

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

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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 materas. 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.

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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 acid 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 acid 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 behaved 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 × 5 factorial arrangement of 3 cultivars of *Urochloa brizantha* (Marandu, Paiaguás and Piatã) and 5 concentrations of aluminum (Al³⁺) in the soil (0.2, 0.4, 0.8, 1.6 and 3.2 cmol Al/dm³), with 4 replications, giving a total of 60 pots. Aluminum chloride (AlCl₃) in solution was used as the source of aluminum.

Each experimental unit was composed of a single pot with 6 dm³ capacity filled with sifted soil, fertilized according to Raij et al. (1996). The soil used in the experiment was classified as Vermelho-amarelo 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	Ca	Mg	H+Al	Al	SB	CEC	BS%	Al%
							(mmol/dm ³)				
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 date 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 Al 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 (F)			
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 F×Al	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).

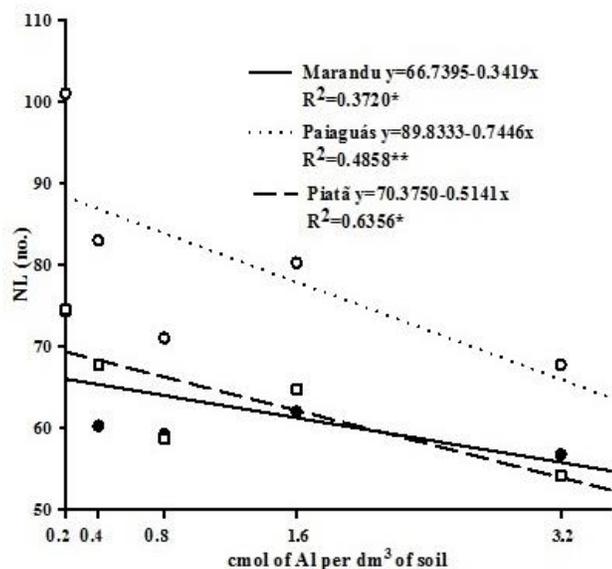


Figure 1. Regression analysis of number of leaves (NL) in 3 cultivars of *Urochloa brizantha* (cvv. Marandu, Paiaguás and Piatã) in relation to level of aluminum in soil.

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/dm³ 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 Al³⁺ concentration in soil increased from 0.2 to 3.2 cmol/dm³.

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/dm³ (Figure 5A).

