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Effects of aluminum on the elongation and external morphology of root tips in two maize genotypes

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ABSTRACT: The objective of this study was to evaluate the effects of toxic levels of aluminum (Al) on the growth and external morphology of root tips in two maize genotypes with differential Al tolerance. The maize genotypes UFVM-100 (Al-sensitive) and UFVM-200 (Al-tolerant) were treated with 0 and 50 µM Al in a 0.5 mM $CaCl_2$ solution at pH 4.5; root elongation, Al content and the external morphology of the root tips were evaluated. Chemical analysis, hematoxylin staining and energy dispersive X-ray spectrometry (EDS) showed greater Al accumulation in the root tips of the UFVM-200 genotype. The inhibition of root elongation in the UFVM-100 genotype, however, was much stronger than in the UFVM-200 genotype. Both maize genotypes exhibited visible and intense alterations in external micromorphology of the root tip, especially the Al-sensitive UFVM-100 genotype. Scanning electron micrographs showed intense cell disorganization and transverse ruptures of the protodermic and outer cortex layers of the cells in both genotypes. The ruptures were deeper and wider and reached the inner cortex layers in the UFVM-100 genotype. The EDS analysis showed that, in addition to Al accumulation, there was a proportional increase in the P concentration in the root tips of the UFVM-200 genotype. This is indicative of possible precipitation and/or immobilization of Al in the root tip apoplast of this genotype, which contributes to symplastic detoxification.

Key words: aluminum tolerance, root growth, root morphology, *Zea mays*.

INTRODUCTION

Aluminum toxicity is one of the most important limiting factors for plant productivity in acidic soils with a pH below 5.5. Many of these soils are located in developing countries with increasing population pressure, leading to food insecurity in these regions (Kochian et al. 2004). In Brazil, there are over 2 million km^2 of acid soils with toxic levels of aluminum (Al), especially in the Brazilian savannah ("Cerrado") (Lopes 1984).

The primary symptom of Al toxicity in plants is the inhibition of root growth, resulting in the reduction of root elongation a few hours after exposure to the mineral (Kopittke et al. 2008; Horst et al. 2010; Arroyave et al. 2011). Despite great efforts in this area of research, the Al-induced mechanism of root inhibition is still not perfectly understood, and it is uncertain whether the primary lesions caused by Al occur in the apoplast or symplasm (Horst et al. 2010).

Numerous studies suggest that the root tip is the primary site of Al toxicity perception (Yang et al. 2008; Horst et al. 2010; Motoda et al. 2011) and of the subsequent expression of Al tolerance (Kollmeier et al. 2000). Aluminum interacts with cells at multiple sites, such as cell walls, plasma membranes, and several structures of the symplasm, resulting in structural damage and/or functional changes that may affect several aspects of plant metabolism (Ma et al. 2004).

The cell walls are the first structures of the roots that are exposed to Al, and most of the total Al accumulated in the roots is strongly linked to carboxylic groups of the pectin constituents of cell walls (Yang et al. 2008). The binding of Al in the cell wall and/or to the apoplastic face of the plasma membrane may impair apoplastic and symplasmic →

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cell metabolism, leading to Al-induced inhibition of root elongation (Horst et al. 2010).

The inhibition of root elongation is accompanied by changes in the architecture and morphology of the roots. A reduction in the formation of lateral roots and root hairs, changes in color, thickening, atrophy and curvature of the roots are common symptoms (Čiamporová 2002). Depending on the duration of exposure and concentration, Al may increase cell wall rigidity, causing the rupture of the rhizodermis and outer cortex of the meristem, which inhibits elongation of the root tips (Blamey et al. 2004; Jones et al. 2006; Kopittke et al. 2008). Although Al effects can occur in all growing regions of the root, the above-mentioned ruptures in pea (Yamamoto et al. 2001), corn (Jones et al. 2006) and bean roots (Kopittke et al. 2008) occurred predominantly in regions within approximately 1 – 2 mm of the root tips, called the distal part of the transition zone (DTZ) (Sivaguru and Horst 1998).

