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Urochloa Cultivated in Soil with Aluminium: A Development and Stomatal Approach

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Authors' contributions

This work was carried out in collaboration between all authors. Author LAML designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GHOD, KBR, HAAS, JVRP, GBR, GGS and ASC managed the analyses of the study. Authors LBP and CRAG managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Some Brazilian soils present low fertility, acidity and also toxicity caused by the presence of aluminium, leading to a lower development of forages. In view of the above, this work aimed to know Urochloa cultivated in soil with aluminium: a development and stomatal approach. The experimental design was a factorial of 3x5, with three cultivars of Urochloa brizantha: Marandu; Paiaguás and Piatã, and five concentrations of aluminium in the soil: 2.0; 4.0; 8.0; 16.0 and 32.0 mmol dm^{-3} of Al, with four replications, a total of 60 plots. Sixty days after the harvest the following parameters were set: number of leaves (NF); plant height (PH); dry mass of the aerial part (DMAP).

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The lower or abaxial epidermal impression of the fragments collected using cyanoacrylate ester was used to determine: Stomata Density (SD) and stomatal functionality (SF). The presence of aluminium in the soil negatively influenced the development of plants. All Urochloa cultivars presented similar responses as exposed to different doses of aluminium in the soil. Doses above 2.0 mmol of aluminium in the soil harmed the development of *U. brizantha*. The cultivar Marandu presented the highest number of leaves and presented better development.

Keywords: Marandu; Paiaguás; Piatã; forage; production.

1. INTRODUCTION

Urochloa is one of the most cultivated species among the forages, since it displays good quality of dry mass, good adaptation and establishment in adverse weather and soil conditions [1]. *Urochloa brizantha,* especially Marandu, Paiaguás and Piatã cultivars, has conquered the market for thirty years, due to their efficient root system, in which their growth and adaptation are easily noticed as planted in soil, allowing physical and chemical enhancement in degraded lands.

Some Brazilian soils present problems with vegetal production, once they present low fertility, acidity [2] and aluminium (Al^{3+}) in their composition, which is found as aluminium oxides or aluminosilicates, however, its availability depends on the soil pH. The main symptom caused by the presence of aluminium in soil is the reduction of the root system, preventing the development of the root as well as its occupation of a more significant amount of substrate, affecting the water and nutrients absorption [3].

When the plant is exposed to high toxicity of aluminium for an extended time, injuries may occur in its tissues, mainly in root cap [4]. Its physiological and biochemical features are changed, entailing the restriction of the growth of aerial part and gain of dry mass [5], which, consequently, can be observed in some deformities on its leaves ultrastructure, mainly in stomata compound present in epidermis of leaves [6,7].

Due to these physiological problems, density and functionality of stomatas are harmed, entailing its efficiency in gases exchange in the relationship atmosphere-plant-soil [8]. That way, the choice of forages cultivars species more tolerant to aluminium toxicity is an important tool in decision-making process on formation of pastures, considering that even in vegetal breeding programs this information can be used as criteria to crossing in future [9].

Therefore, this work aimed to know the development and stomata parameters of *Urochloas* as cultivated in presence of aluminium in soil.

2. MATERIALS AND METHODS

2.1 Installing the Experiment

The experiment was carried out in February, 2018, at Integrated College Stella Maris (FISMA), located in Andradina, São Paulo State. The experimental design was a 3x5 factorial scheme, with three cultivars of Urochloa brizantha: Marandu, Paiaguás and Piatã, and five aluminium concentrations in the soil: 2.0; 4.0; 8.0; 16.0 and 32.0 mmol dm^{-3} Al, with four repetitions, a total of 60 plots.

Each plot was composed of one vase with 6.0 dm^{-3} capacity filled with sifted soil, fertilised according to [10]. Soil pH was maintained at 4.7 letting aluminium available to the plants, as Table 1 shows. The soil used in the experiment was classified as Vermelho-amarelo distrófico férrico, according to [11]. Five seeds were sowed per vase, fifteen days after three plants, the more developed ones, were selected, making up the plots.

