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Nitrogen compounds, proteins and amino acids in corn subjected to doses of aluminum

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Corn (*Zea mays* L.) is highly adaptable, but it has difficulties in expressing its productive potential in soils with high aluminum content, since this element is directly related to high acidity in the soil. The objective of this study was to evaluate the nitrogen compounds, proteins and amino acids of two corn cultivars subjected to increasing doses of aluminum. The experiment was carried out in a greenhouse using one of the corn plants from varieties BRS 106 and BRS 4157. The experimental design was entirely randomized, in a factorial design of 5x2, and the factors were composed of five doses of Al^{3+} (0; 50; 100; 150 and 200 mmol L^{-1}), with five repetitions. The variables analyzed were the concentration of nitrate, the activity of the nitrate reductase, concentration of ammonium, amino acids and proteins. There was a decrease in nitrate, activity of the Reductase enzyme of the Nitrate and protein in the highest dose of aluminum (200 mmol L^{-1}) for both cultivars. There was an increase in ammonium and amino acids in the leaves of cultivars BRS 106 and BRS 4157. Cultivars BRS 106 and BRS 4157 were affected by the increasing doses of aluminum, but cultivar BRS 106 showed to be more tolerant.

Key words: Aluminum, toxicity, corn, metabolism.

INTRODUCTION

Maize (*Zea mays* L.) belongs to the botanical family Poaceae and originating in Mexico as one of the most cultivated cereal in the world, and is considered one of the most efficient crops in energy storage. This culture is used as human food and as animal feed, due to its good nutritional qualities. The corn grain has a mean content of 8% protein providing approximately 63 million tons of protein in the world, but these have lower levels of lysine and tryptophan (Vasal, 1994).

Although it is highly adaptable, this crop has some barriers in productive terms in soils with high aluminum content, which is an element directly related to acidity in

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soil. Generally, pH values (in H_2O) of the soil below 5.5 can already cause serious aluminum toxicity problems for the plants. This is one of the problems of the Amazonian soils (Silva et al., 2006). Estimates show that approximately half of the arable land and with great potential for production of food and biomass are acidified, that is, they are subject to toxicity by aluminum (Kochian et al., 2004).

In addition, aluminum can cause harmful effects in the assimilation of nitrogen in the plants (Pal'ove-Balang and Mistrik, 2011). That causes a reduction in the concentration of nitrate in the presence of aluminum (Souza et al., 2014). Thus, high concentrations of this element cause alterations in the biochemical process of nitrogen, which is considered important in the production of the protein precursor (Camargo and Almeida, 1983; Sphear and Souza, 2004).

The aluminum affects in an expressive way both the absorption and the assimilation of the nitrate reductase activity (NRA) in sorghum (Cambraia et al., 1989). Purcino et al. (2003) found a similar result in corn crops. Although there are techniques such as the lining, which reduces the effects of the aluminum, there are still several gaps regarding the behavior of the species and the variety of plants subjected to high concentrations of aluminum. Therefore, it is necessary to have researches about the maximum dose that the corn crop can tolerate in a certain soil, resistant variety, and also to understand and analyze in which physiologic and biochemical mechanism this metal is involved in the plant. That way, it enables techniques, handling and cultivars that can decrease, mitigate or tolerates acid soils, increasing productivity.

It is known that the aluminum affects the plants negatively, specially the more sensitive ones. However, the studies that involve the operation of the nitrogen metabolism subjected to stress by Al^{3+} are incipient. Taking into account the agronomic and economic importance of corn for animal production, this study aims at evaluating the nitrogen compounds, proteins and amino acids of two corn cultivars subjected to increasing doses of aluminum.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse at the Rural Federal University of Amazon - Capitão Poço Campus. The plants used were from BRS 106 and BRS 4157 varieties, without control of the environment and with only monitoring of temperature and air relative humidity through a digital thermo hygrometer. 5 seeds per vase were used in the sowing. After 5 days of germination, a thinning was carried out, leaving only one plant per vase. The application of the stress by aluminum started on the 15th day after germination, and the biochemical analyses were done in the 30th day (vegetative stage) after germination. The vases were placed with a space of 0.60 m between the rows and 0.40 m between the plants.