The intensity of damage induced by Al depends on plant age, metal concentration and plant tolerance (Ma et al. 2004). The tolerance of plants to Al appears to be the result of a combination of exclusion and/or internal mechanisms (Kochian et al. 2004). These mechanisms allow plant roots that are exposed to toxic levels of Al to reduce or prevent the expression of the toxic effects of this element, such as the inhibition of root elongation.

Therefore, the objective of this study was to evaluate the effects of toxic levels of Al on the elongation and external morphology of root tips in two maize cultivars with differential tolerance to Al.

MATERIALS AND METHODS

Plant material and growth conditions

Two maize genotypes (*Zea mays* L.) differing in Al tolerance — UFVM-100 (Al-sensitive) and UFVM-200 (Al-tolerant) —, provided by the Department of Plant Science of the Federal University of Viçosa (MG), Brazil, were used in the experiments.

Seeds of both maize genotypes, selected for size and shape uniformity, were treated with 70% (v/v) sulfuric acid for 1 min and then washed under running tap water and deionized water. Next, the seeds were surface sterilized with 3% (v/v) sodium hypochlorite for 15 min, then washed under running tap water and deionized water. The seeds were germinated in rolls of neutral pH paper, dipped in 0.5 mM $CaCl_2$ solution at pH 4.5 and maintained under continuous aeration at 25 °C. Four days later, plants were selected for size and shape uniformity, transplanted to 1.6-L polyethylene pots containing 0.5 mM $CaCl_2$ solution at pH 4.5 and exposed to the Al treatment. All plant growth was conducted in a temperature-controlled growth room (25 ± 1 °C) under 230 µmol of irradiance/m^2·s and a photoperiod of 16 h.

Root elongation under different exposure times and aluminum concentrations

Seedlings of both maize genotypes were exposed to 0, 25, 50, 100 and 200 µM Al, supplied as $AlCl_3$, in 0.5 mM $CaCl_2$ solution at pH 4.5. The primary root length was measured before and after 12, 24, 36 and 48 h of plant treatment with Al and the elongation was expressed relative to the control.

Detection of accumulated aluminum in root tips using hematoxylin staining

After plant exposure to 0 or 50 µM Al in 0.5 mM $CaCl_2$ solution at pH 4.5 for 24 h, the root systems of 6 randomly selected seedlings were dipped in 0.2% iron hematoxylin and 0.02% KIO_3 solution for 15 min (Polle et al. 1978). The excess dye was eliminated by washing the roots in deionized water under aeration for 15 min. Finally, the root tips were excised and photographed under a stereomicroscope (Zeiss Stemi model DV4).

Micromorphology analysis of root tips by scanning electron microscopy and energy dispersive X-ray spectrometry

After plant exposure to 0 or 50 µM Al in 0.5 mM $CaCl_2$ solution at pH 4.5 for 24 h, the root tips of 6 randomly selected seedlings, approximately 0.5 cm long, were fixed in 2.5% glutaraldehyde. Next, they were dehydrated in an ethylic series, dried with CO_2 at critical point (Baltec model CPD 030, Liechtenstein), fixed in an aluminum bracket and covered with carbon in a metallizer. The photographic documentation was conducted using a scanning electron microscope (Leo model 1430VP, Cambridge, England) operated at 20 kV with an X-ray probe attached. Sodium →

(Na), aluminum (Al), phosphorus (P) and sulfur (S) contents were estimated in the meristematic zone located just above the root cap (1 mm from the root tip) using energy dispersive X-ray spectrometry (EDS). From the ED spectrum, the relative elemental composition was calculated.

Determination of aluminum content

Aluminum contents were determined in root tips of approximately 1 cm, weighing approximately 0.1 g in fresh weight. The root tips were incubated in 2 M HCl for 48 h (Ma et al. 2004), and the Al content in the extract was determined using inductively coupled plasma-optical emission spectrometry (ICP-OES) at a wavelength of 394.401 nm.

Experimental design and statistical analysis

The treatments were arranged in a randomized factorial block design with three replicates. The results were subjected to analysis of variance, and the means were compared using the Scott-Knott test at $p < 0.05$.

