Research Paper

Urochloa brizantha cultivated in aluminum-toxic soil: Changes in plant growth and ultrastructure of root and leaf tissues
Cambios en el crecimiento y en las ultraestructuras de tejidos radiculares y foliares de Urochloa brizantha cultivado en suelo con niveles tóxicos de aluminio

LUCAS APARECIDO MANZANI LISBOA1,2, GUSTAVO HENRIQUE DE OLIVEIRA DIAS1, HIAGO AUGUSTO AMARAL SACCO1, JOÃO VITOR RODRIGUES PADOVAN1, GABRIEL BANOS RODRIGUES1, KAUÊ BARBAROTTO RIBEIRO1, GABRIEL GEMINIANO DA SILVA1, ALAN DOS SANTOS CARDOSO1, LEANDRO BARRADAS PEREIRA1 and PAULO ALEXANDRE MONTEIRO DE FIGUEIREDO2

1Fundação Educacional de Andradina, Andradina, SP, Brazil. fca.br
2Faculdade de Ciências Agrárias e Tecnológicas, Universidade Estadual Paulista, Dracena, SP, Brazil. dracena.unesp.br

Abstract

Brazilian soils destined for fodder production are infertile and acidic and contain toxic levels of aluminum (Al), which cause a reduction in growth of the root system and aerial plant parts. The main aim of the present pot trial was to determine ultrastructural and developmental changes in root and leaf tissues of Urochloa brizantha, when grown in an acid Oxisol containing varying levels of Al. The experimental design was a 3 × 5 factorial arrangement, involving 3 cultivars of U. brizantha (Marandu, Paiaguás and Piatã) and 5 concentrations of Al in the soil (0.2, 0.4, 0.8, 1.6 and 3.2 cmol/dm³), with 4 replications; a total of 60 pots. All cultivars responded negatively to increasing Al concentration in the soil, even in small amounts. Root ultrastructures were damaged even at concentrations of 0.4 cmol Al/dm³, primarily in the conducting tissues (xylem and phloem) and epidermal cells. Shoot development and leaf tissues were also negatively affected. In general, plant development and ultrastructure of root and leaf tissues in all 3 cultivars of U. brizantha were impaired when grown in the presence of Al at doses >0.2 cmol/dm³ in the soil.

Keywords: Dry matter production, forage, plant morphology, tropical grasses.

Resumen

Los suelos dedicados a la producción de pastos en Brasil se caracterizan en general por baja fertilidad, alta acidez y altas concentraciones de aluminio (Al), lo que conlleva a una reducción del sistema radicular y de las partes aéreas de las plantas. En un experimento de invernadero conducido en Andradina, São Paulo, Brasil, en un Oxisol se evaluaron los efectos de varios niveles de Al en el desarrollo y las ultraestructuras de los tejidos radiculares y foliares de tres cultivares de Urochloa brizantha (cvs. Marandu, Paiaguás y Piatã). El diseño experimental fue en un esquema factorial 3 × 5, con los tres cultivares y cinco concentraciones de Al en el suelo: 0.2; 0.4; 0.8; 1.6 y 3.2 cmol Al/dm³, con cuatro repeticiones, para un total de 60 matas. Los tres cultivares respondieron negativamente al aumentar la concentración de Al en el suelo, incluso en las concentraciones bajas. Las ultraestructuras radiculares sufrieron daños incluso a concentraciones de 0.4 cmol Al, principalmente los tejidos conductores y las células epidérmicas. También el desarrollo de los brotes y los tejidos foliares fueron afectados. En general, el desarrollo de las plantas y las ultraestructuras de tejidos radiculares y foliares se deterioraron cuando los tres cultivares de U. brizantha se cultivaron en presencia de Al en el suelo en dosis superiores a 0.2 cmol/dm³.

Palabras clave: Forraje, hojas, morfología de la planta, producción, raíces.
Introduction

The genus *Urochloa* originated from the African continent, evolving on soils very similar to the infertile soils of the Brazilian Cerrado with a good rainy season and the presence of toxic aluminum (Nunes et al. 1984). *Urochloa* spp. are the most cultivated grass species in Brazil, and display good adaptation and establishment under adverse conditions of soil and climate, producing high yields of dry mass (Ramos et al. 2012; Pezzopane et al. 2015). *Urochloa brizantha*, especially cvv. Marandu, Paiaguás and Piatã, has been popular for more than 30 years. Due to its efficient root system, it easily adapts to acidic soils, with fast and steady growth, has good nutritional value and has been claimed to improve physical and chemical attributes of soil degraded by mining (Stumpf et al. 2016a).

Many Brazilian soils are infertile and acidic (Cantú et al. 2016), as well as containing toxic levels of aluminum (Al), which is found as aluminum oxides or aluminosilicates. Al is one of the most limiting abiotic factors affecting plant production in acidic soils (Stumpf et al. 2016a; 2016b).

The main symptom of toxicity caused by Al in plants is reduction of the root system and altering of the morphology of its tissues (Duressa et al. 2010; Derré et al. 2013). The injuries to the roots hamper the absorption of water, which influences morphophysiological and biochemical characteristics of the plants, restricting both the development of aerial parts and increase in dry mass, with deformation of shoot growth and chlorosis of leaves (Cantú et al. 2016; Jesus et al. 2016).