The corn plants were grown in modified Leonard vases containing sand substrate: vermiculite (1-2) and irrigated with modified nutrient solution of Hoagland and Arnon (1950). The plants exposed to light intensity in the greenhouse were 700 lx, and the pH of the nutrient solution was 4.8. The experimental design was entirely randomized, in a factorial design of 5x2, and the factors were composed of five doses of 0; 50; 100; 150 e 200 mmol L^{-1} de Al^{3+} for the both corn cultivars, with five repetitions, totaling 50 experimental units. Each experimental unit was composed of a plant.

The concentration of nitrate was carried out by a method proposed by Cataldo et al. (1975), in which the samples of 50 mg of leaves previously lyophilized were added to test tubes containing 5.0 ml of distilled water, and those were incubated in water bath for 30 min at 100°C. After that, it was centrifuged at 3,000 rpm for 10 min, and removing the supernatant. The reaction was prepared in a test tube containing 100 µL of the extract + 200 µL of salicylic acid solution 5% (p/v), in concentrated sulfuric acid. After agitation, the tubes were added with 4700 µL of NaOH 2 N. After that, the tubes were left at rest until they reached room temperature for about 20 min. The readings were carried out in spectrophotometer at 410 nm. The nitrate reductase activity (NRA) was obtained by the method described by Hageman and Hucklesby (1971). Leaf discs of 0.5 cm^2 in diameter were removed and weighted in approximately 200 mg of the discs. Right after, they were transferred to test tubes containing 5.0 ml of phosphate buffer and, next, were taken to water bath at 30°C for 30 min. The test tubes were added with 2.0 ml of buffer + 1.0 ml of reaction extract +1.0 ml of sulfanilamide 1% + 1.0 ml of NNEDA 0.02%. They were put at rest for 15 min. After that, the reading went to the spectrophotometer at 540.

The concentration of free ammonium was determined by using the method described by Weatherburn (1967). 50 mg of dry mass (DM) of the leaves was weighted and, right after, put on test tubes with the addition of 5 ml of distilled water and taken to water bath for 30 min at 100°C. After the extraction of the samples, they were centrifuged at 1000 rpm to obtain the total extract. There was an addition of 400 µL of total extract + 2.5 ml of A solution (5 g phenol + 0.025 g Sodium nitroprusside/ 500 mL distilled water) and homogenized in vortex, adding another 2.5 ml of B solution (2.5 g of NaOH + 12.6 mL of sodium hypochlorite/ 500 mL of distilled water), and taking them to water bath for 20 min at 37°C. The tubes were removed from water bath and left to rest for 40 min, and then taken to a spectrophotometer reading at 625 nm.

The total soluble amino acids were obtained by the method described by Peoples at al. (1989). 50 mg lyophilized DM was transferred to test tubes, and 5 ml of distilled water was added. Then, they were taken to water bath for 30 min at 100°C. After the extraction, the samples were centrifuged at 1000 rpm to obtain the total extract. Aliquots of 100 100 µL of the extract + 400 µL of distilled water were added. After that, 250µL of the citrate buffer 0.2 M pH 5.0 and 250 µL of Ninhydrin reagent was added. Then, they were taken to water bath for 15 min at 100°C. Next, the reaction was interrupted in ice bath and 1.5 ml of ethanol 50% (v/v) was added.

The tubes remained at room temperature for 20 min, and the readings were done in a spectrophotometer at 570 nm. The total soluble proteins were obtained by using the method described by Bradford (1976). In test tubes, there was an addition of 100 mg lyophilized DM/ 5.0 ml of the extraction buffer (Tris-HCl25 mM pH 7.6). Then, they were agitated for 2 h in the shaker. After the extraction, the tubes were centrifuged at 2000 rpm for 10 min. The test tubes were added with 100 µL of the sample + 2.5 mL of the Bradford reagent. After that, the tubes were manually agitated taking care to not denature the proteins. After 15 min, the readings were carried out at 595 nm.

The results were subjected to the variance analysis, when significant by the F test, and the effect of the nitrogen doses analyzed by regression, adjusting the equations to express the behavior of the variables being studied.

Table 1. Analysis of the variance for nitrate, nitrate reductase activity (NRA), ammonium, amino acids and proteins in corn cultivars in function of Al^{3+}doses.