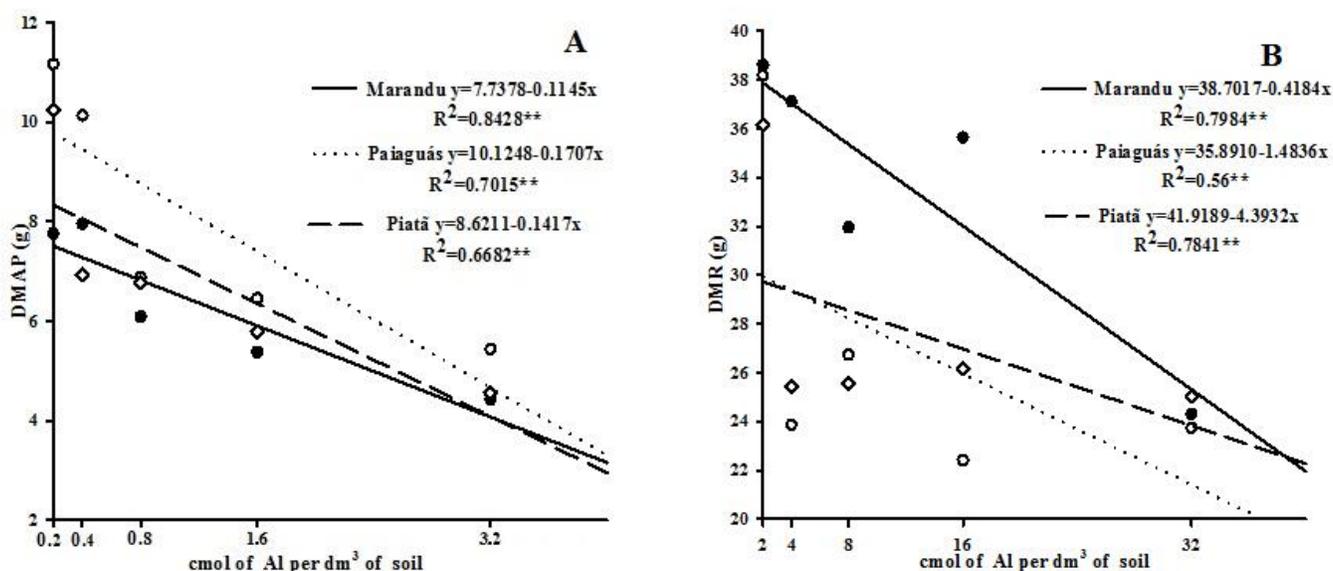


Figure 2. Regression analysis of: **A** – dry mass of aerial parts (DMAP); and **B** – dry mass of roots (DMR) of 3 cultivars of *Urochloa brizantha* (cvv. Marandu, Paiaguás and Piatã) in relation to level of aluminum in soil.

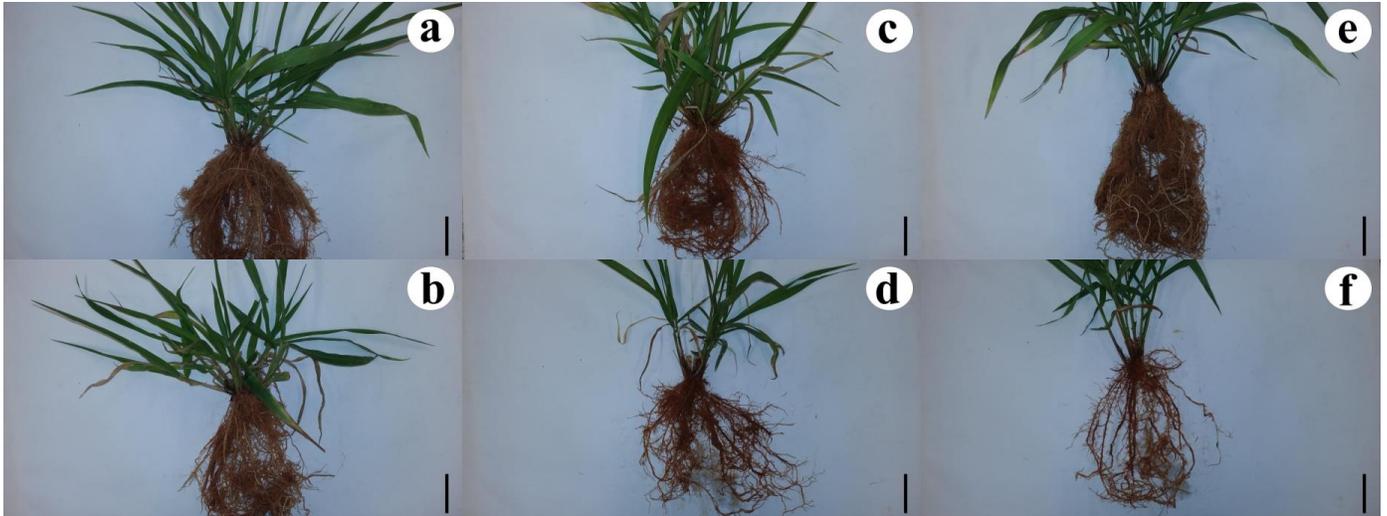


Figure 3. *Urochloa brizantha* plants grown in soils with different concentrations of aluminum: **a** – cv. Marandu in soil with 0.2 cmol Al/dm³; **b** – cv. Marandu in soil with 3.2 cmol Al/dm³; **c** – cv. Paiaguás in soil with 0.2 cmol Al/dm³; **d** – cv. Paiaguás in soil with 3.2 cmol Al/dm³; **e** – cv. Piatã in soil with 0.2 cmol Al/dm³; and **f** – cv. Piatã in soil with 3.2 cmol Al/dm³. 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.

	SD (no./mm ²)	SF	RXD (µm)	RPD (µm)	RET (µm)
Forage (F)					
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)					
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					
Marandu					
P value	0.065	0.0112	0.0059	0.0218	0.0002
Regression	L**	L*	L**	L*	Q**
CV (%)	10.6	18.2	11.1	15.9	16.5
Paiaguás					
P value	0.5968	0.1729	0.0216	0.0002	0.0054
Regression	ns	ns	Q*	Q**	Q**
CV (%)	22.9	20.1	17.3	10.6	23.9
Piatã					
P value	0.7292	0.0121	0.0214	0.0212	0.0001
Regression	ns	Q*	Q*	L*	Q**
CV (%)	16.6	15.9	15.5	27.6	18.1

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.

