

Aluminum-induced stress differently modifies *Urochloa* genotypes responses on growth and regrowth: root-to-shoot Al-translocation and oxidative stress

Felipe Furlan · Lucelia Borgo · Flávio Henrique Silveira Rabêlo ·
Monica Lanzoni Rossi · Adriana Pinheiro Martinelli · Ricardo Antunes Azevedo ·
José Lavres 

Received: 27 March 2018 / Accepted: 8 May 2018 / Published online: 21 May 2018
© Brazilian Society of Plant Physiology 2018

Abstract Forage grasses belonging to the *Urochloa* (*Brachiaria*) genus present tolerance to Al toxicity, however there are intra- and interspecific differences among the species, which in turn should be better depicted. Here we evaluate genotypic differences in Al tolerance in four *Urochloa* (*U. decumbens* cultivar Basilisk; *U. brizantha* cultivar Marandu; *U. brizantha* cultivar Piatã and *U. brizantha* cultivar Xaraés) cultivated in nutrient solution, during growth and regrowth. We analyzed the effect of Al uptake on epidermal and cell membrane damage, and lipid peroxidation in shoots and roots. Exposure of genotypes to Al concentration up to 1.33 mmol L⁻¹ led to different degrees of shoot yield, mainly during the growth period. Increased Al concentration decreased dry matter production in shoots and roots, reduced leaf

area (LA), relative root growth, increased Al accumulation in the roots and root-to-shoot Al translocation, notably during the first growth period. However, Al translocation from roots to shoots augmented massively in all genotypes, during the regrowth. Plant roots exposed to Al were damaged, exhibiting ruptures in the epidermis and reduced number of root hairs. Lipid peroxidation in shoots ranged in all genotypes exposed to Al, however, the oxidative stress was 2–5 times higher in shoots than in roots, notably in Marandu that accumulated 95% more Al than *U. decumbens*. This suggests that in the genotypes that are more tolerant to Al there is maintenance of metabolic activities, including upregulated and efficient antioxidant activity, root growth, LA growth and biomass yield.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s40626-018-0109-2>) contains supplementary material, which is available to authorized users.

F. Furlan · F. H. S. Rabêlo · M. L. Rossi ·
A. P. Martinelli · J. Lavres (✉)
Centro de Energia Nuclear na Agricultura, Universidade
de Sao Paulo, Avenida Centenario, 303,
PO Box 96, Piracicaba, São Paulo CEP 13416-000, Brazil
e-mail: jlavres@usp.br

L. Borgo · R. A. Azevedo
Escola Superior de Agricultura “Luiz de Queiroz”,
Universidade de Sao Paulo, Avenida Padua Dias, 11,
PO Box 9, Piracicaba, São Paulo CEP 13418-900, Brazil

Keywords *Brachiaria* · GeoChem ·
Malondialdehyde · Lipid peroxidation · Relative root
growth

Abbreviations

DM	Dry matter
H ₂ O ₂	Hydrogen peroxide
LA	Leaf area
MDA	Malondialdehyde
PVPP	Polyvinylpyrrolidone
RRG	Relative root growth
SEM	Scanning electron microscope
TBA	2-Thiobarbituric acid

TCA Trichloroanisole
TRL Total root length

1 Introduction

Soil acidity is a limiting factor to crop production in tropical regions, and also in some temperate areas. Acidic soils account for 30% of the world's ice-free land (Von Uexküll and Mutert 1995). At pH lower than 5.0, solubilization of phytotoxic species of available aluminum (Al^{3+}) occurs, which reduces plant growth considerably (Nogueirol et al. 2015; Tamás et al. 2003). Thus, Al is considered one of the major limiting factors to plant growth in acidic soils (Kochian 1995; Von Uexküll and Mutert 1995). Although progress has been made to better understand the physiological and molecular mechanisms of tolerance, mainly related to Al exclusion capacity of roots, very little is known about these mechanisms in species considered Al resistant, such as *Urochloa*—formerly known as *Brachiaria*—(Ramos et al. 2012; Watanabe et al. 2006; Wenzl et al. 2001, 2002). The mechanisms of these adaptations can be divided into those that facilitate the exclusion of Al^{3+} from root cells (exclusion mechanisms) and those that enable plants to tolerate Al^{3+} once it has entered the root and shoot apoplast and symplast, characterizing internal tolerance mechanisms (Ma et al. 2001; Horst et al. 2010; Kopittke et al. 2017). Furthermore, the physiological and molecular basis of these mechanisms have been intensively investigated in a plenty of plant species, including *Urochloa* (*Brachiaria*) genus (Brunner and Sperisen 2013; Arroyave et al. 2018).

For many crop species, Al tolerance rely on exclusion of this element at the rhizosphere scale, notably in the root apical region through the release of anionic organic acids, such as citrate, malate, succinate and oxalate (Delhaize and Ryan 1995; Ma et al. 2001). These organic acids sequester the Al, preventing its entry into the root cells (Horst et al. 2010). Once Al^{3+} has entered the root, there is an internal or symplastic detoxifying mechanism, which includes Al-organic acid complexation and eventually accumulation in, for example, vacuoles (Delhaize and Ryan 1995; Kochian 1995; Ma et al. 2001; Matsumoto 2000).

Secondly, another external Al resistance mechanism is the alkalization of the rhizosphere, which shifts the concentrations of mononuclear Al species in favor of less toxic Al hydroxides. However, Wenzl et al. (2001, 2002) pointed out that Al tolerance of *Urochloa* species based on external detoxification of Al by chelating ligands or rhizosphere alkalization do not adequately account for the outstanding resistance of signalgrass to Al, suggesting that other physiological strategies could be responsible for the high resistance level of this species. Lastly, Arroyave et al. (2013) suggested that the presence of a multi-seriate exodermis might contribute to efficient Al exclusion in *U. decumbens* because these tissues are present in Al-tolerant *U. decumbens* and absent in Al-sensitive *U. ruziziensis*. Although this tolerance process is still unknown for *Urochloa* genus.

The plasma membrane is a subsequent target for Al toxicity. This toxic metal may halts the plasma membrane functioning by inducing reactive oxygen species (ROS), such as oxygen ($^1\text{O}_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH), resulting in lipid peroxidation, which has been well documented in the literature (Vitoria et al. 2001; Gratão et al. 2005, 2015; Guo et al. 2007; Capaldi et al. 2015). To fight these harmful effects to cells, plants rely on the antioxidant protective system composed by enzymes (e.g., superoxide dismutase—SOD, catalase—CAT, ascorbate peroxidase—APX, and glutathione reductase—GR), or by organic compounds (e.g., phenols, sugars, organic acids and non-protein thiols), which are able to balance intracellular concentrations of ROS (Yamamoto et al. 2003; Gratão et al. 2005, 2015; Lee et al. 2007; Nogueirol et al. 2015). Lastly, Arroyave et al. (2018) pointed out that few proteins such as phenylalanine ammonium lyase (PAL), methionine synthase (MS), and deoxymugineic acid synthase (DMAS) decreased, while acid phosphatase (APase) abundance increased in response to Al toxicity, suggesting these phenomena as the initial response to Al toxicity in *U. decumbens*.