Table 1. Soil chemical parameters

pH	MO	P	K	Ca	Mg	H+Al	Al	SB	CTC	V%	m%	B	Cu	Fe	Mn	Zn
$CaCl_2$	g dm^{-3}	mg dm^{-3}				$mmol_c$ dm^{-3}								mg dm^{-3}		
4.7	8	1	0.5	7	6	20	2	13.5	33.5	40	13	0.12	0.4	59	4.1	0.2

SB: Sum of bases; V%: Base Saturation; m%: Saturation Al

2.2 Development Parameters and Stomatal Analysis

Sixty days after the planting the following parameters were set: number of leaves (NF); plant height (PH); dry mass of the aerial part (DMAP). The lower or abaxial epidermal impression of the fragments collected using cyanoacrylate ester was used to determine: Stomata Density (SD) and stomatal functionality (SF) [12,13]. Ten measurements were done for all characteristics in each microscope slide. Average value obtained on each characteristic represented plots. All slides were observed with a Bioval optical microscope, model BIO1600BA-L-BAT, with an attached camera in order to perform the photographs of the cuts. Pictures were used to measure anatomic parameters through the software S-Eye that was calibrated with a microscopic ruler in the same gains, as described by [14].

2.3 Statistical Analyzes

All variables were subjected to the F test ($p<0.05$) and the regression analysis was applied to the aluminium doses, in which their models were tested: linear, quadratic and cubic, for the Urochloa cultivars the Tukey test was applied at 5% probability. [15], by using Assistat 7.7 static software [16].

3. RESULTS

3.1 Development Parameters

Table 2 shows the average values of: number of leaves (NL); plant height (PH); dry mass of the air part (DMAP); stomata density (SD) and stomata functionality (SF) of the analyzed species as cultivated in soil with different concentration of aluminium.

Only in parameter number of leaves was observed a difference among the *Urochloa* cultivars, highlighting Marandu, which presented greater averages. However, a significant difference was observed in all of the analyzed parameters, as the concentration of aluminium is considered, in which the analysis of variance of aluminium doses are presented by using the tested models: linear, quadratic and cubic, as Table 3 shows.

A linear negative response was observed regarding the number of leaves by increasing the concentration of aluminium in the soil, as Fig. 1 shows.

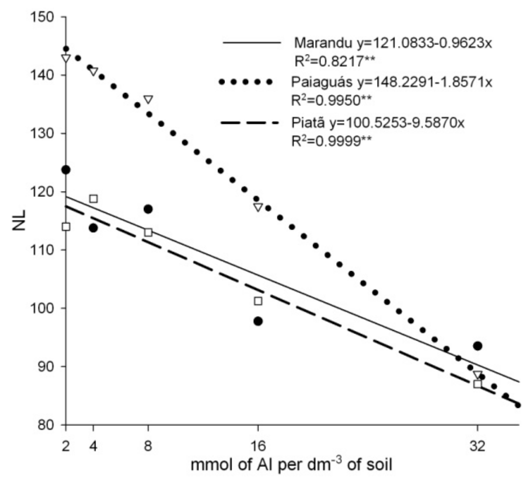

Fig. 1. Regression analysis of the *Urochloa brizantha* cv. Marandu; Paiaguás and Piatã of the number leaf (NL) cultivated in soil with aluminium in 60 days. Andradina, 2018

Table 2. Average values of number of leaves (NL); plant height (PH); Dry mass of the air part (DMAP); Stomata density (SD) and Stomata functionality (SF) of as cultivated in soil with different concentration of aluminium

Forage	NL	PH (cm)	DMAP (g)	SD (n°S/mm^2)	SF
Marandu	125.20a	47.85	12,36	97.10	1.96
Paiaguás	109.15b	46.52	12.27	97.00	1.91
Piatã	106.75b	44.47	11.04	93.30	1.86
MSD	12.69	4.37	1.63	9.32	0.19
CV%	14.53	12.30	17.83	12.67	12.98
GA	113.70	46.28	11.89	95.80	1.91
f	7.37**	1.78ns	2.42ns	0.64ns	0.77ns

*S: stomata. MSD: minimum significant difference. CV: coefficient of variation. GA: general average. f: value of the F calculated in the analysis of variance; **significant at 1%-probability (p<0,01); *significant at 5%-probability (0,01=<p<0,05); ns – non-significant (p>=0,05). Averages followed by the same letter do not statistically differ each other. Turkey test at 5% probability was used. Source: Research data, 2016*

Table 3. The analysis of variance of the regressions of the aluminium doses applied in the soil, where the models were tested: linear, quadratic and cubic