The improvement of plant tolerance of Al toxicity in the soil has become a strategy to supply more adapted materials to areas of the Brazilian savannas, where *Urochloa* spp. are the basis of forage improvement programs (Figueiredo et al. 2019). Major research efforts have been made to improve plant tolerance of Al toxicity through selection and breeding. According to Bitencourt et al. (2011), different genotypes within the same species showed differences in their development, and how they behave when exposed to different doses of Al in solution, mainly in terms of the diameter and length of the main root.

Many researchers have endeavored to characterize the responses of forage plants to presence of Al in the soil, and currently molecular markers are being used to confirm these responses (Worthington et al. 2020). However, it is also necessary to know what changes in morphology of plant organs occur when they are grown in soils containing Al.

The main aim of the present pot study was to determine ultrastructural and developmental changes in root and leaf tissues of grasses, when grown in acid soil containing varying levels of aluminum. Since *Urochloa brizantha* cultivars are so widely grown they were chosen as the test plants.

Materials and Methods

Experimental design

The study was carried out as a pot experiment between February and May 2018, at the Integrated Faculties Stella Maris (FISMA), located in Andradina, São Paulo State, Brazil (20°53' S, 51°22' W; 413 masl). The experimental design was a $3 \times 5$ factorial arrangement of 3 cultivars of *Urochloa brizantha* (Marandu, Paiaguás and Piatã) and 5 concentrations of aluminum (Al$^{3+}$) in the soil (0.2, 0.4, 0.8, 1.6 and 3.2 cmol Al/dm$^3$), with 4 replications, giving a total of 60 pots. Aluminum chloride (AlCl$_3$) in solution was used as the source of aluminum.

Each experimental unit was composed of a single pot with 6 dm$^3$ capacity filled with sifted soil, fertilized according to Raij et al. (1996). The soil used in the experiment was classified as Vermelho-amarello distrófico férrico (Santos et al. 2013) and soil pH was maintained at 4.7 to make Al readily available to the plants (Table 1). Five seeds were sown per pot, and 15 days later the most developed 3 plants were selected and others removed. Sixty days after sowing, a standardization cut was performed at 2 cm above soil level. Evaluations were made on the regrowth of the grass after another 30 days, i.e. when aerial plant parts (shoots) were 30 days old and roots 90 days old. By this a maximum expression of the toxic effects of Al was expected.

Plant growth attributes and ultrastructural changes

Shoot attributes measured were number of leaves/pot (NL) and dry mass of the aerial part (DMAP; g/pot). Roots were recovered from soil, washed and dry mass of roots (DMR; g/pot) was determined. Furthermore, fragments of totally expanded leaves and fragments of roots (1 cm in size) were collected.

Table 1. Chemical attributes of the soil used in the experiment.

| pH (CaCl$_2$) | SOM (g/dm$^3$) | P (mg/dm$^3$) | K (mmol/dm$^3$) | Ca | Mg | H+Al | Al | SB | CEC | BS% | Al% |
|-------------|---------------|--------------|----------------|----|----|------|----|----|-----|-----|-----|
| 4.7         | 8.0           | 1.0          | 0.5            | 7.0| 6.0| 20   | 2.0| 13.5| 33.5| 40  | 13  |

SOM = soil organic matter; SB = sum of bases; BS% = base saturation; Al% = saturation of Al; CEC = cation exchange capacity.
The samples were transported to Laboratory of Vegetal Morphophysiology and Forages at College of Agricultural and Technological Sciences – São Paulo State University. The collected material was immersed in FAA 70 (37% formaldehyde, acetic acid and 70% ethanol in the ratio of 1:1:18; V/V). Twenty-four hours later the fragments were washed and stored in 70% ethanol until the day of the analyses, as described by Kraus and Arduin (1997). All fragments of plant tissues were treated using the relevant procedures for dehydration, diaphanization, inclusion and embedding.

By using a Leica microtome that contains steel razors, 8 μm transverse sections were obtained from each embedded fragment. The first undamaged transverse section was chosen for preparation of the histological slides. These sections were fixed with patches (albumin), tinted with safranin with a 1% ratio and set in microscope and glass slides with Entellan® patch (Kraus and Arduin 1997).

All slides were examined with an Olympus optical microscope, model BX 43, with an attached camera in order to photograph the sections. Pictures were used to measure anatomical parameters through the software cellSens Standard, which was calibrated with a microscopic rule, as described by Figueiredo et al. (2013).

By using transverse sections, the following ultrastructural variables were measured: root xylem diameter (RXD; μm); root phloem diameter (RPD; μm); root endoderm thickness (RET; μm); leaf xylem diameter (LXD; μm); leaf phloem diameter (LPD; μm); adaxial epidermal thickness (ADET; μm); and abaxial epidermal thickness (ABET; μm), according to the methodology of Figueiredo et al. (2013). The lower or abaxial epidermal impression of the fragments, collected using cyanoacrylate ester (Segatto et al. 2004), was used to determine density of stomata (SD) and stomatal functionality (SF) according to Castro et al. (2009). Ten measurements were done for all characteristics on each microscope slide. Plots were represented by average values obtained for each characteristic.

Statistical analyses

All variables were submitted to an F test (P<0.05); the Tukey test was applied at 5% probability and regression analysis was applied to the AI doses, in which their models were tested for linear, quadratic and cubic relationships (Banzatto and Kronka 2013), by using Assistat 7.7 statistic software (Silva and Azevedo 2016).

