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Aluminum negative impact on nitrate reductase activity, nitrogen compounds and morphological parameters in sorghum plants

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Abstract

Aluminum toxicity represents a limiting factor for yield in crops, and study linked to aluminum impact on nitrate reductase and nitrogen compounds is fundamental to explain decreases in growth and development of sorghum plants. Aim of this study was to investigate (i) aluminum impact in sorghum plants, and to measure (ii) nitrate reductase activity, nitrogen compounds, and morphological parameters. Experimental design was completely randomized with 4 aluminum concentrations (0, 50, 100, and 150 μM Al). Parameters measured were leaf area, nitrate reductase activity in leaf and root, proline, amino acids, and total soluble proteins. Significant decreases in nitrate reductase activities were showed in leaf and root tissues. Results linked to total soluble proteins demonstrated that plants treated with aluminum suffered reduction in this parameter promoted by aluminum. This study revealed two relationships between nitrate reductase in leaf and total soluble proteins, as well as between total soluble proteins and shoot dry matter, and also low tolerance of sorghum plants to aluminum

Keywords: *Sorghum bicolor*, abiotic stress, aluminum, nitrogen compounds, nitrate reductase, proteins.
Abbreviations: NO_3^- - nitrate, Al - aluminum, R^2 - determination coefficient, *r* - correlation analysis, photosynthetic active radiation - PAR, cv - cultivar, ATP - Adenosine-5'-triphosphate, DNA - deoxyribonucleic acid, ROS - reactive oxygen specie.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench.] plants are frequently used in areas with irregular rainfall distribution, due to it be more tolerant for drought that other crops such as maize and wheat (Torrecilas et al., 2011), and this characteristic is associated your capacity in water use of efficient form and translocation/mobilization of organic solutes (Ali et al., 2009; Beheshti and Behboodi fard, 2010). Shoot of sorghum can be an option aiming silage production due to adequate production of dry matter and high nutritional value (Bramel-Cox et al., 1995). Sorghum contributes with about 10 to 12% of cultivated total area with silage in Brazil, therefore this culture exercises strong influence over agricultural activity of this country (Rocha Jr. et al., 2000). Aluminum toxicity represents a limiting factor for yield in crops (Valle et al., 2009), because aluminum contained in soil and available to plant promotes reduction in root growth (Tang et al., 2001) and inhibits simulation and transport of nutrients, influencing negatively on yield (Kochian et al., 2005). Study linked to aluminum impact on nitrate reductase and nitrogen compounds is fundamental to explain decreases in growth and development previously reported (Kariuki et al., 2007). In addition, this enzyme is first into nitrogen metabolism, and it is responsible by nitrate (NO_3^-) assimilation. In sorghum is unknown proline role under Al toxicity situation, and to evaluate behavior of this amino acid can contribute in breeding programs focusing tolerance to aluminum. This compound is accumulated during abiotic stresses induced through water, temperature, and nutrients (Hare and Cress, 1997), including stress by aluminum (Khan et al., 2000). Proline action is intensively linked to osmotic adjust process, contributing also to establish membranes and proteins, neutralizing action of free radicals, besides to act as buffer during regulation of the cell redox potential under inadequate conditions (Ashraf and Foolad, 2007). Aim of this study was to investigate (i) aluminum impact in sorghum plants, and to measure (ii) nitrate reductase activity, nitrogen compounds, and morphological parameters.

Materials and methods

Experimental conditions

Study was implemented in Instituto de Ciências Agrárias (ICA) of the Universidade Federal Rural da Amazônia (UFRA), Pará state, Belém city, Brazil (01º27'S and 48º26'W). Experiment was conducted in greenhouse under natural conditions day/night (air temperature minimum/maximum and relative humidity were 24.5/39.7°C and 40/89%, respectively, during experimental period). Photoperiod medium was of 12 h of light, and Photosynthetic Active Radiation (PAR) was 968 μmol m^{-2} s^{-1} (at 12:00 h).