Variation sources	GL	Nitrate	NRA	Ammonium	Amino acid	Protein
Doses of Al^{3+} (Al^{3+})	4	**	**	**	**	**
Cultivars (C)	1	**	**	**	**	**
Al^{3+} x C	4	**	**	**	**	*
CV (%)	-	5.91	2.39	3.39	2.54	6.81

CV = variation coefficient; * = significant ($p < 0.05$); ** = significant ($p < 0.01$), by the Tukey test.

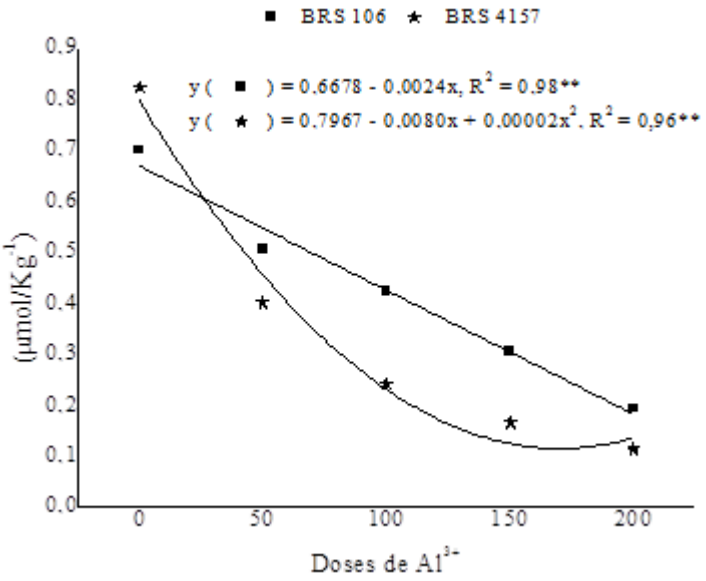

Figure 1. Concentration of nitrate in the leaves of corn cultivars BRS 106 and BRS 4157 in function of the aluminum doses. **significant ($p < 0.01$) by the t test.

RESULTS AND DISCUSSION

The aluminum doses (Al^{3+}) influenced ($p < 0.01$) the biochemical variables (Table 1). The cultivars presented a different behavior regarding nitrate, nitrate reductase activity (NRA), ammonium, amino acids and proteins. For biochemical variables, in the aerial part there was a significant effect ($p < 0.01$) of the interaction between doses Al^{3+} cultivars.

Regarding the concentration of nitrate, corns BRS 106 and BRS 4157 presented a polynomial and linear behavior, respectively, in function of the aluminum doses. For the concentration of nitrate, the control treatment presented 0.69 NO^{3-} kg^{-1} DM and the maximum doses of aluminum presented contents of 0.19 NO^{3-} kg^{-1} DM of nitrate for cultivar BRS 106. While cultivar BRS obtained 0.82 NO^{3-} kg^{-1} DM in the control and 0.11 NO^{3-} kg^{-1} DM maximum doses of aluminum (Figure 1). That is, there was a decrease in the nitrate in the highest dose of aluminum (200 mmol L^{-1}). When there is stress by excess of Al^{3+} in the corn crop, the acidification capacity of the roots, as well as the accumulation of nitrate, are reduced (Lidon et al., 1998; Ahn et al., 2001). Al^{3+} promotes the increase in permeability of the roots membrane, which causes an excess of this metal in the root system. The excess of this metal resulting from the increase in the permeability of the membrane limits the nitrate absorption rate due to the inhibition of its carriers (Simon et al., 1994).

This decrease may be related to the lack of nutrients in the plant, because one of the characteristics of the plant subjected to stress by aluminum is the shortening of the root. Thus, the plants will have difficulties in absorbing water and nutrients, and transport them to the leaves. Therefore, the content of substrates (nutrients, among them the Nitrate) will be compromised, causing the reduction of this substrate in the plant, besides contributing to the decrease in the activity of nitrate