RESULTS AND DISCUSSION

The root elongation decreased with increasing Al concentration and exposure time in both maize genotypes (Figure 1). Regardless of the Al concentration or exposure time, the UFVM-100 genotype, considered Al-sensitive, consistently showed a higher reduction in root elongation compared with the UFVM-200 genotype. Significant differences between the genotypes were detected in plants treated with 25 µM Al as early as 12 h after plant exposure to aluminum. The genotypic differences increased with exposure time and Al concentration, reaching a maximum after plant treatment with 50 µM Al for 24 h; under this condition, the UFVM-200 showed 20% greater root elongation than the UFVM-100 genotype. At longer exposure times and higher Al concentrations, however, genotypic differences decreased.

The inhibition of root elongation is one of the most important and visible effects of toxic concentrations of Al in plants. These Al effects on root growth may be detected as early as a few hours after plant exposure to toxic levels of Al (Kochian et al. 2004) and increase with exposure time. The mechanism of Al-induced inhibition of root elongation is a complex process involving physiological, anatomical and morphological modifications as well as cell division (Silva 2012).

The Al content in root tips increased 14.5 and 15.3 times relative to the control in the UFVM-100 and UFVM-200 genotypes, respectively, after 24 h of plant treatment with 50 µM Al (Figure 2). The roots of the Al-tolerant UFVM-200 genotype exhibited an Al content approximately 19% higher than the Al-sensitive UFVM-100 genotype. Studies with other →

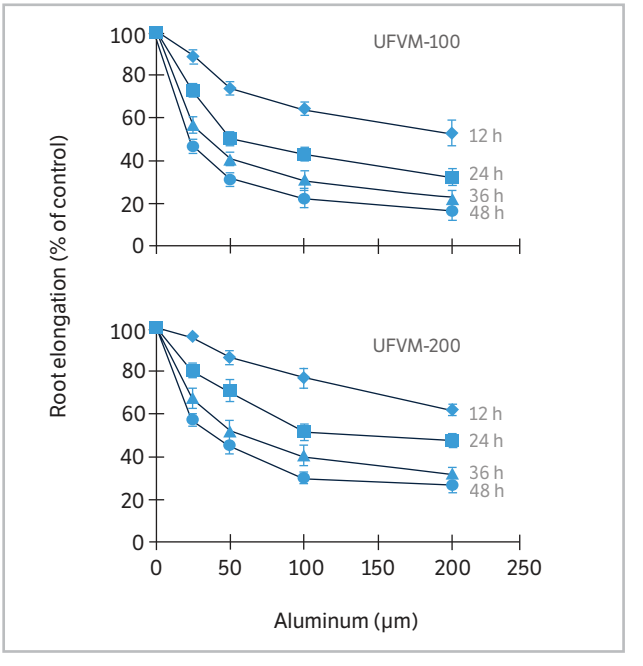

Figure 1. Root elongation in two maize genotypes: UFVM-100 and UFVM-200 treated with increasing Al concentrations in 0.5 mM $CaCl_2$, pH 4.5, for 12, 24, 36 and 48 h. Bars represent standard deviation (N = 3).

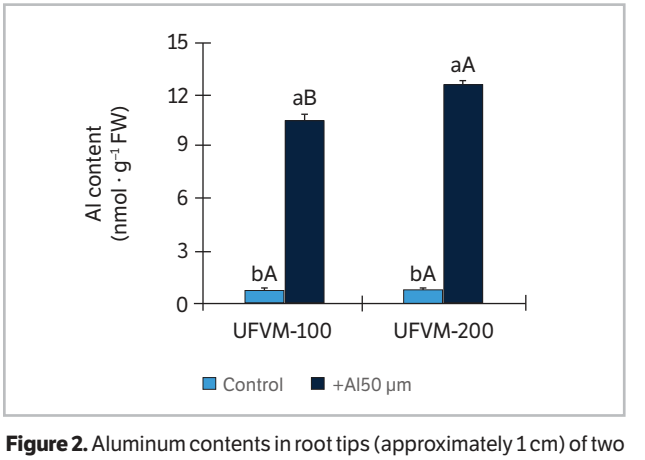

Figure 2. Aluminum contents in root tips (approximately 1 cm) of two maize genotypes: UFVM-100 and UFVM-200, exposed to 0 or 50 µM Al in 0.5 mM $CaCl_2$, pH 4.5, for 24 h. Means followed by same capital letter, between genotypes within the same Al treatment, and by same small letter, between Al treatments within the same genotype, do not differ by the Scott-Knott test at $p < 0.05$. Bars represent standard deviation (N = 3). FW = fresh weight.