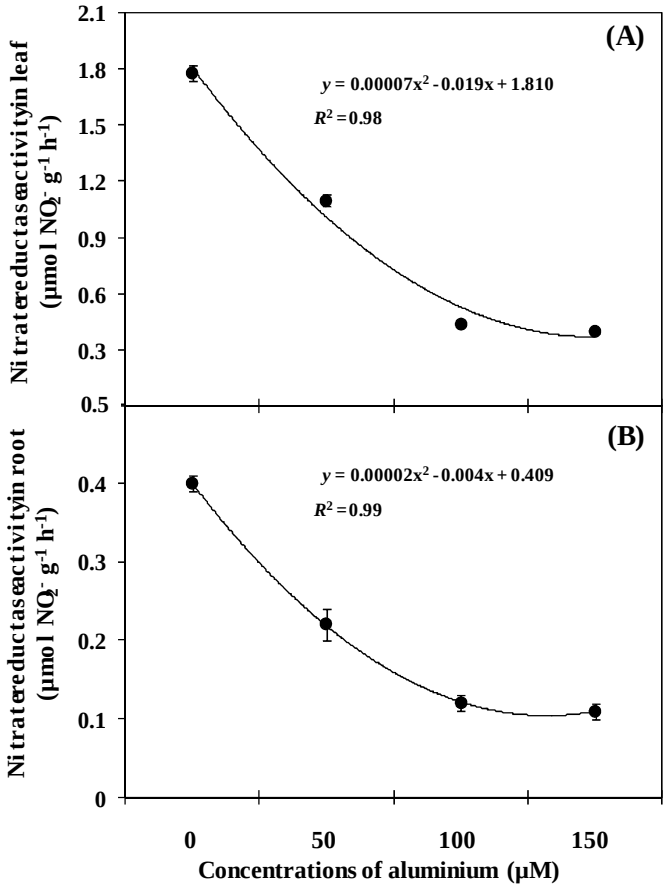

Fig 1. Nitrate reductase activity in leaf (A) and root (B) of *Sorghum bicolor* plants (cv. BR-700) exposed to 0, 50, 100 e 150 µM of aluminum. The bars represent the mean standard errors.

Plant material and aluminum treatment

Seeds of *Sorghum bicolor* (L.) Moench (cv. BR-700) were used in this study, in which it were developed and obtained from Empresa Brasileira de Pesquisa Agropecuária – Milho e Sorgo (Embrapa/Brazil), harvested in 2006 season. Aluminum source used in this study was aluminum sulphate ($Al_2(SO_4)_3.18H_2O$), being dissolved in nutritive solution under 4 concentrations (0, 50, 100, and 150 µM of Al).

Seedling and pots

Seeds were placed to germinate into substrate composed by sand washed and autoclaved at 120°C by 40 min, and after 5 days were selected seedlings with aspect and size similar. Subsequently, these selected seedlings were transferred to pots with individual capacity of 1.2 L, using as substrate sand and silica gel in proportion of 2:1 (v:v), respectively.

Experimental design and treatments

Experimental design was completely randomized with 4 treatments (0, 50, 100, and 150 µM of Al). Experiment was composed by 10 replicates and 40 experimental units, where each experimental unit was constituted by 1 plant.

Plant conduction

During 5th until 10th day after experiment implementation the seedlings were acclimatized with nutritive solution presenting 25% of ionic force. In addition, 11th until 15th day was applied 50% of ionic force, and 16th until 30th day was imposed 100% (total) of ionic force, being this solution changed in constant intervals of three days. During this study, solution pH was kept in 4.5±0.2, and when necessary HCl and NaOH were added.

Harvest and foliar area

Plants were harvested and divided in shoot and root, with cut located in plant collar, being removed branch and determined foliar area from leaves. Subsequently, fresh leaf and fresh root were used for biochemical evaluation.

In vivo nitrate reductase activity in leaf and root

Extraction of the nitrate reductase enzyme (E.C. 1.6.6.1) was carried out with leaf and root until to reach the weight of 200 mg, in which the samples were incubated in 5 mL of extraction buffer (KH_2PO_4 at 0.1M, KNO_3 at 50 mM, isopropanol at 1% (v/v) and pH 7.5) by 30 minutes at 30 ºC, and all the procedures carried out in dark. The quantification of the enzyme activity was makes by the method of Hageman and Hucklesby (1971) with absorbance at 540 nm and using spectrophotometer (Quimis, model Q798DP).

Dehydration and dry matter measurements

Leaves and root harvested were placed in an oven with forced air circulation at 70º C by 96 h. After this period, shoot dry matter and root dry matter were measured and triturated, with the powder resulting kept in glass containers. These containers were remained in the dark at temperature of 15ºC until the moment to carry out biochemical analysis.

Total soluble amino acids

Determination of amino acids was performed using 50 mg of leaf dry matter powder, and was incubated with 5 mL of sterile distilled water at 100 ºC by 30 minutes, after the homogenized was centrifuged to 2.000 *g* by 5 minutes at 20 ºC and supernatant was removed. Quantification of the total soluble amino acids was carried out at 570 nm according to Peoples et al. (1989), and was used L-asparagine + L-glutamine (Sigma Chemicals) as standard.

Proline

Proline level was determined with 50 mg of leaf dry matter powder, which was incubated with 5 mL of sterile distilled water at 100 ºC by 30 minutes, after the homogenized was centrifuged to 2.000 *g* by 5 minutes at 20 ºC. Quantification of proline was carried out at 520 nm according to Bates et al. (1973), in which was utilized L-proline (Sigma Chemicals) as standard.

Total soluble proteins

Determination of the total soluble proteins was carried out with 100 mg of powder, being incubated with 5 mL of extraction buffer (Tris-HCl at 25 mM and pH 7.6). Homogenized was kept in agitation by 2 h, after this period was centrifuged to 2000 *g* by 10 minutes at 20 ºC. Quantification of the total soluble proteins was carried out at 595 nm in agreement with Bradford

(1976), as well as was used albumin bovine (Sigma Chemicals) as standard.

Data analysis

Results were subjected to regression analysis, and equation more adequate was defined using as main criterion the significant effect, and secondary criterion the determination coefficient (R^2). In addition, standard errors were calculated in all points and treatments, as well as correlation analysis (r) was performed by the Pearson parametric method using SAS (Steel et al., 2006).