Therefore, to our knowledge, there is still not enough information in the literature on how these mechanisms occur in tropical forage grasses of the genus *Urochloa*. Thus, less lipid peroxidation of the cell membrane as well as an effective antioxidant system may result in increased Al tolerance or a combination of strategies involving the use of antioxidants such as reported by Alcântara et al. (2015).

Thus, here we investigate the occurrence of differential tolerance to Al between genotypes of *U. brizantha* (cv. Marandu, cv. Piatã and cv. Xaraés) and *U. decumbens* (cv. Basilisk) through stress indicators such as lipid peroxidation, Al accumulation in roots, and root-to-shoot Al translocation. In addition, we evaluate relative root growth, morphological changes in the roots, and shoot and root biomass yields, considering two periods of growth in plants exposed to Al.

2 Material and methods

2.1 Seedlings, Al treatment and nutrient solution

Seeds of *Urochloa decumbens* cv. Basilisk, *Urochloa brizantha* cv. Marandu, *Urochloa brizantha* cv. Piatã and *Urochloa brizantha* cv. Xaraés were placed to germinate in a tray with vermiculite, moistened with calcium sulphate solution (CaSO_4 , 10^{-4} mol L^{-1}), for 12 days. On the 13th day, five seedlings of each genotype (with uniform root length) were transferred to individual 2.2 L pots, with four replications per treatment, containing 2 L of the modified nutrient solution proposed by Clark (1975), diluted to 20% of maximum ionic strength for the seven remaining days. Subsequently, the initial solution was replaced by another with 100% ionic strength and Al was added (0, 0.44, 0.89 and 1.33 mmol L^{-1}) provided with $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$. The pots were rearranged within each block every 3 days for uniformity.

The nutrient concentrations in the solution corresponded to (in mmol L^{-1}): 2.50 Ca, 1.88 N- NO_3 , 0.57 N- NH_4^+ , 2.17 K, 0.032 P, 0.89 Mg, 0.54 S, and (in $\mu\text{mol L}^{-1}$): 50 Fe, 20 B, 7.0 Mn, 1.83 Zn, 0.47 Cu, and 0.62 Mo. The pH of the nutrient solution was initially adjusted to 4.0 ± 0.1 and monitored every 2 days until the end of the experiment. The nutrient solution was renewed every 7 days. During the experimental period, the solutions were constantly aerated. Estimates of Al species were obtained using Geochem-EZ[®] software, according to pH variation of the nutrient solution (Shaff et al. 2010). Each pot (2.2 L) with five plants were maintained at 14 h photoperiod, photosynthetic photon flux density around $1700 \mu\text{mol m}^{-2}\text{s}^{-1}$, 28 ± 5 °C and relative humidity (RH) of $70 \pm 20\%$,

for 35 days of Al exposure (first growth), and for 36 days after cut, for regrowth.

2.2 Plant tissues harvest—roots and shoots

The experiment was evaluated in two periods: growth and regrowth. Shoots were harvested 35 days after Al exposure and for regrowth 36 days after the harvest, with shoots being cut 3 cm above plant collar. Immediately after the second harvest, the roots were washed in running deionized water, and collected in two 0.25 x 1.00 mm sieves. In both harvests, the plant aerial part was separated into leaf blades, and stalks plus leaf sheaths. During both harvests, observations were made to detect early senescence of mature leaves, or toxicity symptoms. The plant material collected in both harvests was dried in a forced-air circulation oven, at 65 °C, for 72 h, and later weighed with a precision scale.

2.3 Leaf area, total root length and relative root growth

The leaf area (LA) at both harvests was determined with a digital area integrator system, LICOR LI 3100[®]. A subsample from each pot, comprising $\pm 20\%$ of total fresh matter of roots, was collected to calculate the total root length (TRL). Then, the subsamples were stained with Gentian violet (50 mg L^{-1}) and stored at approximately 10 °C. Subsequently, the roots were placed in clear blades (3 M[®], 210 x 297 mm), avoiding overlapping, and digitized. The root images were obtained by using a HP[®] Scanjet 2400 scanner. TRL was measured in plants grown under the conditions previously described for 36 days after the first harvest, after which subsamples of roots were scanned for subsequent image analysis using the 3rd version of SIARCS (Integrated System for Analysis and Covering of Soil, Embrapa, Sao Carlos, Brazil). After determining TRL, the subsamples were placed in a forced-air oven, at 65 °C, for 72 h, and their dry matter (DM) yield values were added to the remaining root DM previously recorded. Values of TRL was expressed through direct count among the values of subsample DM with the total DM roots. Relative root growth (RRG) was calculated as $[(\text{length} + \text{Al}^{3+})/(\text{length} - \text{Al}^{3+}) \times 100]$ (Ryan et al. 2011).

2.4 Al-accumulation in plant tissue

The remaining plant parts were collected, washed and dried, at 65 °C, for 48 h in a forced-air oven before grinding them in a stainless steel mill. The samples (0.25 g DW) were microwave-assisted acid digested. A closed vessel microwave oven (ETHOS 1600[®], Milestone, Italy) was used according to the following procedure: 250 mg of ground material was accurately weighed in the TFM vessels and then 6.0 mL of 65% v/v HNO₃ and 1.0 mL of 30% v/v H₂O₂ were added. Thereafter, the residual solutions were transferred to 25 mL volumetric flasks and the volume made up with high purity deionized water (resistivity 18.2 MΩ cm). The final solutions were analyzed by a radially viewed ICP-OES (Vista RL[®], Varian, Australia). Al accumulation in plant tissue was obtained by multiplying Al content (Supplementary data) by DM yield. The Al transport factor was calculated by dividing the Al accumulation in the shoots (sum of the two growth periods) by the Al accumulation in the roots (Kabata-Pendias and Pendias 2001).

2.5 Determining hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) content

Thirty days after plants were exposed to Al, one plant from each pot was collected, preserved in liquid N, and stored in a freezer at – 80 °C. The measurement of H₂O₂ concentration was performed in the leaf blades and roots, following the method described by Alexieva et al. (2001), with modifications. Firstly, 0.2 g of frozen samples was macerated in 2 mL of 0.1% (w/v) trichloroacetic acid (TCA) in the presence of 20% (w/w) polyvinyl polypyrrolidone (PVPP). After complete homogenization, 1.4 mL of the extract was centrifuged at 10,000 rpm for 5 min, at 4 °C. An aliquot of 0.2 mL was withdrawn from supernatant, and 0.2 mL of 100 mmol L⁻¹ potassium phosphate buffer (pH 7.0) and 0.8 mL of 1 mol L⁻¹ potassium iodide were added. Then, the solution was kept for 1 h in the dark for stabilizing the reaction and the readings were taken in a spectrophotometer at 390 nm. Three independent replicates from each sample—one plant per pot—were used.