maize genotypes with differential tolerance to Al, however, have almost invariably shown a greater Al accumulation in the genotypes that are considered more sensitive (Piñeros et al. 2005; Giannakoula et al. 2008). The same has been observed in other grasses such as wheat (Ye et al. 2011), signal grass (*Brachiaria decumbens* Stapf) and palisade grass (*B. brizantha* Hochst. ex A. Rich. Stapf) (Arroyave et al. 2011). Nevertheless, similar to our findings, a maize genotype (Gaume et al. 2001) and a rice variety (Miftahudin et al. 2007) tolerant to Al accumulated higher amounts of Al than the respective genotype or Al-sensitive variety. It is apparent that, in addition to genotypic differences, the Al contents in the roots may depend on several factors including the nutrient solution composition.

The root tips of the plants of both genotypes treated with 50 µM Al for 24 h and stained with hematoxylin produced a dark purple coloration, especially in the region of the root tip (approximately 1.8 – 2.0 mm) (Figure 3), viz. in the meristematic and elongation regions located just above the root cap. The root tip of the UFVM-200 genotype exhibited greater staining than the UFVM-100 genotype in the presence of Al.

Hematoxylin staining is a simple and easy method to detect Al in plant tissue and is widely used to discriminate plant genotypes with respect to Al tolerance (Miftahudin et al. 2007; Castilhos et al. 2011). Cross sections of Al-treated roots showed that Al did not enter beyond the subepidermal layer of the root tissue (Miftahudin et al. 2007). Moreover, this method cannot discriminate between the Al in the apoplast or in the symplast and sometimes failed to differentiate rice cultivars according to their sensitivity to Al (Miftahudin et al. 2007; Macêdo et al. 2009).

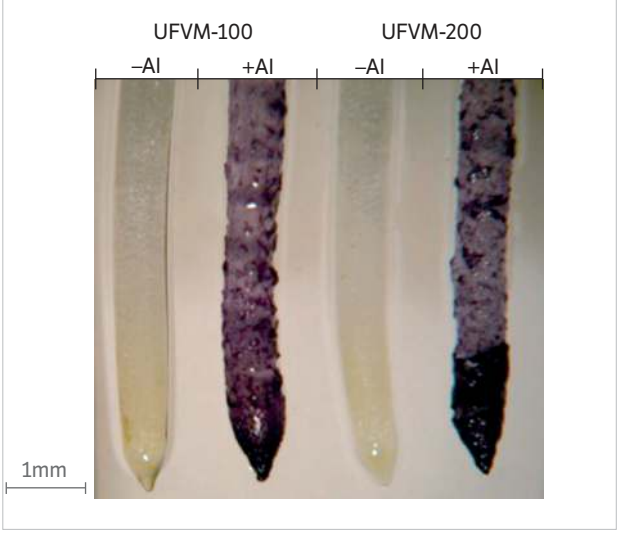

Figure 3. Root tips of two maize genotypes after plant treatment with 0 or 50 µM Al for 24 h stained with hematoxylin (N = 6).

Most of the Al accumulated in the roots is bound to pectin constituents of cell walls (Yang et al. 2008). Al not only binds to the pectin matrix but can also modify the composition and important properties of the cell wall, such as its extensibility (Ma et al. 2004; Jones et al. 2006). These factors may have contributed to the morphologic effects observed in this experiment.

Scanning electron micrographs did not show any abnormality in the root tips of the control plants of both maize genotypes (Figure 4). In the Al-treated plants, however, there was intense cell disorganization that started at approximately 0.95 (UFVM-100) and 1.16 mm (UFVM-200) from the tips. From this point forward, transverse ruptures of the protodermic and outer cortex layers of the cells became evident in the root tips. These transverse ruptures, however, were not observed closer than ca. 1 mm to the root tip. Apparently, the ruptures were formed by the breaking and/or tearing of individual cells and not by the separation of intact cells. A closer examination of these ruptures using a higher magnification scanning electron microscopy (481×) showed that some of the root tip ruptures in the UFVM-100 genotype were much deeper and wider than in the UFVM-200 genotype and reached the inner cortex layers (Figure 5).