Results

Plant growth attributes

Cultivar Paiaguás had the highest average number of leaves (NL) with more dry mass of aerial parts (DMAP) than cv. Marandu (P<0.05). On the other hand, dry mass of roots (DMR) for cv. Marandu was greater than those of Paiaguás and Piatã (Table 2).

There was a negative linear relationship between the number of leaves per plant and the concentration of Al in the soil, with the lowest concentration of Al in soil resulting in the greatest number of leaves in all 3 cultivars (Figure 1).

Table 2. Analysis of variance of plant growth characteristics of 3 Urochloa brizantha cultivars (cvv. Marandu, Paiaguás and Piatã) grown in soils with different concentrations of aluminum.

|         | NL (no.) | DMAP (g/pot) | DMR (g/pot) |
|---------|----------|--------------|-------------|
| Forage  |          |              |             |
| Marandu | 62.5b    | 6.31b        | 33.5a       |
| Paiaguás| 80.6a    | 8.00a        | 27.0b       |
| Piatã   | 64.0b    | 6.86ab       | 27.7b       |
| SMD     | 8.45     | 1.43         | 3.34        |
| P value | 0.0001   | 0.0205       | 0.001       |
| cmol Al/dm³ (Al) |
| 0.2     | 83.3a    | 9.71a        | 37.6a       |
| 0.4     | 70.3b    | 8.33ab       | 28.8b       |
| 0.8     | 63.0b    | 6.57bc       | 28.1b       |
| 1.6     | 69.0b    | 5.87c        | 28.1b       |
| 3.2     | 59.6b    | 4.80c        | 24.4b       |
| SMD     | 12.80    | 2.17         | 5.06        |
| P value | 0.0001   | 0.0001       | 0.001       |
| CV (%)  | 15.9     | 26.4         | 14.8        |
| Overall mean | 69.0 | 7.06        | 29.4        |
| P value FxAl | 0.8364 | 0.6001      | 0.0239      |

Analysis of regression variance

|         | Marandu |              |              |
|---------|---------|--------------|--------------|
| P value | 0.0138  | 0.0001       | 0.0015       |
| Regression | L*     | L**          | L**          |
| CV (%)  | 12.1    | 16.6         | 14.7         |
| Paiaguás|         |              |              |
| P value | 0.0066  | 0.0037       | 0.0012       |
| Regression | L**    | L**          | L**          |
| CV (%)  | 17.3    | 34.9         | 17.9         |
| Piatã   |         |              |              |
| P value | 0.0154  | 0.0001       | 0.0005       |
| Regression | L*     | L**          | L**          |
| CV (%)  | 15.2    | 16.6         | 11.4         |

NL = number of leaves; DMAP = dry mass of aerial parts; and DMR = dry mass of roots. SMD = significant minimum difference; L = polynomial of 1st degree.

In the same way as for number of leaves, dry mass of aerial parts (shoots) was negatively related to concentration of Al in the soil (Figure 2A). The lower development of aerial parts may be a response to the reduced development of roots, which also decreased as the level of Al in soil increased (Figure 2B).

Large differences in development of roots of all 3 cultivars occurred when Al concentrations in soil increased from 0.2 to 3.2 cmol Al/dm³ (Figure 3).
Figure 1. Regression analysis of number of leaves (NL) in 3 cultivars of *Urochloa brizantha* (cvv. Marandu, Paiaguás and Piatã) in relation to level of aluminum in soil.

**Ultrastructural changes in leaf and root tissue**

Table 3 presents analyses of variance of the ultrastructural characteristics of leaves and roots of the 3 *U. brizantha* cultivars grown in soils with different concentrations of Al. Stomatal density (SD) on leaves of all 3 cultivars was similar, but stomatal functionality (SF) for cv. Piatã was greater (*P = 0.007*) than that of cv. Paiaguás. The pattern for roots was different with diameter of root xylem (RXD) for Marandu and Paiaguás being significantly greater than that of Piatã (*P=0.0001*), while diameter of root phloem (RPD) for Marandu was significantly greater than those of Paiaguás and Piatã (*P=0.0001*).

Stomatal development on the abaxial surface of leaves of some of the cultivars was impaired as Al concentrations were increased, as shown in Figure 4. Stomatal density (SD) and stomatal functionality (SF) in Marandu declined in a linear fashion as Al concentrations in soil increased, but Al concentration had no impact on these parameters for Paiaguás or on SD for Piatã (Table 3). For Piatã, SF increased initially as Al concentration in soil increased but then declined giving a quadratic response. Peak activity occurred at approximately 1.9 cmol Al/dm$^3$ in soil.

This negative response to concentration of Al in soil is an important factor in understanding the anatomical responses of plants to varying concentrations of the metal in soil. Figure 5 shows that the morphology of the inner tissues of roots was impaired as Al$^{3+}$ concentration in soil increased from 0.2 to 3.2 cmol/dm$^3$.

Root xylem diameter (RXD) of Marandu showed a linear negative response to the concentration of Al, while Paiaguás and Piatã showed quadratic responses, reaching peaks between 0.8 and 2.1 cmol/dm$^3$ (Figure 5A).