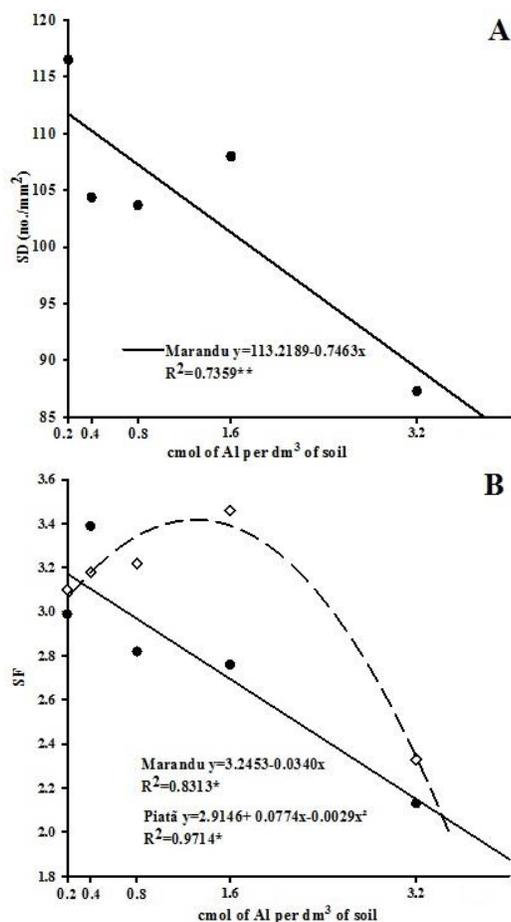


Figure 4. Effects of aluminum concentration in soil on: **A** – stomatal density (SD); and **B** – stomatal functionality (SF) in leaves of *Urochloa brizantha* cvv. Marandu and Piatã.

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 $\text{cmol Al}/\text{dm}^3$ in soil, while Paiaguás and Piatã peaked at 0.9 and 1.3 $\text{cmol Al}/\text{dm}^3$, 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 $\text{cmol Al}/\text{dm}^3$ 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).