The ruptures in the root tip observed in both maize genotypes were similar to those observed in pea (Yamamoto et al. 2001; Motoda et al. 2010, 2011), cowpea (Kopittke et al. 2008) and maize roots (Jones et al. 2006). These transverse ruptures may apparently be caused by the increase in root diameter and the tearing of external cortex and rhizodermic cells of the elongation zone (Blamey et al. 2004; Kopittke et al. 2008; Motoda et al. 2010). According to these authors, root elongation inhibition and rupturing is the result of Al linkage to cell wall components and increased lignin biosynthesis and cell wall rigidity. In addition, it was observed that the epidermal and cortical cells were shorter and wider than the cells in the roots of control plants (Čiamporová 2000). The cells of this root region, termed the DTZ (distal part of the transition zone), switch from cell division to cell elongation, and this zone is considered the preferential site of Al accumulation and the most Al-sensitive region of the →

root (Sivaguru and Horst 1998). In this root zone, Al was detected in the cell walls of epidermal cells after a few seconds of exposure to this mineral, and there was a gradual increase in Al accumulation in the inner root tissue with exposure time (Čiamporová 2002). Independent of the Al-tolerance mechanism, tolerant species should be able to protect this sensitive root zone from the effects of Al.

The elemental composition of the outer layers of the root tips was estimated using EDS (Figure 6). The Na content was not changed by Al treatment and the maize genotypes differed only with respect to the control plants. The Al content was low and approximately the same in the control plants of both maize genotypes but increased with Al treatment, especially in the UFVM-200 genotype, which reached approximately 26% higher than in the UFVM-100. Similarly, the P content also increased in both maize genotypes with the Al treatment; the Al-tolerant genotype UFVM-200 showed approximately 15% higher P content than the UFVM-100. Sulfur also

