

Tolerance of marandu grass to excess iron and manganese

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ABSTRACT

In soaked soils there is an increase in the availability of iron and manganese. Thus, it becomes important to investigate the tolerance levels of plants to excess of these nutrients. The objective was to evaluate the tolerance of *Urochloa brizantha* cv. Marandu cultivated in nutrient solution with different levels of iron and manganese. The experimental design was completely randomized with seven treatments and four replications. The treatments consisted of a nutrient solution and consisted of: 1- adequate concentration of Fe (8.22 mM) and Mn (2.05 mM); 2- Fe, four times the adequate concentration; 3- Fe, eight times the adequate concentration; 4- Mn, four times the adequate concentration; 5- Mn, eight times the adequate concentration; 6- Fe and Mn, four times the appropriate concentration, and; 7- Fe and Mn, eight times the appropriate concentration. The variables evaluated were visual diagnosis (symptomatology), shoot height, root volume, Fe and Mn content in shoots and dry mass production. Grass plants were not tolerant to excess Fe and Mn. The excess of nutrients promoted less development of the root system, with a reduction in volume and dry mass, and the treatment with the highest dose of nutrients resulted in the lowest values. Grass plants were sensitive to the increase in nutrient concentrations, showing reductions in total dry mass production of 55% for treatment with the application of the highest concentration of nutrients in relation to the adequate dose of Fe and Mn.

Keywords: Nutritional Disorder, Micronutrient, Toxicity, *Urochloa brizantha*.

Tolerância do capim marandu ao excesso de ferro e manganês

RESUMO

Em solos encharcados há aumento na disponibilidade de ferro e de manganês. Dessa forma, torna-se importante investigar os níveis de tolerância das plantas ao excesso desses nutrientes. We aimed to evaluate *Urochloa brizantha* cv. Marandu tolerance when grown in nutrient solution with different levels of iron and manganese. The experimental design was completely randomized with seven treatments and four repetitions. Os tratamentos foram constituídos por solução nutritiva e consistiram em: 1- concentração adequada de Fe (8,22 mM) e de Mn (2,05 mM); 2- Fe, quatro vezes a concentração adequada; 3- Fe, oito vezes a concentração adequada; 4- Mn, quatro vezes a concentração adequada; 5- Mn, oito vezes a concentração adequada; 6- Fe e Mn, quatro vezes a concentração adequada, e; 7- Fe e Mn, oito vezes a concentração adequada. As variáveis avaliadas foram a diagnose visual (sintomatologia), altura de parte aérea, volume de raízes, teor de Fe e Mn na parte aérea e produção de massa seca. As plantas de capim não foram tolerantes ao excesso de Fe e Mn. O excesso dos nutrientes promoveu menor desenvolvimento do sistema radicular, com redução no volume e massa seca, sendo que o tratamento com a maior dose dos nutrientes foi o que resultou nos menores valores. As plantas de capim foram sensíveis ao aumento das concentrações dos nutrientes, apresentando reduções na produção de massa seca total de 55% para tratamento com aplicação da maior concentração dos nutrientes em relação à dose adequada de Fe e Mn.

Palavras-chave: Distúrbio nutricional, Micronutriente, Toxicidade, *Urochloa brizantha*.



1. Introduction

The Brazilian cattle herd is estimated at 196.47 million head, occupying an area of 163.1 million hectares of pasture, with a stocking rate of 0.9 animal units per hectare. Mato Grosso has the largest Brazilian cattle herd, with 27.8 million head (14.15% of the total Brazilian herd) (ABIEC, Brazilian Beef Exporters Association, 2022). In this sense, adequate nutrition of pastures is of great importance to ensure high productivity, enabling an increase in the animal stocking rate (Caione and Prado, 2021).

Among the main forage grasses planted in Brazil and especially in the Legal Amazon is *Urochloa brizantha* cv. Marandu, also known as Marandu grass, *brachiarão* or *brizantão*. However, the emergence of the sudden death syndrome of this forage caused many areas to be reformed, requiring the implantation of a new forage. Some reports indicate that the cause of sudden death syndrome is related to excessive soil moisture and fungal attack on the roots of Marandu grass, mainly due to the low tolerance of this grass to excess water in the soil (Dias-Filho and Carvalho, 2000). Sudden death syndrome has become the main symptom of pasture degradation, particularly in Acre, Pará, Mato Grosso, Amazonas, Rondônia, Tocantins and Maranhão (Dias-Filho, 2011). In these regions, soaking and poor soil drainage are relatively common, due to the intense rainfall regime.

Under low pH conditions (Bambolim et al., 2015) and soil soaking, the forms of Fe^{3+} , Mn^{4+} and Mn^{3+} present in the soil are reduced to the divalent forms Fe^{2+} and Mn^{2+} , which are preferably absorbed by plants, and may cause toxicity, since the elements will be made available in large quantities (Melo and Alleoni, 2009; Raij, 2011).