Lipid peroxidation was also determined in the leaf blades and roots of plants, in which metabolites that were reactive to 2-thiobarbituric acid (TBA) were used to estimate the MDA concentration (Heath and

Packer 1968). The initial procedures for MDA measurements were the same for H₂O₂ measurements as described above. Following centrifugation, 0.25 mL of supernatant was added to 1 mL of 20% (w/v) TCA containing 0.5% TBA. The mixture was placed in a water bath at 95 °C for 30 min and then on ice. After 20 min on ice, the samples were centrifuged at 10,000 rpm for 10 min in order to separate some residue formed during heating and to clarify the samples. Readings at 535 and 600 nm were measured using a spectrophotometer, and MDA concentration was determined using the following Eq. 1 (Alcântara et al. 2015). Three independent replicates from each sample—one plant per pot—were used.

$$\text{Concentration} = \left(\frac{\text{Absorbance}(535 - 600)}{155000} \right) \times 10^3. \quad (1)$$

2.6 Microscopical analysis of secondary roots

Root sampling was determined based on visual manifestations of toxicity in the roots and shoots (growth reduction, morphological changes and senescence) of *Urochloa* genotypes depending on the treatments (0 and 1.33 mmol L⁻¹ Al).

For scanning electron microscopical observations, secondary root apices fixed in the modified Karnovsky solution (Lavres Junior et al. 2009, 2010) for 48 h were rinsed three times, for 10 min each, in sodium cacodylate buffer (0.1 mol L⁻¹). Samples were dehydrated in an ethanol series (35, 50, 60, 70, 80 and 90%, 15 min each, and 100% three times, 20 min each), critical point dried in liquid CO₂ (CPD 300, Leica Microsystem, Vienna, Austria), mounted on metal stubs and sputter coated (MED 010, Balzers Union, Balzers, Liechtenstein) with gold for 260 s (Lavres Junior et al. 2009, 2010). Observations were made at 20 kV, in a scanning electron microscope (LEO 435 VP, Zeiss, Cambridge, UK), and digital images were obtained.

2.7 Experimental design and statistical analysis

Experimental units were set up in a completely randomized block design, with four replications. A two-way analysis of variance (ANOVA) was performed in order to evaluate the interaction between Al

and the *Urochloa* genotypes, followed by Duncan's multiple range test (DMRT; $P \leq 0.05$) in order to determine the significant difference between treatments—genotypes within each Al concentration—using the *Statistical Analysis System* software (v. 8.0; SAS Institute Inc., Cary, NC).

3 Results

3.1 Ionic interactions predicted by Geochem-EZ

The changes in pH of the nutrient solution had little interference with Al availability, with a small percentage of bonding of the metal with the following chemical species: PO_4^{3-} , SO_4^{2-} , $\text{B}(\text{OH})_4^-$ and OH^- (Table 1). During the first growth period, the pH of the nutrient solution ranged from 3.0 to approximately 4.5. For nutrient solutions with nominal Al concentrations of 0.44, 0.89 and 1.33 mmol L^{-1} , the variation in pH values was also of 3.0–4.5. In both plant growth periods, with nutrient solution at pH 3.0, 83.4% of Al was available in the solution as Al^{3+} , and about 1.5% Al was bound to PO_4^{3-} , 14.6% linked to SO_4^{2-} , 0.02% bound to $\text{B}(\text{OH})_4^-$, and 0.41% linked to OH^- (Table 1).

3.2 Biomass production, LA, surface and total length of roots

After 35 days of Al exposure in the first growth period, all the genotypes exhibited a progressive decrease in LA (Fig. 1) and dry matter (DM) production (Fig. 2) with the increase of Al concentration. However, when grown under 1.33 mmol L^{-1} , *U. brizantha* cv. Xaraés exhibited lower relative reduction of both LA (54%) and shoot DM (63%) when compared to the other genotypes, which, nevertheless, exhibited LA reduction rates between 67 and 76% when grown under 1.33 mmol L^{-1} Al.

This response pattern was also observed during re-growth of *U. brizantha* cv. Xaraés, which exhibited the lowest rate of relative reduction of LA (21%) (Fig. 1b) and smaller relative reduction of shoot DM (29%) (Fig. 2b) when grown under 1.33 mmol L^{-1} Al. At 1.33 mmol L^{-1} Al, *U. brizantha* cv. Marandu and *U. decumbens* cv. Basilisk exhibited the highest values of relative reduction of LA and shoot DM, both in the first and second growth periods.

Table 1 Ionic interactions predicted by Geochem-EZ[®]

	Al (mmol L^{-1})		
	0.44	0.89	1.33
pH = 3.0			
As a free metal (Al^{3+}) (%)	80.73	83.72	85.81
Complexed with PO_4^{3-} (%)	1.85	1.46	1.21
Complexed with SO_4^{2-} (%)	17.00	14.39	12.55
Complexed with $\text{B}(\text{OH})_4^-$ (%)	0.02	0.02	0.02
Complexed with OH^- (%)	0.41	0.41	0.41
pH = 4.0			
As a free metal (Al^{3+}) (%)	74.42	78.57	81.14
Complexed with PO_4^{3-} (%)	4.66	2.83	2.04
Complexed with SO_4^{2-} (%)	16.81	14.39	12.62
Complexed with $\text{B}(\text{OH})_4^-$ (%)	0.18	0.18	0.17
Complexed with OH^- (%)	3.93	4.03	4.04
pH = 5.0			
As a free metal (Al^{3+}) (%)	45.80	49.00	50.49
Complexed with PO_4^{3-} (%)	6.67	3.46	2.35
Complexed with SO_4^{2-} (%)	10.94	10.07	9.24
Complexed with $\text{B}(\text{OH})_4^-$ (%)	0.93	0.78	0.67
Complexed with OH^- (%)	35.66	36.70	37.25
pH = 6.0			
As a free metal (Al^{3+}) (%)	2.07	2.16	2.23
Complexed with PO_4^{3-} (%)	5.85	3.23	2.25
Complexed with SO_4^{2-} (%)	0.56	0.56	0.57
Complexed with $\text{B}(\text{OH})_4^-$ (%)	0.50	0.46	0.41
Complexed with OH^- (%)	91.02	93.59	94.54
pH = 7.0			
Complexed with PO_4^{3-} (%)	0.08	0.07	0.07
Complexed with OH^- (%)	99.92	99.92	99.92

Primary percentage distribution output for Clark's nutrient solution (Clark 1975), considering only Al (0.44, 0.89, 1.33 mmol L^{-1}) at pH 3.0–7.0, showing the speciation of the individual Al and ligands as a percentage of the total

The production of root dry biomass (Fig. 3a) and relative root growth (Fig. 3b) were reduced with the increase of Al concentrations for all genotypes. In general, *U. brizantha* cv. Xaraés showed the lowest relative reduction values for these parameters.

