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Differential aluminum resistance in *Brachiaria* species

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ABSTRACT

Brachiaria are increasingly cropped in the tropics because these species combine good fodder quality and yield with high resistance to aluminum (Al) toxicity, an important stress factor in acidic soils. The mechanisms for the extraordinarily high resistance to Al toxicity in *Brachiaria decumbens* remain unclear. It has been suggested that the presence of a multiseriate exodermis might contribute to efficient Al exclusion in *B. decumbens*. However, no data concerning the root structure of less Al-resistant *Brachiaria* species have been reported. The aim of the present study was determine whether the exodermis is a distinctive feature of Al hyper-resistant *B. decumbens* compared with *Brachiaria* species with lower Al resistance. *B. decumbens*, *B. brizantha*, and *B. ruziziensis* were grown in nutrient solution without (control) or with 200 μM Al (32 μM Al^{3+} activity) for 96 h. Differences in the Al resistance were assessed using various indicators: Al-induced inhibition of root elongation, membrane damage, and the maintenance of nutrient homeostasis. Transversal root sections were examined using fluorescence microscopy to reveal the presence of an exodermis through auto-fluorescence. Aluminum resistance decreased in the order *B. decumbens* > *B. brizantha* > *B. ruziziensis*. Both the hyper-resistant *B. decumbens* and the moderately resistant *B. brizantha* were more efficient in Al-exclusion than the sensitive *B. ruziziensis*. Apoplastic barriers, in the form of a multiseriate exodermis, were constitutively present in *B. decumbens*, but not in Al-sensitive *B. ruziziensis*. Under control conditions, *B. brizantha* exhibited slightly auto-fluorescent epidermal cell walls, while under Al exposure auto-fluorescent deposits were observed in the intercellular spaces between the epidermal and sub-epidermal cell layers. The results provide circumstantial evidence of a role for apoplastic barriers in the Al resistance of *B. decumbens* and, to a lesser extent, in *B. brizantha*. Nonetheless, additional research is required to determine a causal relationship between the exodermal barrier and Al resistance.

1. Introduction

Brachiaria (Poaceae), a genus of African forage grasses, comprises approximately 100 species. Some of these species are currently gaining considerable relevance as fodder crops in tropical regions worldwide (Boddey et al., 1996). Moreover, *Brachiaria* species have been studied for potential phytoremediation applications (Kopittke et al., 2010). The economic interest in these grasses is greatest in tropical America, where the extensive adoption of *Brachiaria* cultivation over the past four decades has had a revolutionary impact on the productivity of vast areas of previously underused or marginal soils (Boddey et al., 1996; Wenzl et al., 2000). Because *Brachiaria* are well adapted to tropical climates with C4-type photosynthesis, these plants are even more attractive for further breeding efforts.

Soil acidity limits crop production in large areas of the world's potentially arable land, primarily in developing countries in Africa, Asia, and South America. Aluminum toxicity is considered among the most relevant abiotic stress factors limiting crop production on acid mineral soils in these regions. In addition to Al toxicity, these acid soils have an excess of H^+ and Mn and are deficient in P, Ca, and Mg (Wenzl et al., 2003; Kochian et al., 2004; Poschenrieder et al., 2008). Certain *Brachiaria* species perform well under harsh conditions. However, there are considerable differences in tolerance to Al-toxicity and P-deficiency among *Brachiaria* species (Rao et al., 1998; Wenzl et al., 2002; Häussler et al., 2006; Arroyave et al., 2011). The mechanisms underlying these characteristics, however, have not been established. The increased Al tolerance in *Brachiaria decumbens* is unrelated to the root tip exudation of organic acids (Wenzl et al., 2001), a mechanism responsible for differences in Al tolerance in several crop species (Ma et al., 2001). The accumulation of organic acids in root tips contributes to Al tolerance in *Brachiaria* species, but this mechanism hardly accounts for differences in Al tolerance among *Brachiaria* species (Wenzl et al.,

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2002). Recently, differences in Al tolerance between the highly Al-tolerant signalgrass (*B. decumbens*) and the somewhat less tolerant palisade grass (*B. brizantha*) have been associated with Al-induced changes in epidermal cell patterning, leading to the formation of abundant root hairs that apparently accumulate Al (Arroyave et al., 2011). It has been suggested that the constitutive presence of a multiseriate exodermis in *B. decumbens* might contribute to more efficient Al exclusion. However, these structural features have not been analyzed in less resistant *Brachiaria* species. Thus, the aim of the present study was to determine whether the exodermis is a distinctive feature of the Al hyper-resistant *B. decumbens* compared with lower Al-resistant Brachiaria species.