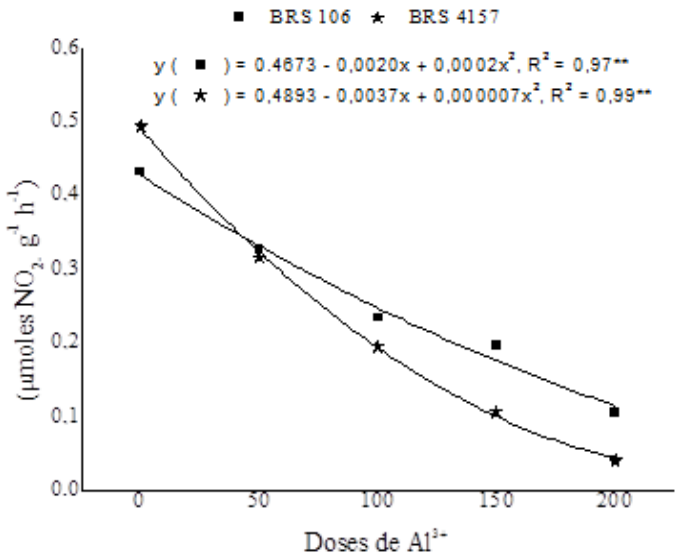

Figure 2. Nitrate reductase activity in the leaves of corn cultivars BRS 106 and BRS 4157 in function of the aluminum doses. **significant ($p < 0.01$) by the t test.

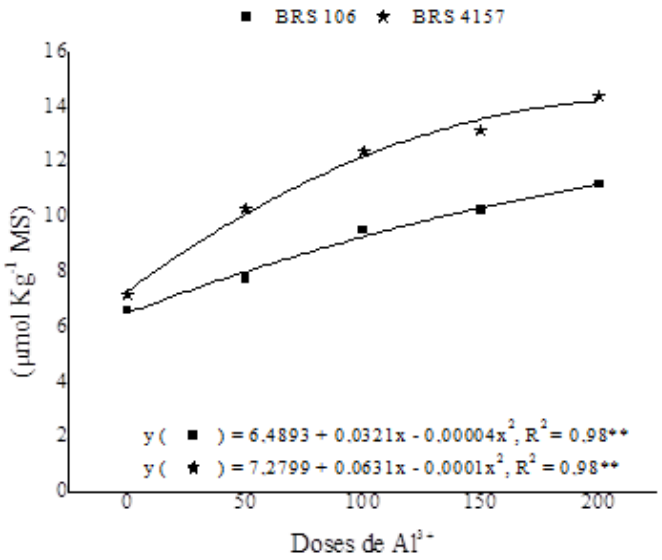

Figure 3. Concentration of free ammonium in the leaves of corn cultivars BRS 106 and BRS 4157 in function of the aluminum doses. **Significant ($p < 0.01$) by the t test.

reductase.

Al^{3+} may cause dramatic effects in the assimilation of N in the plants (Pal'Ove-Balang and Mistrik, 2011), which leads to the decline in the concentration of nitrate in the presence of Al^{3+}. In the presence of aluminum, the rice cultivar Fernandes reduced the concentration of nitrate (Justino et al., 2006). Apparently, the effects of Al^{3+} on the absorption of nitrate depend on the species studied, on the concentration of Al^{3+} in the absorption medium, on the duration of the treatment applied and, probably, on its later interference on the process of nitrate reduction and/or assimilation of nitrogen in organic compounds. Al^{3+} has an effect on the absorption of nitrate, and there are no physiologic explanations for the several conflicting results.

The results showed that there was a decrease in the activity of the Nitrate Reductase activity of 0.43 µmols of NO_2^- g MF^{-1} h^{-1} in the control treatment for 0.1 µmoles of NO_2^- g MF^{-1} h^{-1} in the maximum doses of aluminum (200 mmol L^{-1}) for cultivar BRS 106. For cultivar BRS 4157, there was a reduction of 0.49 µmols of NO_2^- g MF^{-1} h^{-1} to 0.04 µmols of NO_2^- g MF^{-1} h^{-1} in the control treatment and in the maximum aluminum doses (200 mmol L^{-1}), respectively (Figure 2). The reduction was probably because the aluminum decreased the root growth, which is shown by the low absorption of nitrate and water by the roots. That can cause the reduction of the transpiration current, leaving the enzyme inactive. In addition, the nitrate reductase suffers a decrease in the plants subjected to acidity (Sharma and Dubey, 2005).