			Middle square				
Forage	FV	GL	NL	PH (cm)	DMAP (g)	SD (n°S/mm²)	SF
Marandu	Concentration	4	2340.900	154.056	145.542	1768.900	2.034
	Residue	15	137.150	32.795	3.965	80.366	0.065
	Regression	1	L**	L*	L**	L**	L**
Paiaguás	Concentration	4	6943.220	680.625	244.827	5856.400	0.441
	Residue	15	574.030	20.204	5.830	95.025	0.083
	Regression	1	L**	L**	L**	L**	Q*
Piatã	Concentration	4	2030.625	493.506	91.506	5347.656	0.439
	Residue	15	111.050	40.791	4.076	196.458	0.027
	Regression	1	L**	L**	L**	L**	Q**

*Ns- p>=0.05; *0.01=<p<0.05; ** p <0.01. L: polynomial of 1st degree. Q: polynomial of 2nd degree. S: Stomata. NL – number leaf; PH – plant height; DMAP – Dry mass of the air part; SD – Stomata density and SF – Stomata functionality. Source: Research data, 2016*

This linear negative response was also observed to the parameter plant height, as Fig. 2 shows. Again, all cultivars presented a negative linear response to the presence of aluminium in the soil.

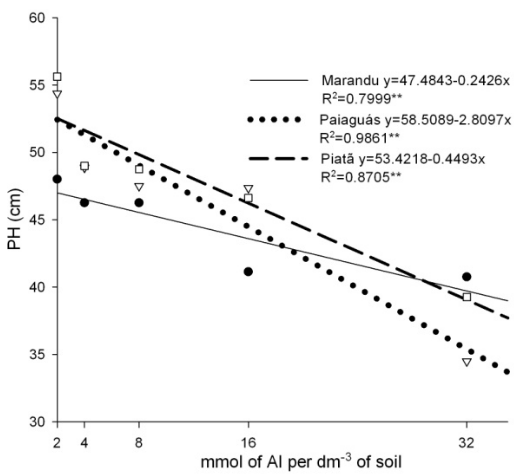

Fig. 2. Regression analysis of the *Urochloa brizantha* cv. Marandu; Paiaguás and Piatã of the plant height (PH) cultivated in soil with aluminium in 60 days. Andradina, 2018

Negative responses in number of leaves may have contributed to a lower accumulation of dry mass in the aerial part of the plant, once this parameter presented linear negative response, as Fig. 3 shows.

3.2 Stomatal Analysis

As the plants developing and staying exposed to the presence of aluminium in the soil, their structures can be modified due to the bad physiological composition caused by the metal. That way, stomata densities of *Urochloa´*s leaves also presented a linear negative response, as Fig. 4 shows.

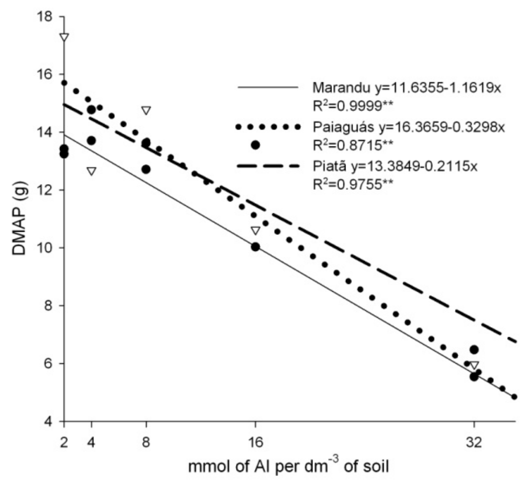

Fig. 3. Regression analysis of the *Urochloa brizantha* cv. Marandu; Paiaguás and Piatã of the dry mass of the aerial part (DMAP) cultivated in soil with aluminium in 60 days. Andradina, 2018

Regarding the parameter stomata functionality, by increasing aluminium doses in the soil, a linear negative response was detected in the Maraandu cultivar, as Paiaguás and Piatã presented quadratic responses, as Fig. 5 shows.

Considering this quadratic response, Paiaguás and Piatã presented their lower reduction at 22.69 and 23.57 mmol of aluminium, highlighting the toxicity of the metal in soil even ninety days after the exposition.