In a study carried out by Caetano and Dias-Filho (2008), Marandu grass in waterlogged condition showed a 55% reduction in the average number of tillers, 74% in root mass and 66% in the relative growth rate. This factor may be related to the excess Fe and Mn available in the soil. This result is similar to that observed by Fageria et al. (2008) that found reduction of dry leaves and roots mass according to the elevation of Fe concentration,

Fe and Mn are abundant elements in tropical soils and play an important role in plant growth and development. However, when made available in excess they can result in toxicity, which can lead to the death of plants. In addition, they may make the plant susceptible to biotic agents (Prado, 2021).

For this reason, studies that seek to evaluate the effects of Fe and Mn toxicity on the growth and accumulation of elements in forage plants are important, as there is little information described in the literature and this may be a factor that contributes to low

productivity and pasture death. We aimed to evaluate *Urochloa brizantha* cv. Marandu tolerance when grown in nutrient solution with different levels of iron and manganese

2. Material and Methods

The experiment was conducted in a greenhouse at the State University of Mato Grosso - UNEMAT, University Campus of Alta Floresta, MT, Brazil. According to the Koppen classification, the climate of the region is type Am, rainy, with a clear dry season, with an average annual rainfall of 3.032 mm and an average annual temperature between 24.6 and 28.3 °C (Alvares et al., 2013).

The sowing was carried out on September 18, 2017, in a plastic tray filled with commercial substrate. The experimental design was completely randomized with seven treatments and four repetitions. The treatments consisted of a classic nutrient solution, adapted from Hoagland and Arnon (1950), varying only the concentrations of Fe and Mn according to each treatment. Treatments consisted of 1- adequate concentration of Fe and Mn; 2- Fe, four times the appropriate concentration; 3- Fe, eight times the appropriate concentration; 4- Mn, four times the appropriate concentration; 5- Mn, eight times the appropriate concentration; 6- Fe and Mn, four times the appropriate concentration, and 7- Fe and Mn, eight times the appropriate concentration. Suitable Fe content was 8.22 mM, and Mn was 2.05 mM, according to the nutrient solution proposed by Hoagland and Arnon (1950).

To obtain the seedlings, encrusted seeds with a cultural value of 90% were sown in a styrofoam tray containing commercial organic substrate. The seedlings were transplanted after the formation of the first two leaves. The vases used in the experiment contained 7 L of nutrient solution. The vases were capped with styrofoam plates, where the plants were allocated. Initially, 50% of the usual concentration was used for adaptation and on the 7th day after the installation of the experiment, the remainder of the solution was added, totaling 100%.

During the growing period of the plants, the nutrient solutions received constant oxygenation, by air compressors (aquarium pumps). The pH of the nutrient solution was monitored daily with the use of a portable pH meter, keeping it between 5.8 and 6.2. When the pH was outside this range, HCl at 1 mol L⁻¹ or NaOH at 1 mol L⁻¹ was used for adjustment. The loss of water by evapotranspiration was replaced by the addition of distilled and deionized water. Every ten days the nutrient solution was discarded, and a new solution was prepared, which was added to the same vases containing

the plants. There was an incidence of caterpillar after 25 days of conducting the experiment. Control was performed using Cypermethrin, at a dosage of 1 mL L⁻¹ following the manufacturer's recommendation.

For the evaluation of the experiment, plants were monitored daily for visual symptoms in order to characterize the toxicity symptoms. For this evaluation, the treatment with the appropriate doses of all elements was considered as standard and the visual aspect of the shoot and roots of the plants of all treatments was compared. We always sought to observe the occurrence of toxicity symptoms described in the literature such as shriveling of leaves, blackened lesions, brittle leaves, spots, and necrotic points (Prado, 2020), among others.

At 35 days, the grass was cut. The height of the shoot was evaluated with the help of a tape measure; the root volume, by immersing the roots in a graduated beaker containing a known volume of water. By subtracting the volume in the presence and absence of roots, the direct response of the root volume was obtained. The collected forage was separated into shoot and root system. The material was packed in Kraft paper packages and sent to a forced air ventilation oven at 65 °C, until the constant mass was obtained and, subsequently, weighed to obtain the accumulation of dry matter from the shoots and roots.

Subsequently, the grinding of the shoot samples and chemical analysis were performed to determine the concentration of Fe and Mn. Fe and Mn content were determined according to the methodology proposed by Embrapa - The Brazilian Agricultural Research Corporation - (2009). Data obtained were submitted to variance analysis using the F-test and means were compared through the Tukey test at 5% probability level, with the help of statistical program SISVAR[®] (Ferreira, 2014).