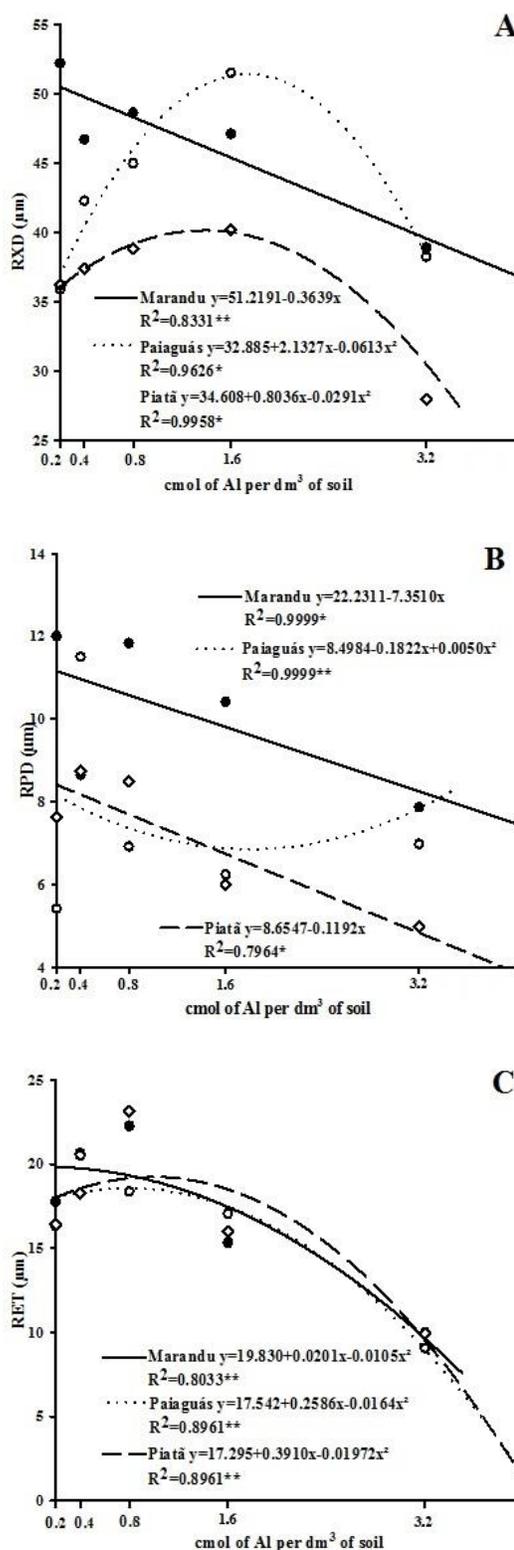


Figure 5. **A** – Root xylem diameter (RXD); **B** – root phloem diameter (RPD); and **C** – root endodermis thickness (RET) of 3 cultivars of *Urochloa brizantha* (cvv. Marandu, Paiaguás and Piatã) grown in soil with different concentrations of aluminum.

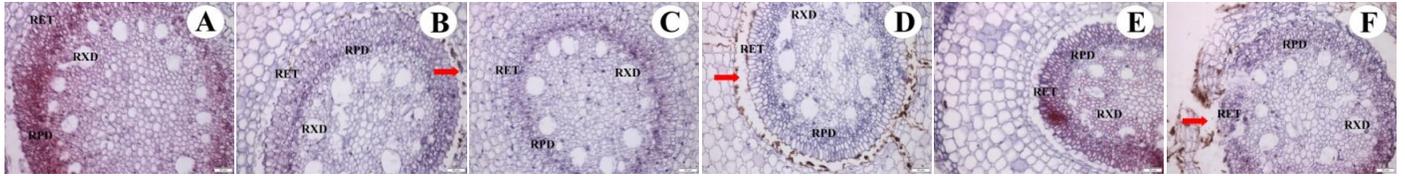


Figure 6. Root ultrastructural changes observed in 3 *Urochloa brizantha* cultivars grown in soil with contrasting levels of aluminum. **A** – cv. Marandu in soil with 0.2 cmol Al/dm³; **B** – cv. Marandu in soil with 3.2 cmol Al/dm³; **C** – cv. Paiaguás in soil with 0.2 cmol Al/dm³; **D** – cv. Paiaguás 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: 400×. 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.

	LXD (μm)	LPD (μm)	ADET (μm)	ABET (μm)
Forage (F)				
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 F×Al	0.0926	0.0807	0.0734	0.1987
Analysis of regression variance				
Marandu				
P value	0.7987	0.0011	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 con-

centrations 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).