Figure 2. Regression analysis of: **A** – dry mass of aerial parts (DMAP); and **B** – dry mass of roots (DMR) of 3 cultivars of *Urochloa brizantha* (cvv. Marandu, Paiaguás and Piatã) in relation to level of aluminum in soil.
Figure 3. *Urochloa brizantha* plants grown in soils with different concentrations of aluminum: a – cv. Marandu in soil with 0.2 cmol Al/dm$^3$; b – cv. Marandu in soil with 3.2 cmol Al/dm$^3$; c – cv. Paiaguás in soil with 0.2 cmol Al/dm$^3$; d – cv. Paiaguás in soil with 3.2 cmol Al/dm$^3$; e – cv. Piatã in soil with 0.2 cmol Al/dm$^3$; and f – cv. Piatã in soil with 3.2 cmol Al/dm$^3$. Bars = 5.0 cm.

Table 3. Analysis of variance of 5 ultrastructural characteristics of 3 *Urochloa brizantha* cultivars (cvv. Marandu, Paiaguás and Piatã) grown in soils with different concentrations of aluminum.

| Forage (F) | SD (no./mm$^2$) | SF | RXD (μm) | RPD (μm) | RET (μm) |
|-----------|----------------|----|----------|----------|----------|
| Marandu   | 104.0a         | 2.82ab | 46.7a    | 10.16a   | 17.2a    |
| Paiaguás  | 114.9a         | 2.52b  | 42.6a    | 7.61b    | 16.7a    |
| Piatã     | 104.6a         | 3.06a  | 36.6b    | 7.37b    | 16.3a    |
| SMD       | 15.09          | 0.39   | 4.81     | 1.20     | 2.45     |
| P value   | 0.1555         | 0.0070 | 0.0001   | 0.0001   | 0.6532   |

| cmol Al/dm$^3$ (Al) | SD (no./mm$^2$) | SF | RXD (μm) | RPD (μm) | RET (μm) |
|---------------------|----------------|----|----------|----------|----------|
| 0.2                 | 112.3a         | 2.97a  | 41.4ab   | 8.35ab   | 16.83b   |
| 0.4                 | 111.4a         | 2.97a  | 42.1ab   | 9.64a    | 19.80ab  |
| 0.8                 | 108.3a         | 2.89a  | 44.1a    | 9.09ab   | 21.25a   |
| 1.6                 | 107.0a         | 3.00a  | 46.3a    | 7.55b    | 16.12b   |
| 3.2                 | 100.1a         | 2.16b  | 35.9b    | 7.37b    | 9.62c    |
| SMD                 | 22.85          | 0.59   | 7.28     | 1.82     | 3.71     |
| P value             | 0.5750         | 0.0007 | 0.0035   | 0.0026   | 0.0001   |

| CV (%)              | 18.21          | 18.19 | 14.92    | 18.70    | 19.06    |
| Overall mean        | 107.8          | 2.80  | 42.0     | 8.38     | 16.73    |
| P value F×Al        | 0.3563         | 0.5295 | 0.1863   | 0.0001   | 0.5518   |

Analysis of regression variance

| Forage (F) | P value | Regression | CV (%) |
|------------|---------|------------|--------|
| Marandu    | 0.065   | L**       | 10.6   |
| P value    |         | L*        | 18.2   |
| Regression |         | L**       | 11.1   |
| CV (%)     |         | L*        | 15.9   |
| Paiaguás   | 0.5968  | L*        | 22.9   |
| P value    |         | Q*        | 20.1   |
| Regression |         | Q**       | 17.3   |
| CV (%)     |         | Q**       | 10.6   |
| Piatã      | 0.7292  | Q*        | 16.6   |
| P value    |         | Q**       | 15.9   |
| Regression |         | L*        | 15.5   |
| CV (%)     |         | Q**       | 27.6   |

SD = stomatal density; SF = stomatal functionality (= ratio polar diameter/equatorial diameter); RXD = root xylem diameter; RPD = root phloem diameter; and RET = root endoderm thickness. SMD = significant minimum difference; L = polynomial of 1st degree; and Q = polynomial of 2nd degree.

*Tropical Grasslands-Forrajes Tropicales* (ISSN: 2346-3775)
Figure 4. Effects of aluminum concentration in soil on: A – stomatal density (SD); and B – stomatal functionality (SF) in leaves of *Urochloa brizantha* cvv. Marandu and Piatã.

For RPD, Marandu and Piatã displayed linear negative effects, while Paiaguás presented a quadratic response, with the greatest effect at 2.3 cmol/dm$^3$ of Al in soil (Figure 5B). For RET, all cultivars showed quadratic responses, as shown in Figure 5C, in which Marandu peaked at 0.1 cmol Al/dm$^3$ in soil, while Paiaguás and Piatã peaked at 0.9 and 1.3 cmol Al/dm$^3$, respectively.

Marked injury to roots of the *U. brizantha* cultivars due to the presence of Al in the soil is depicted in Figure 6 with red arrows indicating the injuries to the endodermis, as the plant was subjected to 3.2 cmol Al/dm$^3$ in soil.

