Morphological and cellular changes in rice roots (*Oryza sativa* L.) caused by Al stress

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**ABSTRACT.** Aluminium toxicity significantly limits crop productivity in acid soils and its effects are primarily root-related. The aim of this paper was to study the effects of aluminium on the morphology and cell structure of rice roots. Different AlCl$_3$ levels were employed to impose stress conditions. Apical root segments were processed using a Progressive Low Temperature Method (PLT) and Lowycril resin, so that the samples could be cut. The stained sections were viewed and photographed using a Zeiss Optic microscope with an attached digital camera. Root elongation was reduced in seedlings exposed to Al, accompanied by deformed roots. Some structural changes were detected in epidermal and cortical cells. Reduced length accompanied by radial expansion contributed to cell elongation inhibition in different cellular types. Thickened cell walls and a vacuole size increase were also observed. These results provide evidence of the structural changes provoked in rice root cells by toxic levels of aluminium, as well as the morphological changes observed in Al-stressed rice roots in our laboratory.

**Keywords:** Aluminium; Rice; Root cells; Structural changes.

**INTRODUCTION**

Aluminum is the most abundant metal and the third most abundant element in the Earth’s crust. A large proportion of Al is incorporated into soil minerals like aluminosilicate, with much smaller quantities appearing in the soluble forms that are capable of influencing biological systems (Guo et al., 2004). In acid soils, however, the release of Al from Al-containing minerals is accelerated, which increases the concentration of phytotoxic forms of Al in the soil and in some farming practices (Zheng and Yang, 2005).

Al has been shown to inhibit both primary root and root hair growth, resulting in poor nutrient acquisition, and consequently leading to shoot nutrient deficiencies and poor crop yields (Taylor et al., 2000; Kochian et al., 2004). The commonly observable symptom of aluminium injury is the inhibition of root elongation resulting from the interactions between aluminium and root cells and their components (Wang and Kao, 2007; Eticha et al., 2005; Ma et al., 2004).

The extent of root growth inhibition always depends on Al concentration, length of plant exposure, and genetically-fixed tolerance or sensitivity of the tested plants. It is largely recognized that root tips are the primary site of Al-induced injury (Delhaize and Ryan, 1995; Sivaguru and Horst, 1995).

Several methods have been developed for evaluating Al tolerance, and these have also contributed to the elucidation of physiological processes (Polle et al., 1978; Ruiz-Torres and Carver, 1992; Moustakas et al., 1993; Zhang et al., 1994). Among these methods, hematoxylin staining has been used as a precocious, non-destructive way to study Al sensitivity in plant species (Polle et al., 1978; Carver et al., 1988; Rincón and González 1992; Delhaize et al., 1993; Wagatsuma et al., 1995), including maize (Guevara et al., 1992; Ryan et al., 1993; Jorge and Arruda, 1997), where it has also been used as a selection phenotypic index for Al resistance (Cançado et al., 1999; Eticha et al., 2005).

Root system architecture can also change after long-term Al application. Seedling roots treated with Al may appear stunted and may undergo a color change. Toxic concentrations of aluminium induced cracking on the root surfaces of pea, soybean, maize and wheat (Wagatsuma et al., 1987; Delhaize and Ryan, 1995; De Lima and Cooperland, 1994; Budíková et al., 1998). Increased Al concentrations in root medium induced severe damage to the root
structure of maize (Ciamporová, 2000). At the cellular level, the possible sites of Al effects occurring are in the cell wall, on the plasma membrane or in the cytoplasm. Possible mechanisms of Al toxicity involve Al interactions with the cell wall constituents and the plasma membrane (Delhaize and Ryan, 1995; Horst, 1995).

During the past few decades, physiologists have contributed to our understanding of the mechanisms of Al toxicity and tolerance in some of the cereals (Matsumoto, 2000; Kochian et al., 2005; Zheng and Yang, 2005; Rubia et al., 2011). Although rice is generally considered the most Al-tolerant species among small grain cereal crops, the mechanisms responsible for the high Al tolerance of rice are not yet understood (Macedo and Jan, 2008).

The aim of this paper is to study the responses to Al toxicity in a Cuban rice cultivar (*INCA LP-7*). The effects of Al on root morphology and on some cellular level changes are also investigated.

**MATERIALS AND METHODS**

**Plant material and growth conditions**

Seeds of *Oryza sativa* L., cv INCA-LP7 were sterilized with 5% sodium hypochlorite and germinated on filter paper for seven days. Two Al concentrations were employed to impose the stress; 0, 65 and 125 µM AlCl3 (pH= 4). The seedlings were under 16 h of light/8 h of darkness at 25°C.

To evaluate root elongation, the primary roots of seedlings (n ≥ 25) were photographed and their length measured with a ruler before microscopic sampling.