2. Materials and methods

2.1. Plant-growth conditions

Seeds of different *Brachiaria* species, including signalgrass *B. decumbens* (Stapf), palisade grass *B. brizantha* (Hochst. Ex A. Rich.) Stapf, and Congo grass *B. ruziziensis* (R. Germ and C.M. Evrard) (Unipasto Marangatu, Brazil and Agrosemillas, Medellin, Colombia) were germinated for 7 days on floating trays in distilled water. The emerged seedlings were transferred to a continuously aerated nutrient solution (plastic beakers with a 5 L capacity; 10 plants per beaker). The solution composition was a modification of the Wenzl et al. (2001) solution, which was designed as an approximation of the chemical soil properties that limit forage productivity on acid mineral soils. Thus, the solution had low ionic strength and low pH. The composition of the solution was (in μM): 106 $(NH_4)_2SO_4$; 100 KNO_3; 20 K_2SO_4; 200 $Ca(NO_3)_2$; 1 $NaH_2PO_4{\cdot}H_2O$; 169 $CaCl_2$; 120 $MgSO_4{\cdot}7H_2O$; 10 Fe-EDTA; 1 $ZnSO_4{\cdot}7H_2O$; 0.2 $CuSO_4{\cdot}5H_2O$; 1 $MnSO_4{\cdot}H_2O$; 0.004 $(NH_4)_2SO_4$; 20 $SiCl_4$; 0.001 $(NH_4)_6MoO_{24}{\cdot}4H_2O$; and 6 H_3BO_3. Solution pH was checked daily and adjusted to pH 4.2 ± 0.1. The solutions were renewed every 2 days. After 15 days in the control nutrient solution, the plants were transferred to treatment solutions supplemented or not (controls) with 200 μM Al as $AlCl_3$. The activity of Al^{3+} in the solution was 31.7 μM (GEOCHEM-EZ, Cornell University, Ithaca, NY, USA). The plants were exposed to Al for different treatment durations of 0, 24, 48 and 72 h. All plants (3 weeks old) were grown for 72 h in the treatment solutions. Controls received solution without Al supply during the entire exposure period, while plants from the 72 h Al treatment received Al-containing nutrient solution over the entire period. The plants exposed to Al for 24 h received control solution for 48 h and Al-supplemented solution for an additional 24 h; plants from the 48-h Al-treatment received control solution for 24 h followed by 48 h Al-exposure. This experimental design ensured that the plants from the different exposure time treatments were the same age at harvest. This exposure time span was selected based on the results obtained from previous studies, demonstrating that *B. decumbens* requires a lag time of 48 h for the full expression of Al resistance. For the analysis of the plasma membrane integrity and SEM (scanning electron microscopy), the experimental runs were prolonged until 96 h of Al exposure. The plants were grown in a growth chamber (Conviron® SH10) under the following conditions: 12/12 h photoperiod, 600 $\mu E\, m^{-2}\, s^{-1}$ photon fluency rate, 27/25 °C day/night temperature, and 60% relative humidity.

2.2. Root elongation and root staining using Evans blue and hematoxylin

At each time-sample, the length of the main root of 20 plants per species and treatment was measured with a ruler. The loss of plasma membrane integrity was evaluated through a spectrophotometric assay using Evans blue stain. The root tips were stained with a 0.25% (w/v) aqueous solution of Evans blue for 15 min, washed three times with distilled water for 10 min each (Baker and Mock, 1994), and photographed. After staining with Evans blue, 1-cm root tips from 15 roots per species and treatment were incubated to determine the extent of membrane damage using 1 mL N,N-dimethylformamide. The optical density was measured spectrophotometrically at 600 nm, according to Kikui et al. (2005). Hematoxylin staining, according Polle et al. (1978), was used to visualize the Al in the roots after a 24-h exposure. The pictures shown are representatives of at least 5 plants per species.

2.3. Scanning electron microscopy (SEM)

The root tips from control or 200 μM Al-treated plants of *B. ruziziensis* were used for SEM studies. The root tips were fixed in a solution of 2% paraformaldehyde and 2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h at 4 °C. All samples were mounted on adhesive carbon films and coated with gold (5-nm particle size) (Julián et al., 2010). The samples were observed using a JMS – 6300 scanning electron microscope (Jeol Ltd., Tokyo, Japan). The pictures shown are representatives of images obtained from at least three plants per treatment.

2.4. Auto-fluorescence

The root apexes (10 mm) were cut with a razor blade from the roots and rinsed with distilled water. Single apexes were placed into a 20mm × 5 mm mold prepared using plastic foil. The root tip samples were embedded in a 2.5% solution of agar–agar at a temperature near the point of solidification (approx. 45 °C). After pouring the agarose solution into the mold, the root tips were carefully positioned in parallel to the longitudinal axis of the mold. The mold was placed on ice for the agarose to solidify. Subsequently, the agarose-embedded root tip samples were manually cut (transversal section) using a razor blade. The cross-sections were observed under a Nikon fluorescence microscope (Optiphot-2, Nikon Corp., Tokyo, Japan) equipped with a high-intensity light source and blue–violet filter (BV-2A). The pictures shown are representatives of images obtained from at least 3 plants per species and treatment.

2.5. Root and shoot concentrations of Al and selected mineral nutrients

The oven-dried (90 °C) samples were ground to fine powder, followed by acid digestion (nitric acid:H_2O_2 69%:30%, 5:2 v/v) in an open hot-block digestion system (Item No.: SC154-54-Well HotBlock™, United Kingdom). The concentrations of the selected elements were determined through inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Jarrell-Ash, model 61E Polyscan, England). The data shown represent the means of three independent samples per species and exposure times. For statistical analysis, variance-stabilizing transformations were performed, where necessary, to conform to the assumptions of ANOVA. Subsequently, the data were analyzed using factorial ANOVA, followed by Tukey's HSD test to identify significant differences among the responses of *Brachiaria* species to Al treatment for different exposure times.

3. Results

3.1. Root elongation and morphology

Clear differences in the response of roots to Al treatment were observed among the *Brachiaria* species. While the elongation of the