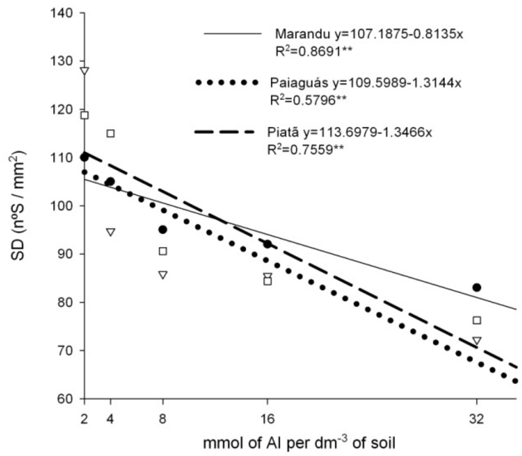

Fig. 4. Regression analysis of the *Urochloa brizantha* cv. Marandu; Paiaguás and Piatã of the Stomata Density (SD) cultivated in soil with aluminium in 60 days. Andradina, 2018

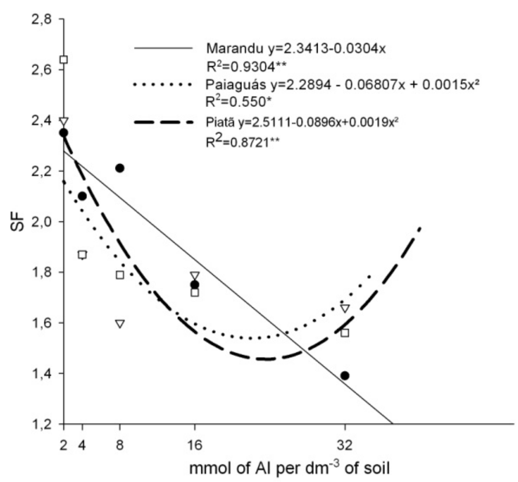

Fig. 5. Regression analysis of the *Urochloa brizantha* cv. Marandu; Paiaguás and Piatã of the stomatal functionality (SF) cultivated in soil with aluminium in 60 days. Andradina, 2018

4. DISCUSSION

4.1 Development Parameters

Due to a reduction in the number of leaves of forages in presence of aluminium in soil, its photosynthetic may have been harmed, leading to a lower accumulation of dry mass in the aerial part of the plant, as Figs. 1 and 3 shows. Aluminium is harmful to the vegetal as its concentration increases in the soil, and its availability is affected by the pH difference in the solution, where, in acid soils, an elevation in the availability of the plant occurs, entailing an elevation in its toxicity [17].

The first negative response to the presence of aluminium in soil is the atrophy of the root system due to the inhibition on cell division present in the root cap or even in small injuries in this area [18,19,4], also in the expression of enzymes, mainly the phytochelatins that are produced as response of the plant to the presence of the metal [20], these damages can be reduced as magnesium availability occurs in the soil [21].

Even with other metals or under concurrency with other vegetal, some plants present bad development in its structure [7,19], once by decreasing the number of leaves, the height of plants as reduced due to lower accumulation of atmospheric carbon in the dry mass, this lower growth can be a negative response to the stress caused by aluminium in the soil [22].

4.2 Stomatal Parameters

Due to the exposition of the vegetal to the toxicity of aluminium in the soil the development of epidemical structures were harmed in the same way between as the studied parameters of *Urochloas* cultivars. Due to the negative response in leaves stomata density, gas exchange between atmosphere and plant was affected, as the structure of the aerial part of the plant was also harmed, as Figs. 1 and 2 shows. This process may have been increased due to the bad physiological composition caused by the presence of aluminium, which starts to ask a greater energy waste from the vegetal to soften the toxicity damages and did not use this metabolic energy to its growth [23,8].

Even the stomata structures were harmed by increasing doses of aluminium in the soil, as Fig. 5 shows. A lower functionality is an indication that the stomata opening was reduced, presenting a smaller area to the gas exchange needed to photosynthetic reactions [13,19,24].

An approach of the knowledge to responses of morphological development as cultivated in acid soils in presence of aluminium is an important tool in the decision-making process regarding the choice of more resistant cultivars or even at the correct moment to set the planting system [9,2].

5. CONCLUSION

All Urochloa cultivars presented similar responses as exposed to different concentration of aluminium in the soil.

Concentration above 2.0 mmol of aluminium in the soil harmed the development of *U. brizantha*. The cultivar Marandu presented the highest number of leaves and presented better development.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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