of Al^{3+} localization were observed between the genotypes through morin fluorescence images. In fact, the results indicated that the Al^{3+} entering the root over the 48 h exposure period had penetrated beyond the first few cell layers of IAC, but not of Caiapó. The presence of Al^{3+} deep within these root cross-sections does not prove any direct effects of Al^{3+} on cell metabolism, nor does it rule out any of the current hypothesis regarding control over Al^{3+} toxicity responses to sites on the root periphery (Yamamoto *et al*. 2001; Cai *et al*. 2011). Nonetheless, it demonstrates the existence of an important

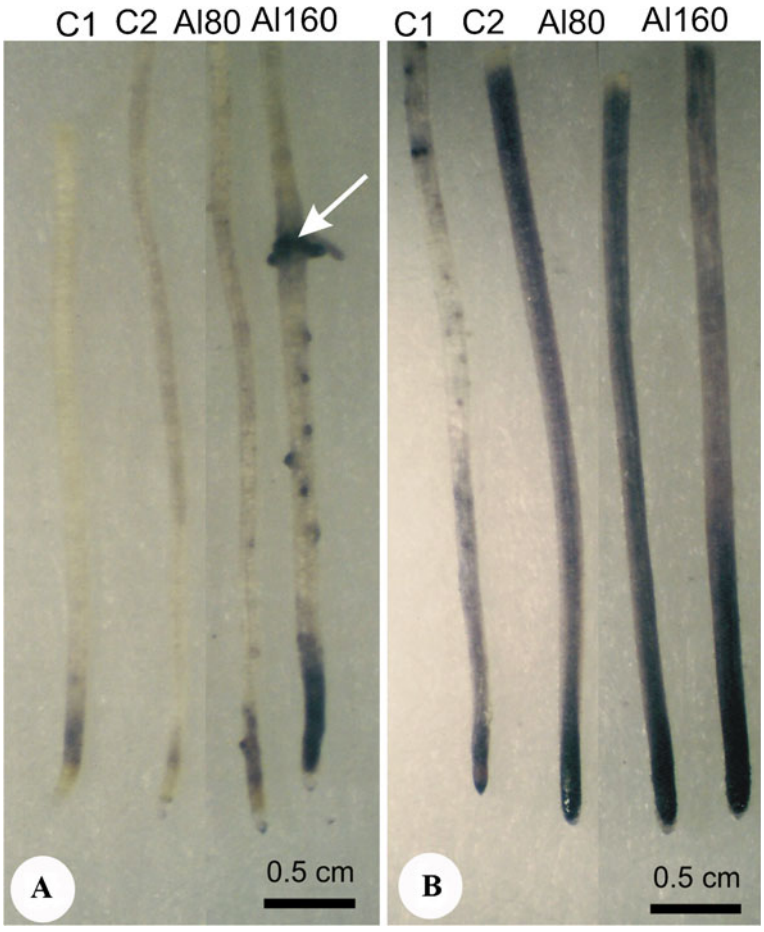

Figure 4. Invertase activity in root tips of rice seedlings of IAC-1289 (**A**) and Caiapó (**B**) genotypes after exposure to 80 and 160 µM Al^{3+} (C1 – medium without sucrose; C2 – medium without glucose oxidase).

barrier mechanism in Caiapó that prevents Al^{3+} penetration into the cortex.

The plasma membrane works as a barrier to Al^{3+} penetration since this metal, as well as other polyvalent ions, are virtually insoluble in lipid bilayers (Delhaize and Ryan 1995). Yet, through many different unknown ways, not only is Al^{3+} somehow able to permeate the plasma membrane but in some cases, as described by Tice *et al*. (1992), half of the total Al^{3+} accumulated in the root apex may be present in the symplasm.

Yamamoto *et al*. (2001) reported that in peas (*Pisum sativum*), prolonged treatment with aluminium caused the formation of cracks in root surfaces which allowed the metal to reach inner root tissues, leading to loss of plasma membrane integrity. Lazof *et al*. (1996) demonstrated that in a mature root section of soybean (*Glycine max*) bearing lateral roots, Al^{3+} penetration and accumulation within the first cell layers of the cortex happens readily and evenly during a 4 h exposure. They also showed that Al^{3+} levels in this region were greater than in the developmental zone in all positions. Silva *et al*. (2001), studying Al^{3+} tolerance in soybean found that Al^{3+} accumulation and growth inhibition were greater in lateral roots than in primary ones. The results shown in figure 3E and F suggest that the protrusion of the lateral roots, which ruptures the epidermis as well as the exodermis, is a phenomenon that allows more Al^{3+} to enter into the roots. These cracks are formed when the primordia of the lateral root, originating from the pericycle, penetrates through cell layers of the cortex, aiming to reach the root surface.