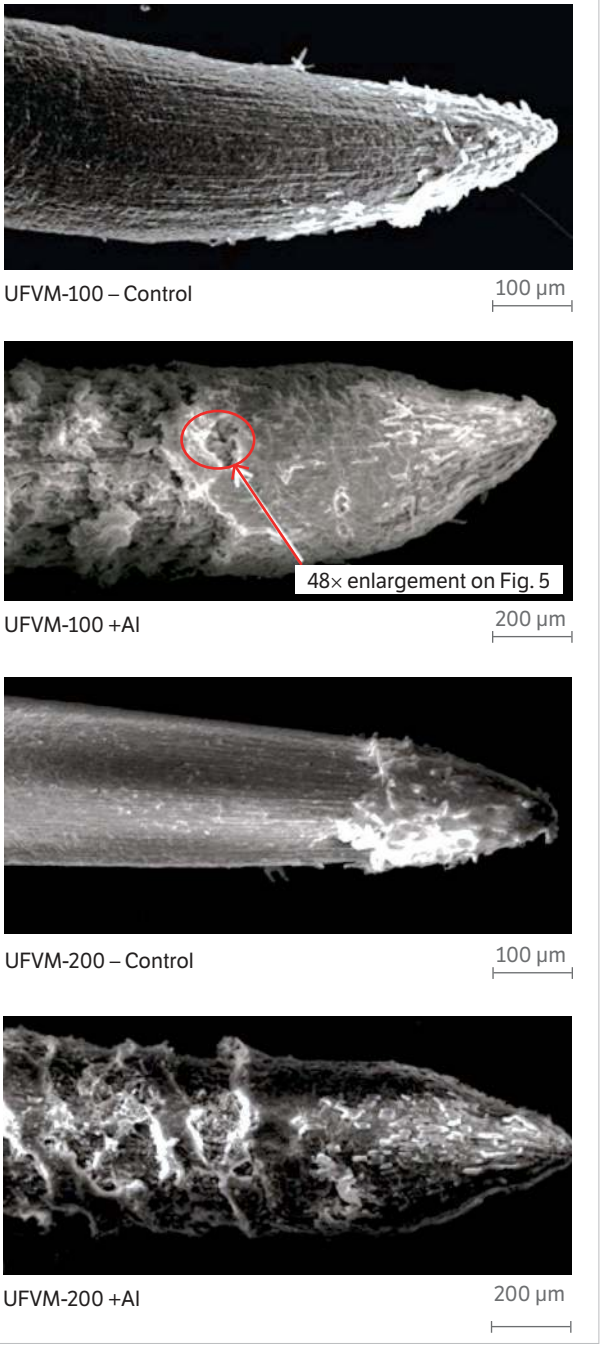

Figure 4. Scanning electron micrographs of the root tips of two maize genotypes, after plant treatment with 0 or 50 µM Al in 0.5 mM $CaCl_2$ for 24 h.

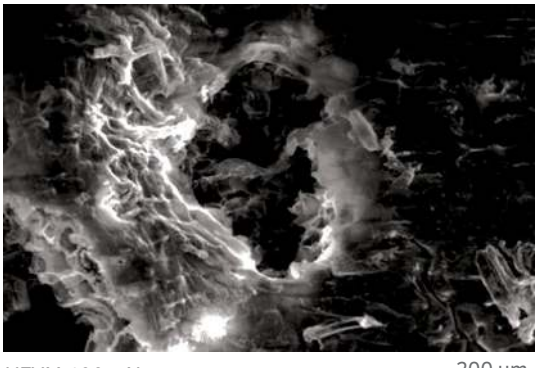

Figure 5. Scanning electron micrograph showing the rupture in the root tips of the UFVM-100 genotype (see arrow in Figure 4; Mag = 481×), after plant treatment with 50 µM Al in 0.5 mM $CaCl_2$ for 24 h.

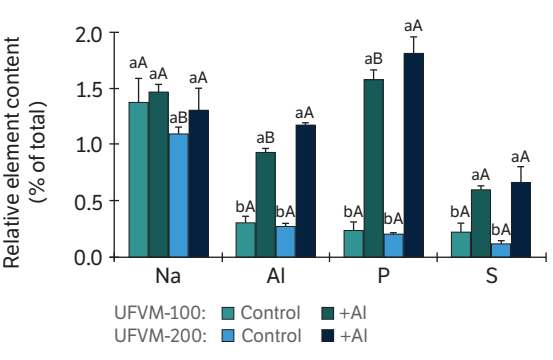

Figure 6. Elemental composition estimated by energy dispersive X-ray spectrometry (EDS) of root tips of the UFVM-100 and UFVM-200 genotypes, after treatment with 0 or 50 µM Al for 24 h. Means followed by same capital letter, between genotypes within the same Al treatment, and by same small letter, between Al treatments within the same genotype, do not differ by the Scott-Knott test at $p < 0.05$. Bars represent standard deviation (N = 3).

increased with the Al treatment, but the genotypes did not differ statistically.

Phosphorus can interact with Al in the apoplast to produce low solubility compounds, such as $AlPO_4$, immobilizing and detoxifying Al in the root tissues (Zheng et al. 2005). Gaume et al. (2001) showed that increasing the P concentration in the nutrient solution decreased the Al effect on root growth. The Al content was consistently higher in the Al-tolerant cultivar independent of P concentration. Results such as these have led some authors to suggest a possible role of P in Al tolerance in wheat (Zheng et al. 2005) and maize (Gaume et al. 2001). Localization of Al using the Al-specific stain Morin with confocal laser scanning microscopy showed that most of the Al was localized in the apoplast (Zheng et al. 2005). Therefore, the higher Al and P contents found in the root tips of the UFVM-200 genotype, not ruling out other possibilities, suggest an external immobilization of Al in the roots, which excludes Al from the symplasm and contributes to the Al tolerance of this genotype.

CONCLUSION

The roots of the UFVM-200 genotype (Al-tolerant) accumulated a higher amount of Al than the UFVM-100 genotype (Al-sensitive). Nevertheless, the Al-tolerant genotype showed lower root elongation inhibition and less intense morphological alterations in the root tips. The higher Al and P accumulation in the root of the Al-tolerant genotype indicates a possible role of P in the precipitation and/or immobilization of Al in the root tip apoplast, enabling symplastic detoxification.

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