A statistical difference was observed between the cultivars for leaf xylem diameter (LXD), where Paiaguás showed higher values, and for leaf phloem diameter (LPD), where the values of Piatã were lower. For abaxial epidermal thickness (ABET) there was no statistical difference between cultivars. However, Piatã showed higher adaxial epidermal thickness (ADET) than both Paiaguás and Marandu. A negative response to increasing Al concentration in soil was also found in the morphology of some internal tissues of leaves (Table 4).

Figure 5. A – Root xylem diameter (RXD); B – root phloem diameter (RPD); and C – root endodermis thickness (RET) of 3 cultivars of *Urochloa brizantha* (cvv. Marandu, Paiaguás and Piatã) grown in soil with different concentrations of aluminum.
Al toxicity in *U. brizantha* cultivars

**Figure 6.** Root ultrastructural changes observed in 3 *Urochloa brizantha* cultivars grown in soil with contrasting levels of aluminum. **A** – cv. Marandu in soil with 0.2 cmol Al/dm³; **B** – cv. Marandu in soil with 3.2 cmol Al/dm³; **C** – cv. Paiaguás in soil with 0.2 cmol Al/dm³; **D** – cv. Paiaguás in soil with 3.2 cmol Al/dm³; **E** – cv. Piatã in soil with 0.2 cmol Al/dm³; and **F** – cv. Piatã in soil with 3.2 cmol Al/dm³. Zoom: 400x. The red arrows indicate the injuries to the root endodermis. RXD = root xylem diameter; RPD = root phloem diameter; and RET = root endoderm thickness.

**Table 4.** Analysis of variance of 4 ultrastructural characteristics of leaves of 3 *Urochloa brizantha* cultivars (cvv. Marandu, Paiaguás and Piatã) grown in soil with different concentrations of aluminum.

| Forage (F)     | LXD (µm) | LPD (µm) | ADET (µm) | ABET (µm) |
|----------------|----------|----------|-----------|-----------|
| Marandu        | 25.5a    | 3.98ab   | 7.26b     | 7.12a     |
| Paiaguás       | 22.0b    | 4.00a    | 6.87b     | 6.45a     |
| Piatã           | 22.3ab   | 3.48b    | 9.10a     | 6.80a     |
| SMD             | 3.47     | 0.51     | 1.47      | 1.12      |
| P value         | 0.0345   | 0.0310   | 0.0014    | 0.3624    |
| cmol Al/dm³ (Al) |          |          |           |           |
| 0.2             | 27.0a    | 4.39a    | 9.39a     | 7.46a     |
| 0.4             | 20.5b    | 3.82ab   | 8.93ab    | 7.54a     |
| 0.8             | 24.1ab   | 3.61ab   | 7.00bc    | 7.31a     |
| 1.6             | 24.0ab   | 3.88ab   | 8.47ab    | 6.56ab    |
| 3.2             | 21.06b   | 3.40b    | 4.92c     | 5.10b     |
| SMD             | 5.36     | 0.78     | 2.22      | 1.70      |
| P value         | 0.0068   | 0.0133   | 0.0001    | 0.0008    |
| CV (%)          | 19.44    | 17.73    | 24.72     | 21.58     |
| Overall mean    | 23.33    | 3.82     | 7.74      | 6.79      |
| P value FxAl    | 0.0926   | 0.0807   | 0.0734    | 0.1987    |

Analysis of regression variance

| Marandu        |          |          |           |           |
|----------------|----------|----------|-----------|-----------|
| P value        | 0.7987   | 0.0111   | 0.1776    | 0.0012    |
| Regression     | ns       | L**      | ns        | L**       |
| CV (%)         | 14.9     | 15.5     | 27.4      | 16.8      |

| Paiaguás       |          |          |           |           |
|----------------|----------|----------|-----------|-----------|
| P value        |          |          |           |           |
| Regression     | ns       | ns       | L*        | L*        |
| CV (%)         | 24.5     | 22.3     | 20.5      | 22.6      |

| Piatã           |          |          |           |           |
|----------------|----------|----------|-----------|-----------|
| P value        | 0.0015   | 0.6044   | 0.0008    | 0.0331    |
| Regression     | L**      | ns       | L**       | L*        |
| CV (%)         | 19.0     | 13.5     | 24.4      | 25.7      |

LXD = leaf xylem diameter; LPD = leaf phloem diameter; ADET = adaxial epidermal thickness; and ABET = abaxial epidermal thickness. SMD = significant minimum difference; and L = polynomial of 1st degree.

Leaf xylem diameter (LXD) of Piatã showed a linear negative response to increasing concentrations of Al in soil (Figure 7A), while leaf phloem diameter (LPD) of Marandu showed a linear negative response (Figure 7B). In a similar way, leaves of Paiaguás and Piatã showed linear negative responses in ADET to increasing concentrations of Al (Figure 7C). For ABET, all 3 cultivars showed linear negative responses as Al concentrations increased (Figure 7D).

Ultrastructural changes in inner tissues of leaves of the cultivars were observed as Al concentration in soil increased (Figure 8).

_Tropical Grasslands-Forrajes Tropicales (ISSN: 2346-3775)_
Figure 7. Leaf xylem diameter (LXD), leaf phloem diameter (LPD), adaxial epidermal thickness (ADET) and abaxial epidermal thickness (ABET) of 3 *Urochloa brizantha* cultivars (cv. Marandu, Paiaguás and Piatã) in soil with different concentrations of aluminum.