3.3 Al accumulation in plant tissues

Al content increased in shoots and roots of all genotypes during the first and second growth periods with the increase of Al in the nutrient solution

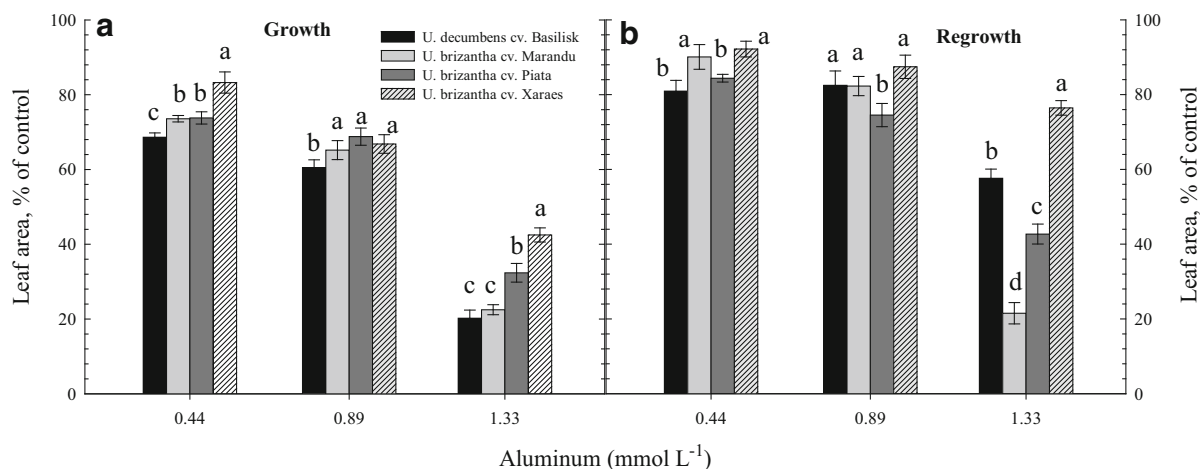


Fig. 1 Relative reduction of leaf area (%) at the first growth (a) and regrowth (b) of *Urochloa* genotypes exposed to Al in nutrient solution. For each Al concentration, different letters indicate significant difference between genotypes (DMRT, $P < 0.05$)

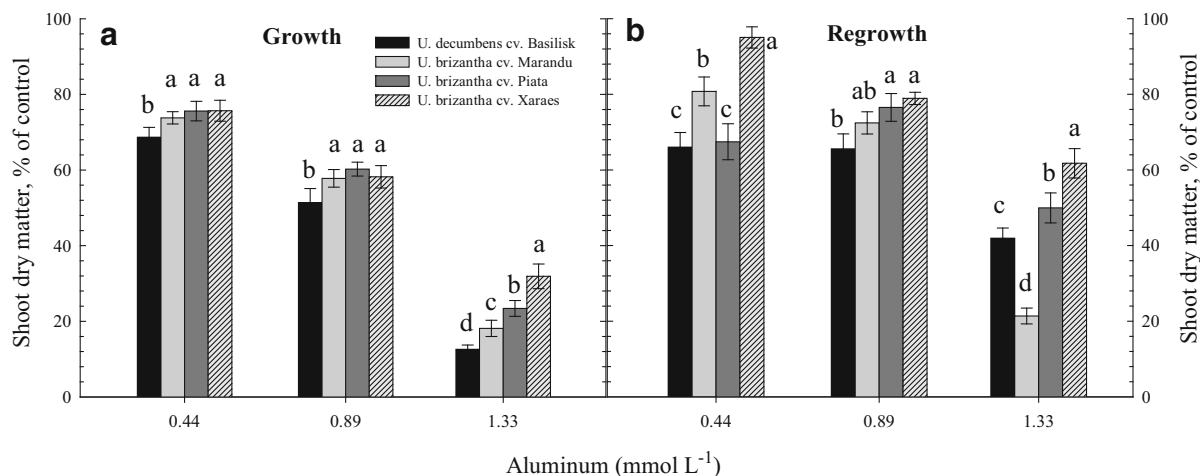


Fig. 2 Relative reduction in dry matter yield of shoots at first growth (a) and regrowth (b) of *Urochloa* genotypes exposed to Al in nutrient solution. For each Al concentration, different letters indicate significant difference between genotypes (DMRT, $P < 0.05$)

(Fig. 4). The highest Al accumulation in shoots occurred for *U. brizantha* cv. Marandu either during growth (Fig. 4a) or regrowth (Fig. 4b). At harvest, the Al accumulation in roots was highest for *U. brizantha* cv. Xaraés especially when grown under 0.89 and 1.33 mmol L⁻¹ Al (Fig. 4c).

At 1.33 mmol L⁻¹, Al accumulation in roots was 57, 72 and 51% higher in Xaraés as compared to *U. decumbens*, *U. brizantha* cv. Piatã, and *U. brizantha* cv. Marandu, respectively (Fig. 4c). The latter suggests that Xaraés retain Al in roots more effectively as compared to the other genotypes. Compared to Al accumulation in shoots at first growth, roots

exhibited higher Al content, on average 17-fold higher. In contrast, the Al accumulation in the shoots at regrowth was very close to its accumulation in the roots at harvest (Fig. 4c).

The distinct genotypes showed a variable root-to-shoot Al translocation, and Marandu showed the lowest Al-transport factor when grown under 1.33 mmol L⁻¹ as compared to the other genotypes, while Piatã and *U. decumbens* showed the highest root-to-shoot Al translocation.

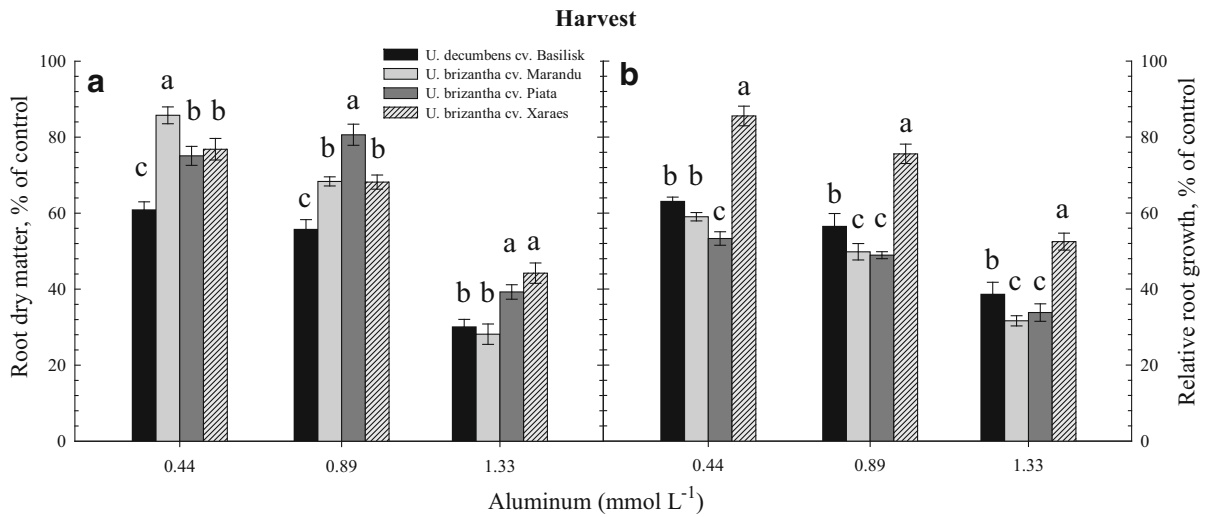


Fig. 3 Relative reduction (%) of dry mass of roots (a) and relative root growth (b) of *Urochloa* genotypes at harvest, in response to increasing Al in the nutrient solution. For each Al

concentration, different letters indicate significant difference between genotypes (DMRT, $P < 0.05$)