**Hematoxylin staining**

Staining protocol was based on Polle et al. (1978). The roots of seedlings cultivated for seven days, in the presence or absence of Al, were gently shaken in 200 ml distilled water for 15 min. The water was then replaced by 200 ml of aqueous hematoxylin solution [0.2% hematoxylin (Merck) and 0.02% potassium iodide, w/v] and left at the same slow agitation for 20 min. The solution was then replaced with 200 ml of water. The root apices were excised and photographed under stereoscopic microscope.

**Light microscopy**

Apical segments obtained from primary roots (3 mm) were fixed with 4% paraformaldehyde. After three washes in distilled water, the roots were dehydrated by Progressive Lowering Methods (PLT) and embedded in Lowicryl resin (Risueño, 2000).

Semi-thin longitudinal and cross-sections were made on a LKB Ultramicrotome and stained with toluidine blue. The sections were viewed with a Zeiss optic microscope and photographed with a digital camera coupled to the microscope.

**Statistical Analysis**

Randomized complete design was employed for the experiments. The experimental results were submitted to ANOVA of simple classification. The statistical package STATGRAPHICS (version 4.1 for Windows) was used to calculate both the SE and to compare the means (Tukey’s test).

**RESULTS**

Root elongation was significantly inhibited in rice seedlings exposed to Al (Figure 1). However, growth inhibition was more severe in seedlings exposed to 125 µM than in seedlings exposed to 65 µM AlCl3. In the most stressed roots, elongation was reduced 24% compared to the control plants, while those exposed to 65 µM AlCl3 exhibited a reduction of 15.5% (Figure 1).

Seminal roots stained with hematoxylin are shown in Figure 2A. In seedlings exposed to Al, the root tips exhibited a red-brown staining, which was more intensive in roots exposed to 125 than to 65 µM Al. Seedlings exposed to 125 µM also exhibited stained spotting. No staining was observed in control plants (Figure 2A).

Roots of stressed seedlings showed symptoms of Al injury at both Al concentrations. Root-apes appeared swollen and irregularly curved in Al-treated seedlings (Figure 2B). A localized thickened zone was observed between 20 and 40 mm from the rice root apex, corresponding to an increased diameter of cross-sections (Figure 2C).

Cross-sections of roots exposed to both Al concentrations were observed as more disordered (Figure 2C) than those of the control roots. Some alterations in the size of epidermal and cortical cells provoked by Al were responsible for this change. The epidermal cells were of typical size in longitudinal sections of the control roots (Figure 3), but lost their tissue features and appeared shorter and wider than the cells in control roots, when exposed to 65 and 125 µM AlCl3. These changes were also observed in cortical cells (Figure 3).
Increases in vacuolar volume were induced by Al (Figure 3). Intensively vacuolated cells were observed in the cortex and the epidermis in longitudinal sections of stressed roots. This increase was more intense in cortex cells than in epidermal cells (Figure 3).

Structural modifications in the cell walls of root-tip cells were observed in seedlings exposed to Al (Figure 4). A thickening of tangential cell walls occurred in the external cortical cells (first-third layer). Seedlings exposed to the highest Al concentration (125 µM AlCl₃) showed the greatest increase in cell wall thickening (Figure 4).

**DISCUSSION**

Aluminium toxicity limits plant growth mainly through its adverse effects on root growth and development. Under acidic soil conditions, active, phytotoxic forms of Al are released into the soil solution at levels that can inhibit root growth and damage roots (Liao et al., 2006).

Root inhibition in seedlings exposed to Al (65 and 125 µM AlCl₃) was modest, causing no more than 22% inhibition in the most stressed roots, compared to the Al control. A similar inhibitory effect of Al on root elongation has been confirmed in most of the experimental work (reviewed by Ciamporová, 2002) and in previous results obtained using different rice cultivars (*Oryza sativa* L.) (Alvarez et al., 2005). This is a major consequence of Al...
toxicity, which subsequently affects nutrients and water uptake (Horst et al., 2010).

These results established that the INCA-LP7 rice cultivar is not only more Al tolerant than other rice cultivars studied (Alvarez et al., 2005), but also more Al tolerant than other crop species reported in the literature (Wenzl et al., 2001; Piñeros et al., 2002; Amenós et al., 2009).

This degree of Al tolerance was also in accord with a low level of Al accumulation (as indicated by the low degree of Al-specific hematoxylin staining [Figure 2]) that was localized, to the first millimeters of the root tip. Differential Al accumulation observed between both cultivars is probably related to Al adsorption and desorption kinetics of the root tip cell walls, where polysaccharides are thought to play an important role in excluding Al specifically from the rice root apex (Yang et al., 2008, Silva et al., 2010).

This finding suggests that the hematoxylin technique could be used to identify tolerant and sensitive rice genotypes after long-term seedling exposure to Al. The technique is also supported in previous works, where an Al-sensitive rice cultivar, J-104, exhibited significant root damage and dramatic Al accumulation under these conditions (unpublished results). Similar results were obtained in wheat, where this technique proved conducive to identifying tolerant and sensitive genotypes after a very short exposure of seedlings to Al, which was well before differences in the seminal root length become detectable (Delhaize et al., 1993).