Figure 8. Ultrastructural changes in leaf tissues of 3 *Urochloa brizantha* cultivars grown in soil with contrasting concentrations of aluminum. A – cv. Marandu in soil with 0.2 cmol Al/dm³; B – cv. Marandu in soil with 3.2 cmol Al/dm³; C – cv. Paiaguás in soil with 0.2 cmol Al/dm³; D – cv. Paiaguás in soil with 3.2 cmol Al/dm³; E – cv. Piatã in soil with 0.2 cmol Al/dm³; and F – cv. Piatã in soil with 3.2 cmol Al/dm³. Zoom: 400x. LXD = leaf xylem diameter; LPD = leaf phloem diameter; ADET = adaxial epidermal thickness; and ABET = abaxial epidermal thickness.
Discussion

This study has provided clear indications of the ultrastructural and developmental changes in plants of some *U. brizantha* cultivars when grown in soils with varying concentrations of Al. The decrease in number of leaves of the grasses as concentration of Al increased could reduce net photosynthesis, thereby lowering accumulation of dry mass in the aerial parts and roots (Figure 2B). Al becomes harmful to plant growth as concentration in soils increases and as its availability is increased in acidic soils, i.e. with lower pH in soil solution (Cai et al. 2011).

It is worth mentioning that the cultivars presented different responses to cultivation in acid soils in presence of Al. It is for such soils in the Brazilian savanna region that cv. Marandu was launched in 1985, followed by cv. Xaraés in 2003. Following further genetic improvement, new cultivars like Pião and Paiaguás have become alternatives to Xaraés and Marandu, as they present a greater accumulation of leaves as reported by Valle et al. (2007). Our findings are in agreement with that earlier work and suggest these new cultivars are a useful alternative for cattle producers on acid soils.

The first negative response to the presence of Al in soil is atrophy of the root system owing to inhibition of cell division in the root cap or to small injuries in this area (Čiamporová 2002; Guo et al. 2014; Wang et al. 2016; Xu et al. 2016). Enzyme activity can be reduced as a response to stress to which the plant is submitted in the presence of Al (Kumari et al. 2008; Duressa et al. 2011).

This reduced development of the root system results in lower absorption of nutrients, which will impair the growth of aerial parts of the plant (Figures 2B and 3). In all situations, plants that display poor development of the root system also display problems in their above-ground structure (Reis et al. 2017; Lisboa et al. 2019).

The impairment of stomatal density and functionality of the grasses with increase in Al concentration in soil could be a result of low availability of nutrients supplied to leaves from the root system. Vegetation may react by activating ALMT (aluminum-activated malate transporter) found in plasma membrane or on the tonoplast of plant cells (Palmer et al. 2016). This response can be impaired in the presence of calcium ions, lightless conditions and even abscisic acid action (Sasaki et al. 2010; Araújo et al. 2011).

Marked changes were detected in conducting tissues of the roots (xylem and phloem), as Figures 5A and 5B show, which may depress cell formation in the aerial parts of the plant (Figure 8). Al is stored in pericycle and can lead to formation of xylem. As the concentration of Al increases in acidic soils, deposition occurs within the plants which starts to interfere with the transport of sap within the xylem and phloem vessels. The displacement of Al inside these conducting vessels occurs in the form of Al-citrate, when Al associates with citric acid, since the metal is found in the root cortex reaching a high internal concentration in the vessels, and producing lesions in these root tissues (Klug et al. 2011; Ma and Hiradate 2011) as Figures 6B, 6D and 6F show.

As the Al-citrate is translocated to leaf cells, the plant may show an anti-oxidative physiological response, possibly even chelating the metal, fixing it as oxalate, which is inactive and may be stored within cells (Souza et al. 2018). This process may produce a hardness in the inner tissues, impairing the development of the axial or radial cells of leaf tissues.

At low Al concentrations in soil, an increase in thickness of root endodermis occurred, peaking between 0.9 and 1.3 cmol Al/dm³, but this was followed by an acute fall in epidermal thickness as concentrations of Al in soil increased. This morphological response is critical for the root system, as root epidermis acts as a barrier to protect the root vessels. While low concentrations of Al had a positive effect, higher concentrations had a marked negative effect, which could interfere with the volume of cytoplasm within the cell (Poschenrieder et al. 2008; Duressa et al. 2011; Ma and Hiradate 2011).

Conclusions

All 3 *U. brizantha* cultivars studied responded negatively to increasing Al concentration in the soil, in amounts >0.2 cmol/dm³, through impairment of plant development and ultrastructure of root and leaf tissues. Both shoot development and leaf tissue production were reduced. However cv. Paiaguás produced more leaves and more above-ground biomass than cv. Marandu at all Al concentrations. Further studies in the field are warranted to determine if these findings can be reproduced on a larger scale.

References

(Note of the editors: All hyperlinks were verified 13 January 2020.)

Araújo WL; Fernie AR; Nunes-Nesi A. 2011. Control of stomatal aperture. Plant Signaling & Behavior 6:1305–1311. doi: 10.4161/psb.6.9.16425

Banzatto DA; Kronka SN. 2013. Experimentação agrícola. 4th Edn. Funep, Jaboticabal, SP, Brazil.