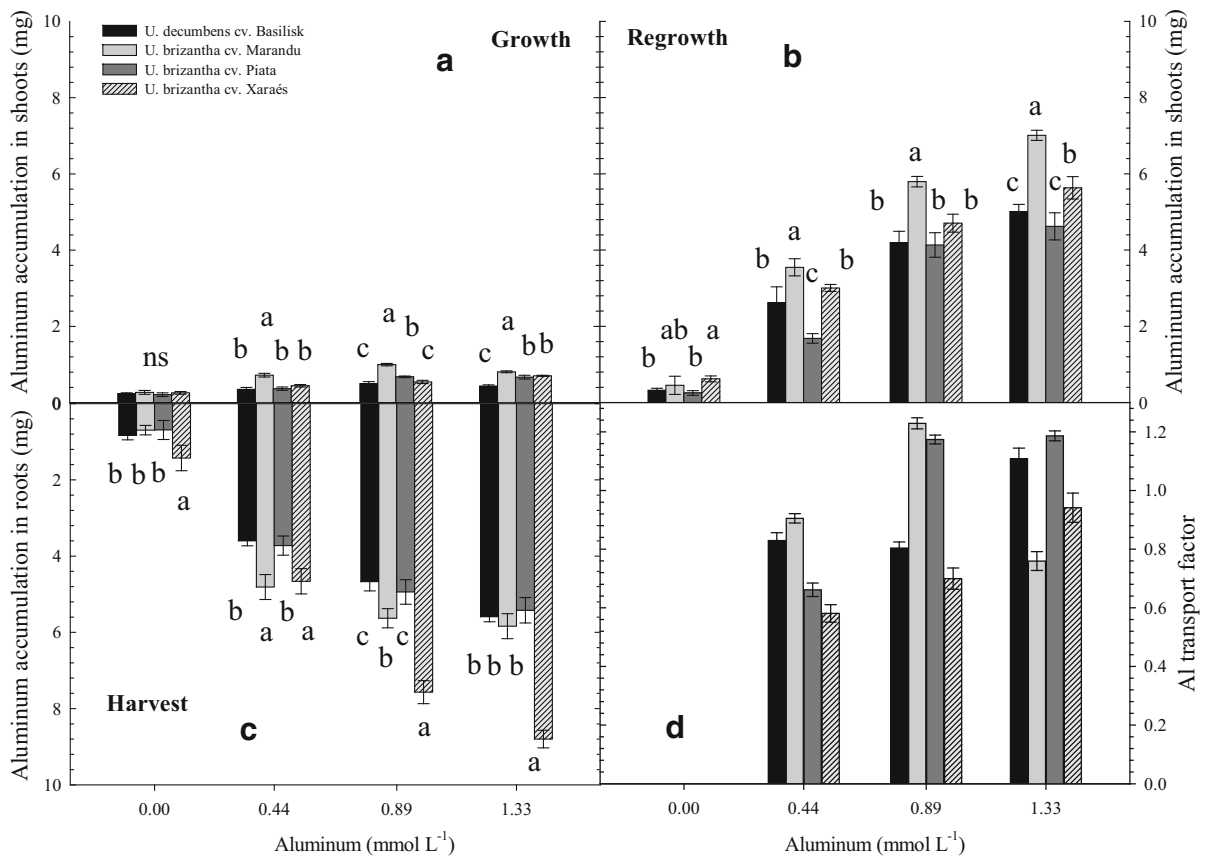


Fig. 4 Aluminum uptake in shoots at first growth (a), at regrowth (b), in the roots at harvest (c) and Al transport factor (d) of *Urochloa* genotypes subjected to aluminum exposure in

the nutrient solution. Different letters indicate significantly different values of genotypes within each Al concentration (DMRT, $P < 0.05$)

3.4 Lipid peroxidation (MDA) and concentration of hydrogen peroxide (H_2O_2)

The genotypes tested in the experiment showed distinct differences in MDA concentration in leaf extracts (Fig. 5a). At the highest Al concentration, MDA content in *U. brizantha* cv. Piatã, Marandu, and *U. decumbens* increased by 15, 12, and 1% when compared to *U. brizantha* cv. Xaraés, respectively. However, the root MDA variation between the four genotypes was not significantly changed by the increase of Al in the solution, except for 1.33 mmol L^{-1} ; at this concentration, root MDA content in Piatã, Marandu and *U. decumbens* was 36, 25, and 34% higher than Xaraés, respectively (Fig. 5c). When grown under 1.33 mmol L^{-1} leaf H_2O_2 increased by 25, 24, and 10% in Piatã, Marandu and *U. decumbens* as compared to Xaraés, respectively (Fig. 5b). In contrast, root H_2O_2 of all genotypes was

not significantly changed by the increase of Al in the solution (Fig. 5d). In general, shoot H_2O_2 was six times higher than in roots.

3.5 Morphological changes in *Urochloa* roots

The roots tips of *Urochloa* genotypes showed the presence of root hairs and epidermal cells well organized when not exposed to Al (Fig. 6a, b, e, f). In the presence of Al, the root tips of *Urochloa* species showed drastic changes in its root morphology (bent and curved root), resulting in roughness of root and exhibiting ruptures in the epidermis as well as a reduced number of root hairs (Fig. 6g, h). Flaking cells were observed on the root surface of Marandu and Xaraés cultivars (Fig. 6g, h), notably on the root apex, and these changes were pronounced in both *U. brizantha* cv. Marandu and *U. brizantha* cv. Xaraés (Fig. 6e, f).