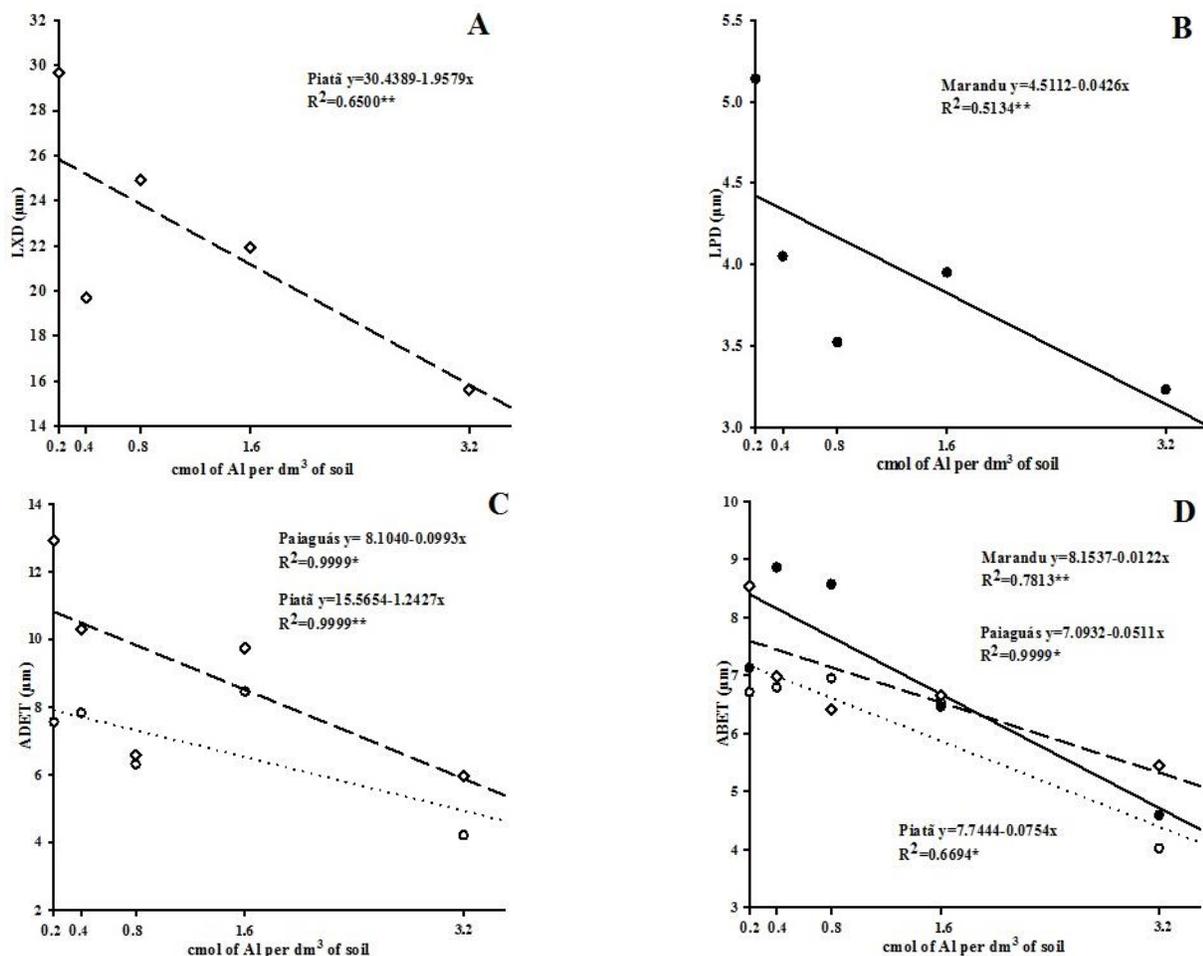


Figure 7. Leaf xylem diameter (LXD), leaf phloem diameter (LPD), adaxial epidermal thickness (ADET) and abaxial epidermal thickness (ABET) of 3 *Urochloa brizantha* cultivars (cvv. Marandu, Paiaguás and Piatã) in soil with different concentrations of aluminum.

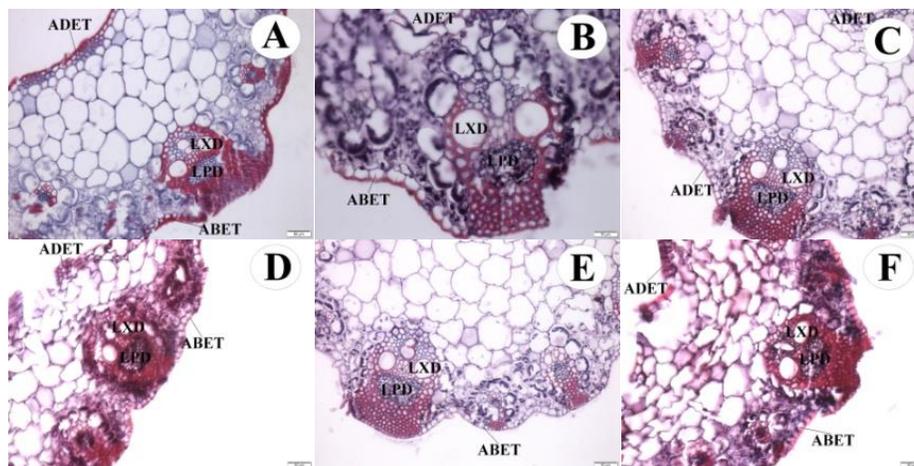


Figure 8. Ultrastructural changes in leaf tissues of 3 *Urochloa brizantha* cultivars grown in soil with contrasting concentrations of aluminum. **A** – cv. Marandu in soil with 0.2 cmol Al/dm³; **B** – cv. Marandu in soil with 3.2 cmol Al/dm³; **C** – cv. Paiaguás in soil with 0.2 cmol Al/dm³; **D** – 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: 400×. 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 ultra-structural 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 acid 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 Piatã 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 Hradate 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 Hradate 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.

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

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