3. Results and Discussion

Grass plants did not present characteristic symptoms of toxicity when visual symptoms were assessed, according to results described in the literature, such as shriveling of leaves, blackened lesions, brittle leaves, spots, and necrotic points (Prado, 2020), among others. However, the development of thinner leaves, more subject to tipping and lower concentration of roots when compared to treatment with the appropriate dose of Fe and Mn was observed. This response (absence of visual symptoms) may be associated with the fact that Marandu grass has tolerance to excess Mn (Nunes et al., 1985), a fact that can also be attributed to Fe since it is an abundant element in tropical soils. In addition, the

symptomatology of nutrient deficiency or excess may vary by culture.

According to Foy (1976), tolerance to high levels of Mn would be related to the ability of this plant to compartmentalize this micronutrient in the apoplast of root cells, decreasing its transport to the shoots. Guirra et al. (2011), evaluating manganese excess (60 and 120 mg dm⁻³) in Marandu grass found similar results, where no typical visual symptoms of nutrient toxicity were observed in the first cut of the grass, which was carried out at 38 days after the installation of the experiment, a time similar to the cut of the present work.

Other toxicity studies with Fe and Mn in other cultures obtained different results. Jucoski et al. (2016), evaluating the excess of iron on the growth of pitangueira (*Brazilian cherry*), submitted to concentrations of 1.0 and 2.0 mM, noted that the plants presented leaf spots of yellow/brownish color that evolved into necrotic lesions, as described in the literature. Stein et al. (2014) observed similar symptoms in different rice cultivars, such as discoloration and necrosis in older leaves. In the present study, another factor that may have contributed to the absence of the appearance of classic symptoms of toxicity was the time spent conducting the experiment, in which the plants were under stress of excess Fe and Mn only in the establishment phase, when the plants have greater vigor.

There was no effect of the treatments on plant height (Table 1), demonstrating that excess nutrients did not decrease shoot height growth. Fageria and Rabelo (1987), evaluating rice tolerance to iron toxicity, noted that plant height was the variable least influenced by nutrient excess. There was an effect of the treatments on root volume (Table 1). The highest root volume was observed in the standard treatment, where the appropriate doses of Fe and Mn were applied, and the lowest root volume was observed with the application of the highest dose of Fe and Mn (8x the recommended dose), not differing from the other treatments.

This result demonstrates that the excess of both nutrients promotes restriction in root growth. A similar result was verified by Camargo and Ferreira (1992), evaluating wheat cultivars in nutrient solution, whereas the concentration of Mn increased from 0.11 mg L⁻¹ to 1200 mg L⁻¹ there was a reduction in root development. Different authors describe that when subjected to high concentrations of Fe, plants of different species can develop tolerance mechanisms, where part of the Fe will not be absorbed, being accumulated in the superficial part of the root and another part destined for the old leaves, and this factor may result in less nutrient absorption (Audebert and Sahrawat, 2000; Zhang et al., 2016).

Table 1. Plant height (PH), root volume (RV) and Fe and Mn content in the shoot, in *B. brizantha* cv. Marandu plants, depending on the application of Fe and Mn concentrations

Treatments	PH --- cm ---	RV -- cm ³ plant ⁻¹ --	Fe content ----- mg kg ⁻¹ -----	Mn content
Adequate Fe and Mn	99.00	22.25 a	132.51 ab	91.81 c
Fe 4x	95.25	11.5 b	136.43 ab	129.75 bc
Fe 8x	87.12	11.12 b	197.62 a	120.99 bc
Mn 4x	95.62	9.25 b	111.84 b	248.97 b
Mn 8x	82.37	10.25 b	178.34 ab	420.65 a
Fe and Mn 4x	90.12	11.88 b	151.68 ab	224.14 bc
Fe and Mn 8x	95.50	8.88 b	168.55 ab	203.57bc
MSD	21.78	7.19	66.96	136.5
F Value	1.52 ns	8.61**	4.56**	15.51**
CV (%)	10.28	25.71	15.61	23.8

** and ns: Significant at 1% probability of error and not significant, respectively. Means followed by distinct letters, in the column, differ from each other by Tukey's test ($P < 0.05$). CV = Coefficient of variation.

Besides the low nutrient absorption the roots may present reddish coloration when subjected to high doses of Fe as observed by Jucoski et al. (2016). According to Siqueira-Silva et al. (2012), the darkening of the roots indicates deposition of iron oxide or phenols. There was an effect of the treatments for the Fe and Mn content in the grass shoots (Table 1). The highest concentration of Fe was observed in the treatment where the highest dose of nutrient was applied (8x the recommended dose), and the lowest concentration was observed in the treatment with application of Mn (4x the recommended dose). This result demonstrates that although plants have mechanisms that reduce absorption in conditions of excess Fe (Becker and Asch, 2005; Siqueira-Silva et al., 2012), this characteristic was not observed in the present study with the application of eight times the adequate dose of nutrient.