Hematoxylin is a dye commonly used to study Al sensitivity in plant species (Polle et al., 1978; Delhaize et al., 1993; Wagatsuma et al., 1995), including maize (Guevara et al., 1992; Ryan and Kochian, 1993; Jorge and Arruda, 1997 and Piñeros et al., 2002). An important aspect of this technique is that the reaction between hematoxylin and Al is specific, such that other stressing factors would exert a minimal effect, if any, on the evaluation processes of the Al effects (Cançado et al., 1999).

Some changes were appreciated in root features of seedlings exposed to Al. Swollen and curved roots with different colors were observed in our research, which are common symptoms of Al stress (reviewed by Ciamporová, 2002). These results suggested that long-term Al application can change the architecture of the root system. In maize seedlings treated with Al, lateral roots sometimes appeared very close to the axial root apex, exhibiting a deformed and thickened structure and a possible color change (Budikóva et al., 1998).

Al exposure triggered different changes in the shape of epidermal or outer cortex cells. A reduction in the length of epidermal and cortex layer root cells was observed in longitudinal sections, accompanied by a cell radial expansion. Similar alterations in other species have been documented (Horst, 1995; Barceló and Poschenrieder, 2002; Gunsé et al., 2003). A reduced length was appreciated in the meristematic and elongation zones of barley root (Kochian, 1995), and the root cortices of wheat (Sasaki et al., 1997) and maize (Budikóva, 1999).

This finding suggests that Al affects cellular growth orientation. In Arabidopsis thaliana, the root changes that were accompanied by disorganization of the microtubule cytoskeleton were induced by protein kinases and phosphatases inhibitors, suggesting the effects of Al on mechanisms of the regulation of cell growth polarity (Horst,
1995). Cell elongation and the direction of cell expansion are linked processes of functional importance in plant development (Sugimoto et al., 2000).

The radial expansion of epidermal and cortex cells may exert mechanical stress causing an increase in cross-sections of rice roots exposed to Al (Alvarez et al., 2005) and destruction of peripheral root tissues (Ciamporová, 2000). This phenomenon has also been observed in maize exposed to Al stress (Wang et al., 2004).

Although we did not determine vacuolar content in our research, an increased vacuolation inside the cells observed in rice roots exposed to both Al concentrations, particularly those in the apical region of the primary roots, suggests that this mechanism may be employed to internal Al detoxification. In maize, this relied on the active transport of Al from the cell wall to vacuoles (Zheng et al., 2005), and in other studies, higher vacuolation was observed in the root cap, the epidermis, and the cortex (Ciamporová, 2002; Vázquez et al., 1999). Vázquez et al. (1999) detected Al in similar vacuolar deposits located in Si-containing vacuoles of maize root cells. The amount of Al in the vacuolar deposits increased with increasing exposure of maize root to Al stress, which might lead to reducing the toxic effects of Al in the cytoplasm and to recovery of the root growth.

Thickened cell walls were frequently observed in Al-treated roots and principally in external cortex cells, which revealed irregularly-thickened cell walls. Although many researchers suggest integrating Al with many cellular sites: cell wall, plasma membrane or DNA (Silva et al., 2000), it seems that most of the Al accumulated in the cell wall. When a plant grows under toxic Al conditions, Al is first introduced through its apoplast. Ample evidence suggests that primary Al accumulation in root tissues is localized in the cell wall (Hossain et al., 2005), which could be the reason for the thickening we observed. It remains unclear, however, whether Al interacts directly with the cell wall or whether these changes are an indirect consequence of other Al-induced changes (Pietraszewska, 2001).

Significant changes are observed in rice roots exposed to Al. Although there is much evidence regarding the effects this metal has on diverse root systems, its effects on the anatomic and histological features are less studied. This point could be addressed in future investigations of Al toxicity in plants.

**LITERATURE CITED**


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铝离子在水稻根所引起的形态上及细胞内之变化

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铝金属离子之毒害在酸性土壤中严重地限制了作物之生产量；而此毒性主要係经由根部发作。本文之目的乃探讨铝离子对水稻根部之形态及细胞构造所造成之损害。我們使用不同浓度之三氧化铝来探讨危害之程度。根冠部位是以逐步低温法 (PLT) 及 Lowycril 樹脂處理；如此一來試樣可被切除。經染色之切片以 Zeiss 光學顯微镜取樣數位相片來検覈並照像。根部之延伸程度经铝离子处理後會減少，同時根會變形。在表皮细胞及皮层细胞可検测到若干構造上之改變。減少之长度伴随著横軸向之膨脹；此乃源于不同型之細胞的延展過程受到阻害。同時也观察到加厚之細胞壁及液泡大小之增加。以上之结果提供证据说明水稻根部細胞受到足以導致傷害之铝离子之影响后所產生的構造上之改變及形态上之变化；此乃我们实验室所观察到的。

關鍵词：铝；水稻；根部細胞；構造之改變。