Bitencourt GA; Chiari L; Laura VA; Valle CB do; Jank L; Moro JR. 2011. Aluminum tolerance of genotypes of signal grass. Revista Brasileira de Zootecnia 40:245–250. doi: 10.1590/ S1516-35982011000200003

Tropical Grasslands-Forrajes Tropicales (ISSN: 2346-3775)
Cai MZ; Wang FM; Li RF; Zhang SN; Wang N; Xu GD. 2011. Response and tolerance of root border cells to aluminum toxicity in soybean seedlings. Journal of Inorganic Biochemistry 105:966–971. doi: 10.1016/j.jinorgbio.2011.04.004

Cantù T; Vieira CE; Piffer RD; Luiz GC; Souza GH. 2016. Transcriptional modulation of genes encoding nitrate reductase in maize (Zea mays) grown under aluminum toxicity. African Journal of Biotechnology 15:2465–2473. doi: 10.5897/AJB2016.15585

Castro EM; Pereira FJ; Paiva R. 2009. Histologia vegetal: Estrutura e função de órgãos vegetativos. Editora UFLA, Lavras, MG, Brazil.

Čiamporová M. 2002. Morphological and structural responses of plant roots to aluminum at organ, tissue, and cellular levels. Biologia Plantarum 45:161–171. doi: 10.1023/A:1015159601881

Derré LO; Custódio CC; Agostini EAT de; Guerra WEX. 2013. Water uptake time courses for coated and uncoated Urochloa brizantha and Urochloa ruziensis seeds. Colloquium Agrariae 9:103–111. (In Portuguese.) doi: 10.5747/ca.2013.v09.n2.a094

Duressa D; Soliman KM; Chen D. 2010. Mechanisms of magnesium amelioration of aluminum toxicity in soybean at the gene expression level. Genome 53:787–797. doi: 10.1139/G10-069

Duressa D; Soliman K; Taylor R; Senwo Z. 2011. Protomecnic analysis of soybean roots under aluminum stress. International Journal of Plant Genomics Vol. 2011, article 282531. doi: 10.1155/2011/282531

Figueiredo PAM; Ramos SB; Viana RS; Lisboa LAM; Heinrichs R. 2013. Morph anatomical changes of sugar cane leaves in phase of establishment under weed competition. Planta Daninha 31:777–784. (In Portuguese.) doi: 10.1590/S0100-83582013000400003

Figueiredo UJ de; Berchimbrock YV; Valle CB do; Barrios SCL; Quesenberry KH; Muñoz PR; Nunes JAR. 2019. Evaluating early selection in perennial tropical forages. Crop Breeding and Applied Biotechnology 19:291–299. doi: 10.1590/1984-70332019v19n3a41

Guo L; Ott DW; Cuthright TJ. 2014. Accumulation and histological location of heavy metals in Phragmites australis grown in acid mine drainage contaminated soil with or without citric acid. Environmental and Experimental Botany 105:46–54. doi: 10.1016/j.envexpbot.2014.04.010

Jesus DS de; Martins FM; Azevedo Neto AD de. 2016. Structural changes in leaves and roots are anatomical markers of aluminum sensitivity in sunflower. Pesquisa Agropecuária Tropical 46:383–390. doi: 10.1590/1983-40632016v4641426

Klub B; Specht A; Horst WJ. 2011. Aluminium localization in root tips of the aluminium accumulating plant species buckwheat (Fagopyrum esculentum Moench). Journal of Experimental Botany 62:5453–5462. doi: 10.1093/jxb/er222

Kraus JE; Arduin M. 1997. Manual básico de métodos em morfologia vegetal. Universidade Rural, Seropédica, RJ, Brazil.

Kumari M; Taylor GJ; Deyholos MK. 2008. Transcriptomic responses to aluminum stress in roots of Arabidopsis thaliana. Molecular Genetics and Genomics 279:339–357. doi: 10.1007/s00438-007-0316-z

Lisboa LAM; Lapaz AM; Spósito THN; Viana RS; Figueiredo PAM de. 2019. Growth, development and foliar ultrastructural parameters of different eucalyptus genetic materials. Floresta 49:21–30. doi: 10.5380/rv.f49i1.52527

Ma JF; Hiradate S. 2000. Form of aluminium for uptake and translocation in buckwheat (Fagopyrum esculentum Moench). Planta 211:355–360. doi: 10.1007/s004250000292

Nunes SG; Boock A; Penteado MIO; Gomes DT. 1984. Brachiaria brizantha cv. Marandu. Documentos 21. EMBRAPA-CNPGC, Campo Grande, MS, Brazil. infoteca.cnptia.embrapa.br/infoteca/handle/doc/317899

Palmer AJ; Baker A; Muench SP. 2016. The varied functions of aluminium - activated malate transporters – much more than aluminium resistance. Biochemical Society Transactions 44:856–862. doi: 10.1042/BST20160027

Pezzopane CG; Santos PM; Cruz PG da; Altoé J; Ribeiro FA; Valle CB do. 2015. Hydric deficiency in genotypes of Brachiaria brizantha. Ciência Rural 45:871–876. (In Portuguese.) doi: 10.1590/0103-8478cr20130915

Poschenrieder C; Gunsé B; Corrales I; Barceló J. 2008. A glance into aluminum toxicity and resistance in plants. Science of the Total Environment 400:356–368. doi: 10.1016/j.scitotenv.2008.06.003

Raij B; Cantarella H; Quaggio JA; Furlani AMC. 1996. Recomendações de adubação e calagem para o Estado de São Paulo. 2nd Edn. Instituto Agronômico - Fundag, Campinas, SP, Brazil.