The high acidity in the soil can cause inhibition of nitrate reductase activity (Sharma and Dubey, 2005). The nitrate reductase activity was negatively affected in corn plants when they were grown in conditions of high acidity (Lin-Xianyong et al., 2002). The increase in the doses of aluminum elevated the concentration of ammonium in the leaves of cultivars BRS 106 and BRS 4157, with a polynomial adjustment. Ammonium increased from 6.57 µmols of NO_2^- g^{-1} MF h^{-1} in the control to 11.15 µmols of NO_2^- g^{-1} MF h^{-1} in the highest dose of aluminum (200 mmol L^{-1}) for cultivar BRS 106. For cultivar BRS 4157 there was an increase from 7.15 µmols of NO_2^- g^{-1} MF h^{-1} (control) to 14.37 µmols of NO_2^- g^{-1} MF h^{-1} (200 mmol L^{-1}) (Figure 3).

This result can be explained because the ammonium needs the glutamine synthetase enzyme to transform into Glutamine and, later on, in glutamate, releasing amino acids that will help the good development of the plant. As soon as it notices that the content of free ammonium increases with the increase of the aluminum doses, the toxicity probably inactivates the activity of the Glutamine Synthetase enzyme, preventing ammonium NH_4^+ from incorporating into the Glutamate amino acid to form glutamine, and consequently enabling its accumulation. Purcino et al. (2003) found a similar result in which the assimilation of NH_4^+ was affected by Al^{3+}, once this metal compromises the process by altering the activity of the enzymes capable of incorporating it in amino acids. One of the factors that can contribute to that is the prevalence of NH_4^+ rather than NO^{3-} in the conditions of acidity and toxicity of Al^{3+} experienced by plants of cowpea (Kerbauy, 2008).

Another relevant factor for the accumulation of ammonium in both cultivars is that in the process of photorespiration (mitochondria) occurs the deamination phenomenon, which is a natural procedure of the plant

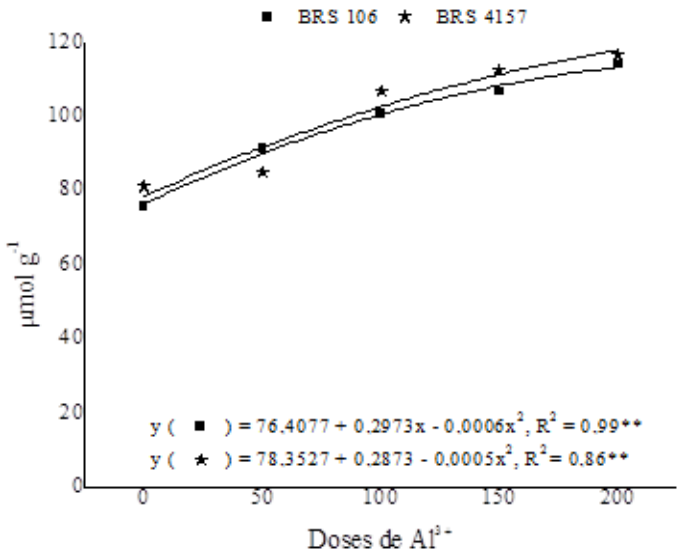

Figure 4. Concentration of amino acids in the leaves of corn cultivars BRS 106 and BRS 4157 in function of the aluminum doses. **significant (p < 0.01) by the t test.

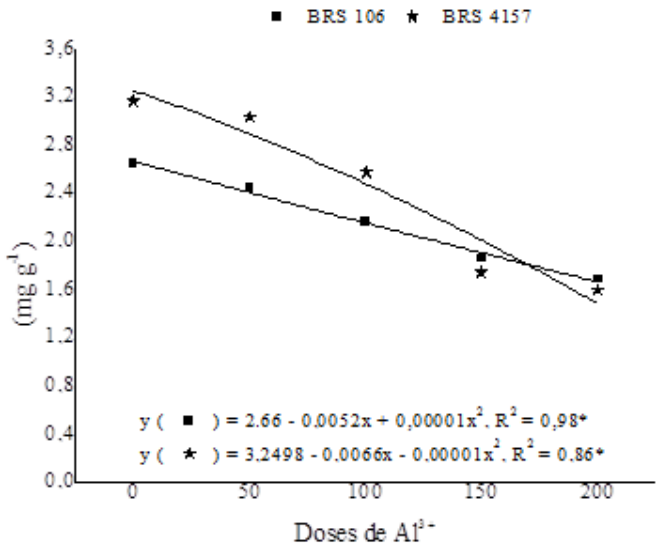

Figure 5. Concentration of protein in the leaves of corn cultivars BRS 106 and BRS 4157 in function of the aluminum doses. *significant (p < 0.05) by the t test

for the release of ammonium. However, with the action of this stress by Al^{3+}, there was possibly a lack of control of this deamination, contributing to the accumulation of ammonium both in BRS 106 and BRS 4157.