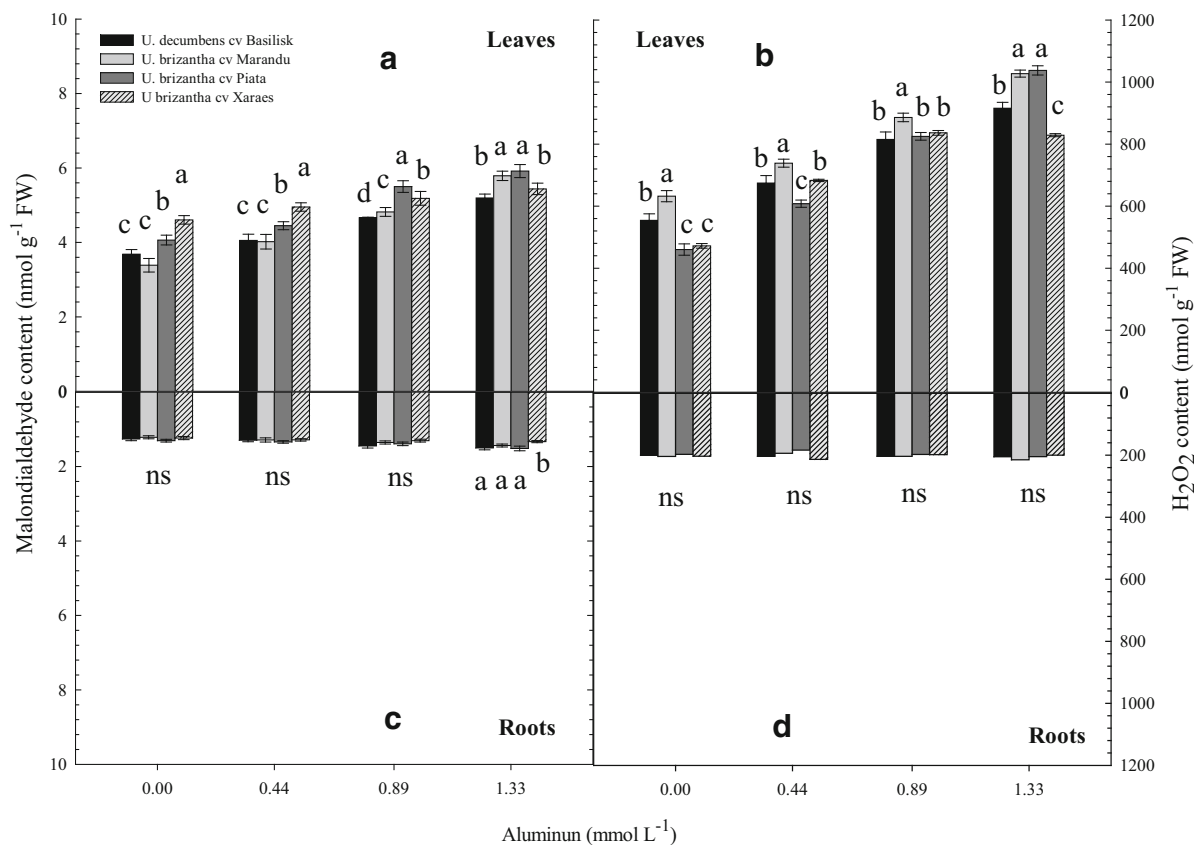
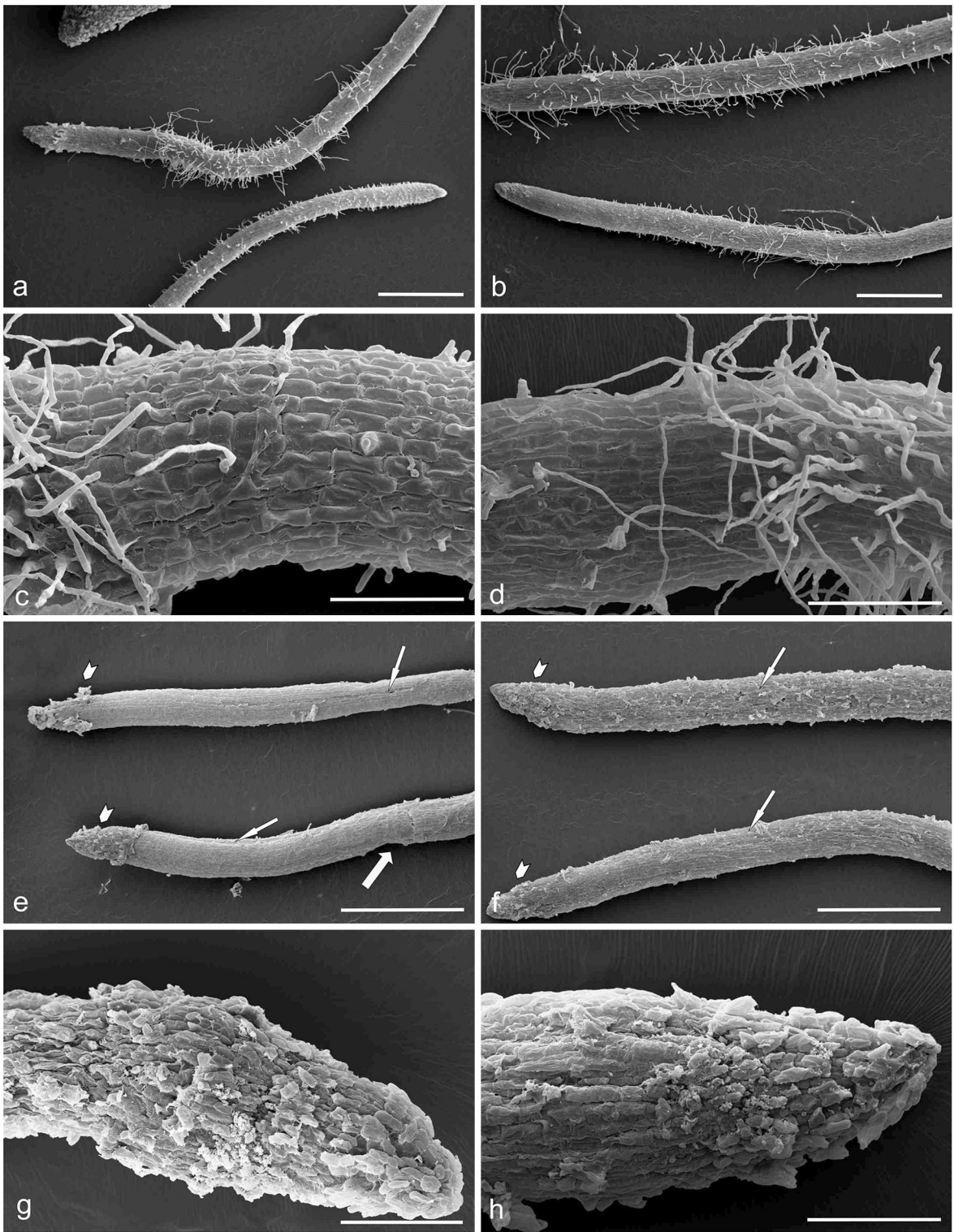


Fig. 5 Malondialdehyde content (MDA) in extract of leaves (a) and roots (b), and hydrogen peroxide (H_2O_2) content in extracts of leaves (c) and roots (d) after 30 days of growth of

Urochloa genotypes exposed to Al in nutrient solution. For each Al concentration, different letters indicate significant difference between genotypes (DMRT, $P < 0.05$)



◀ **Fig. 6** Root apex of *Urochloa brizantha* cv. Marandu (**a, c, e, g**), and *U. brizantha* cv. Xaraés (**b, d, f, h**), observed under scanning electron microscope, showing a smooth surface and root hair development when not exposed to Al (**a–d**), and absence of root hairs when exposed to Al (**e–h**). Yellow arrow indicate crater and throttled surface in Marandu (**e**). Arrow heads indicate the shortening or absence of the cap and cracks on the surface cells adhering to the root tip surface (**e, f**) and arrows indicate ruptures on the root surface of *U. brizantha* cv. Marandu (**e**). Bars: **a, b, e, f** 500 μm ; **c, d, g, h** 100 μm . (Color figure online)

4 Discussion

Aluminum caused physiological stress in *Urochloa* genotypes because approximately 81% of the monomeric Al species did not form solid-phase Al (precipitant) in the nutrient solution. These results confirmed the continuous Al availability and toxicity of Al^{3+} ions in all solutions used.

Between genotypes, we were able to detect different levels of Al tolerance in the following order: *U. brizantha* cv. Xaraés > *U. decumbens* cv. Basilisk \geq *U. brizantha* cv. Piatã > *U. brizantha* cv. Marandu. Thus, although regarded as an Al-tolerant genus (Arroyave et al. 2011, 2013), there is a gradient of tolerance among genotypes of *U. brizantha* (palisadegrass) and *U. decumbens* (signalgrass). In general, we have observed for all genotypes a greater decrease in LA, SDM (shoot dry matter), relative root growth and root dry matter at the first growth, in contrast to the regrowth period. On the other hand, Al-translocation from roots to shoots as well as Al-accumulation in shoots increased massively in all genotypes, during the regrowth.