Ramos FT; França MGC; Alvim MN; Rosselio ROP; Zonta E. 2012. Aluminum tolerance measured by root growth and mucilage protection in Urochloa brizantha and Urochloa decumbens. Journal of Plant Interactions 7:225–229. doi: 10.1080/17429152012.603207

Reis AR dos; Barcelos JQP; Osório CRWS; Santos EF; Lisboa LAM; Santini JMK; Santos MJQ dos; Furlani Jr E; Campos M; Figueiredo PAM de; Lavres J; Gratão PL. 2017. A glimpse into the physiological, biochemical and nutritional status of soybean plants under Ni-stress conditions. Environmental and Experimental Botany 144:76–87. doi: 10.1016/j.envexpbot.2017.10.006

Santos HG dos; Almeida JA; Oliveira JB de; Lumbrares JF; Anjos LHC dos; Coelho MR; Jacomine PTK; Cunha TJF; Oliveira VA de. 2013. Sistema brasileiro de classificação de solos. 3rd Edn. Embrapa Solos, Rio de Janeiro, RJ, Brazil. bit.ly/2HfIUO

Sasaki T; Mori IC; Furuichi T; Munemasa S; Toyooka K; Matsuoka K; Murata Y; Yamamoto Y. 2010. Closing plant stomata requires a homolog of an aluminum-activated malate transporter. Plant and Cell Physiology 51:354–365. doi: 10.1017/cpcp016

Segatto FB; Bisognin DA; Benedetti M; Costa LC da; Rampelotto MV; Nicoloso FT. 2004. A technique for the anatomical study of potato leaf epidermis. Ciência Rural

Tropical Grasslands-Forragens Tropicais (ISSN: 2346-3775)
Al toxicity in U. brizantha cultivars

34:1597–1601. (In Portuguese.) doi: 10.1590/S0103-84782004000050042
Silva FAS; Azevedo CAV de. 2016. The Assistat Software Version 7.5 and its use in the analysis of experimental data. African Journal of Agricultural Research 11:3733–3740. doi: 10.5897/AJAR2016.11522
Souza MC de; Scalon MC; Poschenrieder C; Roser Tolrà R; Venâncio T; Teixeira SP; Costa FB da. 2018. Aluminium detoxification in facultative (Passovia ovata (Pohl ex DC.) Kuijt and Struthanthus polyanthus Mart. - Loranthaceae) and dependent (Psittacanthus robustus (Mart.) Marloth - Loranthaceae) Al-accumulating mistletoe species from the Brazilian savanna. Phytochemistry 153:58–63. doi: 10.1016/j.phytochem.2018.05.020
Stumpf L; Pauletto EA; Pinto LFS; Pinto MAB; Dutra Jr LA; Scheunemann T. 2016a. The root system of Urochloa brizantha: Development and influence on the attributes of a degraded soil. Interciencia 41:334–339. (In Portuguese). bit.ly/3sCM2H4
Stumpf L; Pauletto EA; Pinto LFS; Garcia GF; Ambus JV; Silva TS da; Pinto MAB; Tuchtenhagen IK. 2016b. Physical condition and root development of grasses in a constructed minesoil after coal mining. Pesquisa Agropecuária Brasileira 51:1078–1087. (In Portuguese) doi: 10.1590/S0100-204X2016000900007
Valle CB do; Euclides VPB; Valério JR; Macedo MCM; Fernandes CD; Dias Filho MB. 2007. Brachiaria brizantha cv. Piatã: Uma forrageira para a diversificação de pastagens tropicais. Seed News 11(2):28–30. alice.cnptia.embrapa.br/alice/handle/doc/969633
Wang P; Deng X; Huang Y; Fang X; Zhang J; Wan H; Yang C. 2016. Root morphological responses of five soybean [Glycine max (L.) Merr] cultivars to cadmium stress at young seedlings. Environmental Science and Pollution Research 23:1860–1872. doi: 10.1007/s11356-015-5424-4
Worthington M; Perez JG; Mussurova S; Silva-Cordoba A; Castiblanco V; Cardoso Arango JA; Jones C; Fernandez-Fuentes N; Skot L; Dyer S; Tohme J; Palma F di; Arango J; Armstead I; Vega JJ de. 2020. A new genome allows the identification of genes associated with natural variation in aluminium tolerance in Brachiaria grasses. Journal of Experimental Botany eraa469. doi: 10.1093/jxb/eraa469
Xu Q; Yu W; Ding Z; Song L; Li Y; Ma D; Wang Y; Shen J; Jia S; Sun H; Zhang H. 2016. Aluminum induced metabolic responses in two tea cultivars. Plant Physiology and Biochemistry 101:162–172. doi: 10.1016/j.plaphy.2016.02.001

(Received for publication 3 August 2018; accepted 21 December 2020; published 31 January 2021)

© 2021

Tropical Grasslands-Forrajes Tropicales is an open-access journal published by International Center for Tropical Agriculture (CIAT), in association with Chinese Academy of Tropical Agricultural Sciences (CATAS). This work is licensed under the Creative Commons Attribution 4.0 International (CC BY 4.0) license.