Fe toxicity can vary according to the species it was submitted. Dobermann e Fairhurst (2000) found results starting at 300 mg kg⁻¹ for rice culture; Jucoski et al. (2016) found symptoms of toxicity in Fe contents in leaves between 120 and 229 mg kg⁻¹ of Fe in *Eugenia uniflora* L, with reduction in both leaves and roots. There was a higher concentration of Mn in the treatment where the highest dose of nutrient was applied (8x the recommended dose), followed by treatment with application of Mn (4x the recommended dose), which differed from treatment with application of the appropriate dose of nutrients (Table 1). This result demonstrates that despite the grass having the ability to compartmentalize this micronutrient in the apoplast of the roots, lowering their transport to the shoot (Foy, 1976), it can be absorbed in large amounts as seen with Fe.

Kohno et al. (2008) observed in bean cultivars, that according to the increase in the concentration of Mn in the nutrient solution, there was a higher content of the nutrient in the plant tissue, similar to the present work.

According to Werner et al. (1996), the Mn leaf content indicated as suitable forage is 40 to 250 mg kg⁻¹. The present study evidences the treatment with the application of the highest dose of Mn, which presented a concentration of 420 mg kg⁻¹ in the shoot, a level much higher than that recommended for forage. Guirra et al. (2011) observed that although the doses of Mn reached high levels in the shoots, it was not enough to decrease the growth of Marandu grass. There was an effect of the treatments on all evaluated dry mass variables ($P < 0.05$). For shoot dry mass, root dry mass and total dry mass, the treatment with the appropriate doses of Fe and Mn provided the highest values, where only for the shoot dry mass it did not differ from the treatments with Fe 8x and Mn 4x (Table 2).

In research conducted by Jucoski et al. (2016) plants exposed to treatment for 45 days with 2.0 mM Fe had reductions of 149, 100, and 139% in leaf dry mass productions, roots and total, respectively, when compared to the control treatment. In the present experiment, for the treatment with the application of the highest doses of nutrients (8x the recommended dose of Fe and Mn), reductions in the production of shoot dry mass of 50%, root dry mass of 76% and total dry mass of 55% were obtained, in relation to the appropriate dose of Fe and Mn.

Olaleye et al. (2001), working with the excess Fe in two rice cultivars, observed that as the concentration increased from 1000 to 4000 mg of Fe²⁺ L⁻¹ there was a decrease in plant dry matter when compared to the control treatment. Similar to the experiment developed by Fageria and Rabelo (1987), evaluating the tolerance of rice cultivars to excess Fe, they noted that the dry weight of roots was more sensitive to differences in nutrient concentration, ranging from 0.89 and 1.78 mM. Also in rice cultivars, Fageria et al. (2008) found a reduction in the leaves and roots dry mass as the concentration of Fe increased.

Table 2. Shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) of *B. brizantha* cv. Marandu plants, depending on the application of Fe and Mn concentrations.

Treatments	SDM	RDM	TDM
		-----g plant ⁻¹ -----	
Adequate Fe and Mn	9.12 a	2.17 a	11.29 a
Fe 4x	5.52 b	0.72 b	6.25 b
Fe 8x	5.73 ab	0.52 b	6.26 b
Mn 4x	5.70 ab	0.57 b	6.27 b
Mn 8x	3.86 b	0.66 b	4.53 b
Fe and Mn 4x	4.69 b	0.87 b	5.56 b
Fe and Mn 8x	4.56 b	0.51 b	5.07 b
MSD	3.55	0.62	3.75
F Value	4.85**	18.89**	7.51**
CV (%)	27.58	31.70	25.25

** Significant at 1% probability of error. Means followed by distinct letters, in the column, differ from each other by Tukey's test (P<0.05). CV = Coefficient of variation.

Cavalcante et al. (2013), evaluating the tolerance of cv. MG5 to the toxicity of manganese (0, 15, 30, 60 and 120 mg dm⁻³) observed that the shoot dry matter of the grass showed reduction in the second cut of the grass; however, for root dry matter there were no symptoms of reduction; nonetheless, the plants showed reductions in all variables evaluated.

4. Conclusions

Grass plants were not tolerant to excess Fe and Mn, presenting lower root system development, reduction in volume and dry mass. With the application of 8x the recommended dose of Fe and Mn there was a reduction in total dry mass production of 55% in relation to the appropriate dose of Fe and Mn.

Authors' Contribution

Gustavo Caione: Orientation, writing and final revision. Luana Marques Campos: Conducting the experiment, analysis and data tabulation. Willian Tsuyoshi Kume: Laboratory analysis and data interpretation. Guilherme Ferreira Ferbonink: Laboratory analysis and data interpretation. Daniele Fátima de Oliveira Caione: Data discussion, review and writing.

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