As the aluminum dose increased, there was also an increase in the concentration of amino acids of both cultivars (BRS 106 and BRS 4157), with a polynomial adjustment. For cultivars BRS 106 and BS 4157, there was an increase from 75.61 µmol of AA/ g MS (control) to 114.01 µmol of AA/ g MS (200 mmol L^{-1} of Al^{3+}) and from 81.03 µmol of AA/ g MS (control) to 116.43 µmol of AA/ g MS (200 mmol L^{-1} de Al^{3+}) respectively (Figure 4). The growth of total soluble amino acid may have probably been caused by the increase in the activity of proteases enzyme, which break the reserve proteins according to the exposition of a plant to any injury, in this case the effect of aluminum toxicity, contributing with the water deficit.

Those effects are observed as a consequence of the inhibition of root growth (Beutler et al., 2001). This fact is probably due to the increase in the activity of the proteases enzyme, which break the reserve proteins in plants exposed to long periods of water deficit, increasing the content of total soluble amino acids, aiming at adjusting osmotically to the stressing medium (Kerbayy, 2004).

Cruz et al. (2011) obtained results that show the opposite, where they observed that the presence of Al^{3+} caused the decrease in the concentration of amino acids and total soluble proteins in sorghum plants, showing that the presence of this element can actually limit the vegetable growth. Balang and Zelinova (2013) when studying the behavior of both cultivars of *Lotus corniculatus* under stress conditions caused by toxic aluminum, observed that the reduction of free amino acids may be related to the low availability of nitrogen due to the inhibition of absorption of nitrate and ammonium under stress conditions.

As the aluminum dose increased, there was a decrease in the concentration of proteins in both cultivars (BRS 106 and BRS 4157), with a polynomial adjustment. There was a decrease in the concentrations of total soluble proteins from 2.64 mg protein/ g DM (control) to 1.68 mg protein/ g DM (200 mmol L^{-1} of Al^{3+}) in cultivar BRA 106 and from 3.16 mg protein/ g DM (control) to 1.59 mg protein/ g MS (200 mmol L^{-1} of Al^{3+}) (Figure 5). During the stress caused by aluminum, this element acts as a limiting factor for the assimilation of nitrogen, once there is a reduction in the nitrate reductase activity, which is the first enzyme associated to the nitrogen metabolism, and the low supply of nitrogen would cause a reduction in the synthesis of protein (Cruz et al., 2011).

Possibly, the decrease in proteins in both cultivars is related to the breaking of these total soluble proteins by the proteases enzymes, starting to form amino acids and, within those amino acids, there is a deamination, forming ammonium. Therefore, with the protein break, there will be a contribution to the increase of the amino acids and ammonium, respectively. The proteins degrade, forming amino acids that adjust osmotically; among those amino acids you can find proline. It starts to work in order to avoid the loss of water in the leaf tissues. Somers et al. (1996) showed that there was a decrease in the content of total soluble proteins (cytoplasm) in plants subjected to treatments with Al^{3+}, both for plants resistant to metal and sensitive to it. However, in a study made by Souza et al.

(2014) with species of Urochloa subjected to aluminum, it was not observed alterations in the concentration of amino acids and proteins.

The presence of aluminum may inhibit the absorption of other ions, such as Mg (Malavolta et al., 1997). This element plays an important role in the metabolic pathways such as glycolysis, Krebs cycle and pentose phosphate pathway. The addition of aluminum made Mg unavailable and the deficit of this element may have reduced the activity of these metabolic pathways such as the Krebs cycle, and it may also have resulted in an alteration of the proteins biosynthesis, because Mg is necessary for the protein synthesis, as a cofactor to several enzymes (Boutler, 1970).

Conclusions

As the aluminum doses increased, there was a reduction in nitrate, nitrate reductase and total soluble proteins. The increasing doses of aluminum provided an increment in the concentrations of ammonium, total soluble amino acids for both cultivars. Cultivars BRS 106 and BRS 4157 were affected by the increasing doses of aluminum, but cultivar BRS 106 showed to be more tolerant.

Conflicts of Interests

The authors have not declared any conflict of interests.

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