Physiological injuries triggered by Al^{3+} stress are closely dependent on membrane injury (Matsumoto *et al*. 1996). Zhang *et al*. (1994) observed an 86% increase in callose content in root tips of a sensitive wheat cultivar at 30 min of 75 µM Al^{3+} supply, but when Al^{3+}-resistant and Al^{3+}-sensitive cultivars were compared at equivalent levels of root growth inhibition, accumulation of callose did not differ between cultivars. Thus, they suggested that deposition

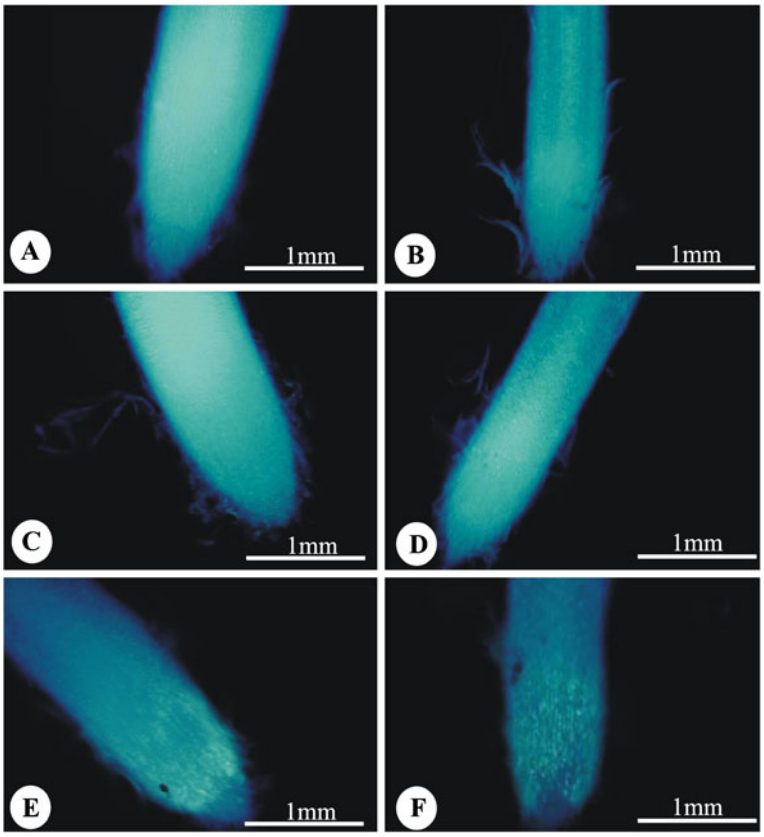

Figure 5. Callose localization on root tips stained with aniline blue 1% and observed under fluorescence microscope. (**A** and **B**) control, (**C** and **D**) after 24 h of exposure to 80 μM Al^{3+}, and (**E** and **F**) after 60 h of exposure to 80 μM Al^{3+}. (**A**, **C** and **E**) Caiapó, (**B**, **D** and **F**) IAC.

and accumulation of callose reflects physiological stress as well as the degree of cumulative cell injury, especially in the Al^{3+}-sensitive cultivars.

The release of Ca^{2+} from internal cell compartments is thought to increase activity of free calcium in the cytoplasm (Bhuja *et al*. 2004), which would act as both a signal of cell damage and an activator of callose synthesis. However, these authors demonstrated that such increases cannot be the sole factor modulating Al^{3+}-induced callose deposition in wheat (*Triticum* sp), and suggested that Al^{3+} presence could activate callose synthase by either altering cellulose synthase conformation or increasing cytoplasmic availability of UDP-glucose, the substrate for callose synthase, by inhibition of the system for its transportation into the vacuole.

The results of the present study demonstrated that there is a time lag of at least 24 h before callose formation in response to Al^{3+} exposure (figure 5). This absence of callose deposition possibly indicates the existence of more immediate mechanisms of Al^{3+} tolerance that could be activated more promptly than callose synthesis. In this way, the genotypic plasticity of the tolerant genotype would not rely on callose deposition as its initial resource to prevent Al^{3+} penetration into the root. In addition, Al^{3+} inhibits cell-to-cell diffusion of molecules through plasmodesmata, by inducing callose formation which rapidly blocks apoplastic and symplastic transport of some substances such as indole-3-acetic acid (IAA), resulting in root growth inhibition (Sivaguru *et al*. 2000). However, callose formation is not an early event in Al^{3+} toxicity syndrome in rice, and for this reason does not seem to play a major role in Al^{3+}-induced inhibition of root growth. The callose deposition in these conditions was not of interest. However, the greater invertase activity in the tolerant cultivar (Caiapó) primary roots indicates cell division and growth under Al^{3+} toxicity. Thus, involvement of other factors in the mechanism of Al^{3+}-toxicity, such as cell wall stiffening and alteration on electrical properties of the plasma membrane, cannot be ruled out (Li *et al*. 2000).