The data obtained in this study showed that, in general, there was a decrease in both LA and DM in all genotypes evaluated when subjected to Al doses (Figs. 1, 2), which is also reported for several species of forage grasses (Poozesh et al. 2007). DM of plant shoots reduced more during the first growth period (Fig. 2a), possibly due to the high-energy requirement for initial growth, morphogenesis and root system establishment (Lavres Junior et al. 2004). Another factor possibly related to the significant decrease in DM in the first growth may be the greater sensitivity to Al presented by young plants in detriment of adult plants, notably on regrowth. On the other hand, DM reduction in roots was more pronounced than in shoots

during the second growth period (Figs. 2, 3), which is expected, since Al hinders the development of the root system (Horst et al. 2010). Although the damage caused by Al to shoot tissues is not thoroughly known, particularly in leaves, significant injuries in roots caused by Al were observed, being pronounced in both Marandu and Xaraés (Fig. 6). Low DM production in the shoots and LA reduction are directly related to the damage caused to the roots, since damages to root tissues result in low water and nutrients uptake (e.g., Ca^{2+} , Mg^{2+} and H_2PO_4^-), consequently limiting plant growth (Giannakoula et al. 2008).

Dry matter yield in all the genotypes was dramatically decreased when Al concentration in solution was increased. However, *U. brizantha* cv. Xaraés exhibited the smallest DM reduction in the roots, while *U. brizantha* cv. Marandu exhibited the highest decrease (Fig. 3). In addition, *U. brizantha* cv. Marandu has also exhibited the greatest decrease of root growth when Al concentration in the solution was augmented.

The results presented here concerning productive (DM production) and morphological parameters (reduction of relative LA, and root length) indicate that *U. brizantha* cv. Xaraés obtained lower values of relative reduction rates, thus being considered more tolerant to Al, different from *U. brizantha* cv. Marandu, which showed less tolerance to Al-stress, among the four genotypes.

The data obtained here also indicated differences between the genotypes studied in terms of Al accumulation in shoots and roots. For shoot Al accumulation it was showed that during the first growth period (Fig. 4a, b), *U. brizantha* cv. Marandu followed by *U. brizantha* cv. Piatã, accumulated larger amounts of Al when grown under almost Al concentrations, with lower DM production (Figs. 2a, b, 3a). The roots of the genotypes evaluated showed values of Al accumulation higher than those recorded in leaf blades (Fig. 4), indicating this phenomena as an important pathway for Al tolerance (Wang et al. 2004; Rangel et al. 2009; Horst et al. 2010). Comparing Al accumulation in roots of the four genotypes exposed to 0.89 and 1.33 mmol L^{-1} , we observed that *U. brizantha* cv. Xaraés showed the highest root Al accumulation (Fig. 4c). These data indicate that tolerance showed by *U. brizantha* cv. Xaraés is probably related to the maintenance of metabolic activities, such as upregulated synthesis of metabolites, like

phytochelatins, glutathione, sugar, and amino acids (Rabêlo et al. 2018), notably in more tolerant genotype.

The analysis of MDA and H₂O₂ concentration in plant tissues showed that substantial differences in oxidative stress occurred in shoot tissues among the four genotypes. Conversely, less difference of MDA and H₂O₂ concentrations appeared in roots, although there was higher Al accumulation in roots, indicating a possible role of efficient antioxidative protection system in this tissue (Fig. 5).

H₂O₂ concentrations in the leaf blades (Fig. 5b) were more than six times higher than in the roots (Fig. 5d), regardless of Al concentration in the nutrient solution. This result can be attributed in some extent to functions of H₂O₂ as opening and closing stomata (Desikan et al. 2004) and regulation of genes encoding antioxidant enzymes in the leaves of plants exposed to stress conditions (Neill et al. 2002). Although H₂O₂ acts in processes related to cellular defense and as signalling molecules in plants, increased ROS production can cause lipid peroxidation (Loix et al. 2017). In this sense, *U. brizantha* cv. Xaraés and *U. decumbens* cv. Basilisk were the only genotypes that kept oxidative stress lower with increased Al concentration in solution (Fig. 5a–c), indicating that these genotypes have more efficient detoxifying mechanisms and tolerance to Al in relation to the other genotypes. Guo et al. (2004) showed similar results, reporting lower MDA concentration in leaves of tolerant genotypes of barley in response to Al and Cd, and higher MDA amounts in sensitive genotypes.

In root tissues of *Urochloa* genotypes investigated in this study, the average MDA content in response to an increase of Al concentrations in *U. brizantha* cv. Xaraés was 25% lower than in *U. brizantha* cv. Marandu, and 36% lower than *U. brizantha* cv. Piatã (Fig. 5c). These data also provide evidence that *U. brizantha* cv. Xaraés has different tolerance to Al compared to other genotypes, possibly for having internal mechanisms that minimize the peroxidation of lipid membranes, thereby decreasing cell damage caused by Al. This fact corroborates the lower root DM reduction presented by this genotype (Fig. 3).

The data obtained in this study provide the basis for better understanding different tolerances between the four *Urochloa* genotypes assessed, highlighting the *U.*

brizantha cv. Xaraés as more tolerant to Al. However, further studies considering responsiveness of the antioxidant protective system, Al-uptake in the apoplast-symplast interface, root ultrastructure and subcellular location of Al are necessary to elucidate the intrinsic mechanisms involved in different tolerance to Al in the genus *Urochloa*.

Acknowledgements This research was supported by São Paulo Research Foundation (Fapesp) (grant #2013/02986-0).

References

- Alcântara BK, Macherer-Noonan K, Silva Junior FG, Azevedo RA (2015) Dry priming of maize seeds reduces aluminum stress. PLoS ONE 12:e0145742. <https://doi.org/10.1371/journal.pone.0145742>
- Alexieva V, Sergiev I, Mapelli S, Karanov E (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant, Cell Environ 24:1337–1344
- Arroyave C, Barceló J, Poschenrieder C, Tolrà R (2011) Aluminium-induced changes in root epidermal cell patterning, a distinctive feature of hyperresistance to Al in *Urochloa decumbens*. J Inorg Biochem 105:1477–1483
- Arroyave C, Tolrà R, Thuy T, Barceló J, Poschenrieder C (2013) Differential aluminum resistance in *Urochloa* species. Environ Exp Bot 89:11–18
- Arroyave C, Tolrà R, Chaves L, de Souza MC, Barceló J, Poschenrieder C (2018) A proteomic approach to the mechanisms underlying activation of aluminum resistance in roots of *Urochloa decumbens*. J Inorg Biochem 181:145–151
- Brunner I, Sperisen C (2013) Aluminum exclusion and aluminum tolerance in woody plants. Front Plant Sci 4:172. <https://doi.org/10.3389/fpls.2013.00172>
- Capaldi FR, Gratão PL, Reis AR, Lima LW, Azevedo RA (2015) Sulfur metabolism and stress defense responses in plants. Trop Plant Biol 8:60–73
- Clark RB (1975) Characterization of phosphatase of intact maize roots. J Agric Food Chem 23:458–460
- Delhaize E, Ryan PR (1995) Aluminum toxicity and tolerance in plants. Plant Physiol 107:315–321
- Desikan R, Cheung MK, Clarke A, Golding S, Sagi M, Fluhr R, Rock C, Hancock J, Neill S (2004) Hydrogen peroxide is a common signal for darkness- and ABA-induced stomatal closure in *Pisum sativum*. Funct Plant Biol 31:913–920
- Giannakoula A, Moustakasa M, Mylonab P, Papadakis I, Yupsanis T (2008) Aluminum tolerance in maize is correlated with increased levels of mineral nutrients, carbohydrates and proline, and decreased levels of lipid peroxidation and Al accumulation. J Plant Physiol 165:385–396
- Gratão PL, Pole A, Lea PJ, Azevedo RA (2005) Making the life of heavy metal-stressed plants a little easier. Funct Plant Biol 32:481–494

- Gratão PL, Monteiro CC, Tiago T, Carvalho RF, Alves LR, Peters LP, Azevedo RA (2015) Cadmium stress antioxidant responses and root-to-shoot communication in grafted tomato plants. *Biomaterials* 28:803–816
- Guo T, Zhang G, Zhou M, Wu F, Chen J (2004) Effects of aluminum and cadmium toxicity on growth and antioxidant enzyme activities of two barley genotypes with different Al resistance. *Plant Soil* 258:241–248
- Guo TR, Zhang GP, Zhang YH (2007) Physiological changes in Barley plants under combined toxicity of aluminum, copper and cadmium. *Colloid Surface B* 57:182–188
- Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* 125:189–198
- Horst WJ, Wang Y, Eticha D (2010) The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminum resistance of plants: a review. *Ann Bot* 106:185–197
- Kabata-Pendias A, Pendias H (2001) Trace elements in soils and plants, 3rd edn. CRC Press, Boca Raton
- Kochian LV (1995) Cellular mechanisms of aluminum toxicity and resistance in plants. *Annu Rev Plant Phys* 46:237–260
- Kopittke PM, McKenna BA, Karunakaran C, Dynes JJ, Arthur Z, Gianoncelli A, Kourousias G, Menzies NW, Ryan PR, Wang P, Green K, Blamey FPC (2017) Aluminum complexation with malate within the root apoplast differs between aluminum resistant and sensitive wheat lines. *Front Plant Sci* 8:1377. <https://doi.org/10.3389/fpls.2017.01377>
- Lavres Junior J, Ferragine MDC, Gerdes L, Raposo RWC, Costa MNX, Monteiro FA (2004) Yield components and morphogenesis of Aruana grass in response to nitrogen supply. *Sci Agric* 61:632–639
- Lavres Junior J, Malavolta E, Nogueira NL, Moraes MF, Reis AR, Rossi ML, Cabral CP (2009) Changes in anatomy and root cell ultrastructure of soybean genotypes under manganese stress. *Rev Bras Cienc Solo* 33:395–403
- Lavres Junior J, Reis AR, Rossi ML, Cabral CP, Nogueira NDL, Malavolta E (2010) Changes in the ultrastructure of soybean cultivars in response to manganese supply in solution culture. *Sci Agric* 67:287–294
- Lee YP, Kim SH, Bang JW, Lee HS, Kwak SS, Kwon SY (2007) Enhanced tolerance to oxidative stress in transgenic tobacco plants expressing three antioxidant enzymes in chloroplasts. *Plant Cell Rep* 26:591–598
- Loix C, Huybrechts M, Vangronsveld J, Gielen M, Keunen E, Cuypers A (2017) Reciprocal interactions between cadmium-induced cell wall responses and oxidative stress in plants. *Front Plant Sci* 8:1867. <https://doi.org/10.3389/fpls.2017.01867>
- Ma JF, Ryan PR, Delhaize E (2001) Aluminium tolerance in plants and complexing role of organic acids. *Trends Plant Sci* 6:273–278
- Matsumoto H (2000) Cell biology of aluminum toxicity and tolerance in higher plants. *Int Rev Cytol* 200:1–46
- Neill SJ, Desikan R, Clarke A, Hurst RD, Hancock JT (2002) Hydrogen peroxide and nitric oxide as signalling molecules in plants. *J Exp Bot* 53:1237–1247
- Nogueiro RC, Monteiro FA, Gratão PL, Borgo L, Azevedo RA (2015) Tropical soils with high aluminum concentrations cause oxidative stress in two tomato genotypes. *Environ Monit Assess* 187:73–89
- Poozesh V, Cruz P, Choler P, Bertoni G (2007) Relationship between the Al resistance of grasses and their adaptation to an infertile habitat. *Ann Bot* 99:947–954
- Rabêlo FHS, Fernie AR, Navazas A, Borgo L, Keunen E, Silva BKA, Cuypers A, Lavres J (2018) A glimpse into the effect of sulfur supply on metabolite profiling, glutathione and phytochelatin in *Panicum maximum* cv. Massai exposed to cadmium. *Environ Exp Bot*. <https://doi.org/10.1016/j.envexpbot.2018.04.003>
- Ramos FT, França MGC, Alvim MN, Rossiello ROP, Zonta E (2012) Aluminum tolerance measured by root growth and mucilage protection in *Urochloa brizantha* and *Urochloa decumbens*. *J Plant Interact* 7:225–229
- Rangel AF, Rao IM, Horst WJ (2009) Intracellular distribution and binding state of aluminum in root apices of two common bean (*Phaseolus vulgaris*) genotypes in relation to Al toxicity. *Physiol Plantarum* 135:162–173
- Ryan PR, Tyerman SD, Sasaki T, Furuichi T, Yamamoto Y, Zhang WH, Delhaize E (2011) The identification of aluminum-resistance genes provides opportunities for enhancing crop production on acid soils. *J Exp Bot* 62:9–20
- Shaff JE, Schultz BA, Craft EJ, Clark RT, Kochian LV (2010) GEOCHEM-EZ: a chemical speciation program with greater power and flexibility. *Plant Soil* 330:207–214
- Tamás L, Huttová J, Mistrík I (2003) Inhibition of Al-induced root elongation and enhancement of Al-induced peroxidase activity in Al-sensitive and Al-resistant barley cultivars are positively correlated. *Plant Soil* 250:193–200
- Vitória AP, Lea PJ, Azevedo RA (2001) Antioxidant enzymes responses to cadmium in radish tissues. *Phytochemistry* 57:701–710
- Von Uexküll HR, Mutert E (1995) Global extent, development and economic impact of acid soils. *Plant Soil* 171:1–15
- Wang Y, Stass A, Horst WJ (2004) Apoplastic binding of aluminum is involved in silicon-induced amelioration of aluminum toxicity in maize. *Plant Physiol* 136:3762–3770
- Watanabe T, Osaki M, Yano H, Rao I (2006) Internal mechanisms of plant adaptation to aluminum toxicity and phosphorus starvation in three tropical forages. *J Plant Nutr* 29:1243–1255
- Wenzl P, Patiño GM, Chaves AL, Mayer JE, Rao IM (2001) The high level of aluminum resistance in signalgrass is not associated with known mechanisms of external aluminum detoxification in root apices. *Plant Physiol* 125:1473–1484
- Wenzl P, Chaves AL, Patiño GM, Mayer JE, Rao IM (2002) Aluminum stress stimulates the accumulation of organic acids in root apices of *Urochloa* species. *J Plant Nutr Soil Sc* 165:582–588
- Yamamoto Y, Kobayashi Y, Devi SR, Rikiishi S, Matsumoto H (2003) Oxidative stress triggered by aluminum in plant roots. *Plant Soil* 255:239–243