A great number of physiological processes could contribute to Al^{3+} exclusion from the meristematic cell region, including increased secretion of mucilage (Sivaguru and Horst 1998). Acid polysaccharides, as the main fraction of

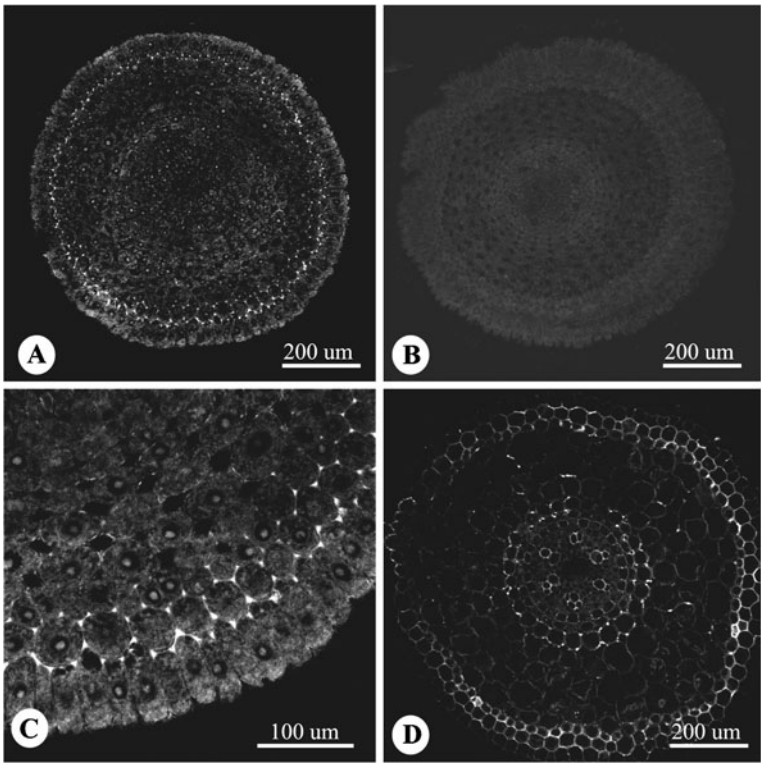

Figure 6. Callose localization on cross-sections of rice root tips stained with aniline blue 1% and observed with confocal laser scanning microscope (**A**) Caiapó 80 µM Al^{3+}, (**B**) IAC control, (**C**) Caiapó 80 µM Al^{3+} and (**D**) IAC 80 µM Al^{3+}.

mucilage, have a significant ability to complex and immobilize Al^{3+} due to their negative carboxyl groups, which prevent the binding of Al^{3+} to cellular components (Cai *et al*. 2011). It has been shown that in rice, the release of Al^{3+}-binding compounds into the apoplastic region and at the root tip surface can effectively chelate Al^{3+}, avoiding to some extent its penetration into root cells (Ma 2000; Ramos *et al*. 2007) and preventing successive cellular component damage. The greater activity of invertase in Caiapó roots, when compared with IAC, is an indicator of changes in primary metabolism and root cell growth, and can be indicative of the resistance mechanism. Both the cell wall and vacuolar invertases maximize the production of hexoses, which promote cell respiration, division and expansion (Koch 2004). Moreover, the high activity of the invertases in tissues with oxidative stress may be indicative of changes in the transport of IAA, responsible for cell hypertrophy (Koch 2004). These symptoms are common in roots under Al^{3+} toxicity. Simon *et al*. (1994) showed that in tomato (*Solanum lycopersicum*) root growth, invertase acid activity was reduced in the presence of Al^{3+}, supporting the role of invertase in root elongation. Alternatively, rice clearly showed an increase in invertase activity, mainly in the tolerant genotype, which could provide support for Caiapó roots due to increased availability of carbon skeleton and energy for growth.

As shown before, Caiapó is more efficient in rendering Al^{3+} to a non-phytotoxic state than IAC (Ramos *et al*. 2007; Marciano *et al*. 2010). This difference is genetically driven and results in more accurate mechanisms to exclude or withstand abnormal concentrations of Al^{3+} in Caiapó. This is understandable, since Al^{3+} resistance is unlikely to be a result of a single mechanism in all plant species. In fact, there seems to be a general consensus among researchers that tolerance to aluminium is an inherited characteristic that may be controlled by one or more major genes and several minor genes (Ezaki *et al*. 2000; Ma 2000; Taylor *et al*. 1997; Degenhardt *et al*. 1998). The results presented here corroborate this hypothesis since Al^{3+} tissue accumulation, resulting in a reduction of growth, correlates with invertase activity and callose accumulation. This shows that maintenance and superior root growth observed in the tolerant genotype in toxic Al^{3+} concentrations were promoted by signal transduction and metabolic processes that contributed to an increase in invertase activity. These processes can contribute to a higher Al^{3+} tolerance in rice, beyond the capability of the other plant species studied. In this way, the expression of the role and activity of specific invertases in apoplastic and symplastic root regions should be better understood.

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