Results and discussion

Changes in nitrate reductase activity of leaf and root

Significant decreases in nitrate reductase activity were obtained in leaf (Figure 1 A) and root (Figure 1 B) of sorghum plants exposed to aluminum, when compared to control plants (0 µM Al). In addition, this parameter in leaf and root presented quadratic behavior, and was showed lower values into each aluminum concentration in root tissue, if compared with leaf. Aluminum concentrations investigated promoted reduction in nitrate reductase activity due to probably lower gene expressions that act in synthesis of nitrate reductase.

These genes are over-expressed by factors such as NO_3^- concentration and light (Epstein and Bloom, 2004). These environment factors induce the synthesis of new enzymes (Sivazankar and Oaks, 1996) and stimulate phosphatase protein that will desphosphoryla several residues of serine found in enzyme nitrate reductase, which this mechanism will keep active. In contrary, factors such as dark and ion Mg^{+2} stimulate kinase protein responsible by the phosphorylation of nitrate reductase activity resulting in enzyme inactivation (Kaiser and Huber, 1994). Study conducted by Purcino et al. (2003) reveals that aluminum in excess promotes negative effects on nitrate reductase activity. However, results described by Rufty Júnior et al. (1995) working with *Glycine max* plants was not showed changes provoked by aluminum.

Aluminum consequences on nitrogen compounds

Concentrations of total soluble amino acids in leaf were significantly reduced ($P < 0.05$) with increase in aluminum levels applied during this study, as well as was found better adjust in linear equation (Figure 2 A). Proline suffered decrease at 50, 100, and 150 µM Al, and equation model more adequate was quadratic (Figure 2 B). Results linked to total soluble proteins demonstrate significant reduction promoted by aluminum, and value lower was found in 150 µM Al (Figure 2 C). Additionally, correlation analysis demonstrated that there is a positive and linear relationship (Figure 3) between nitrate reductase in leaf and total soluble proteins (r=0.97; $P < 0.05$). Reduction in total soluble amino acids can be explained by decrease of protein synthesis, because the aluminum promotes fall in ATP levels (Lorenc-Plucinska and Ziegler, 1996), and this restriction will affect DNA transcription during to protein synthesis. In other words, aluminum promoted indirect negative effect on total soluble amino acids due to reduction in ATP production that will supply energy to process protein synthesis, decreasing consequently degradation to proteins from amino acids. Study conducted by Amaral et al. (2000) evaluated responses of *Stylosanthes guianensis* and *Stylosanthes macrocephala*

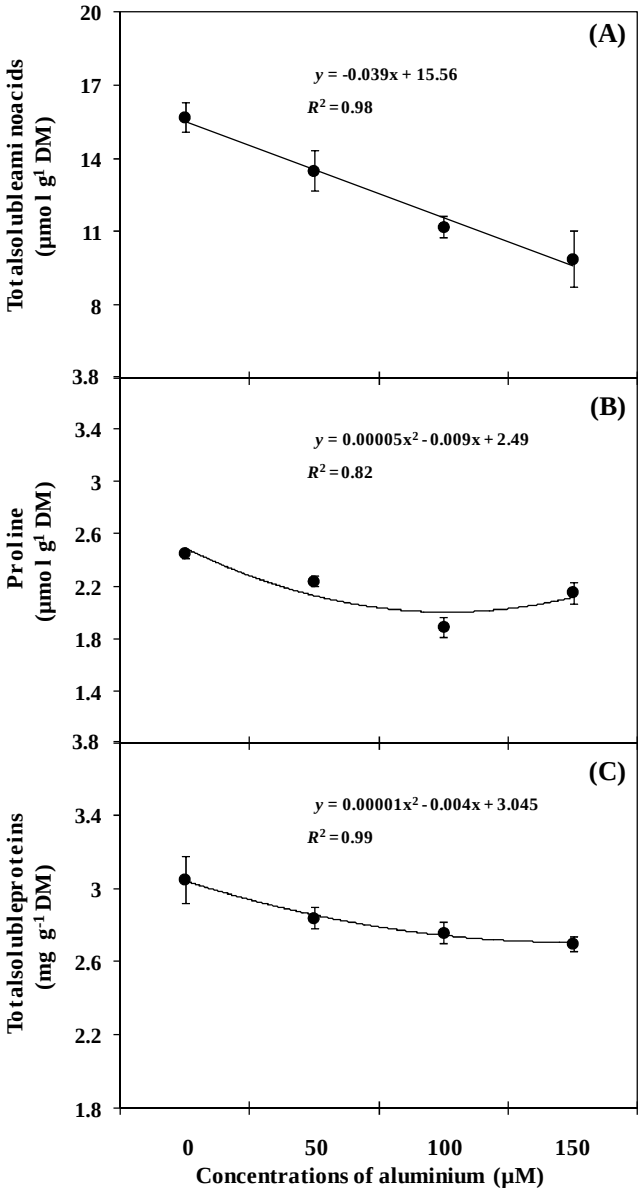

Fig 2. Total soluble amino acids (A), proline (B), and total soluble proteins (C) in leaf of *Sorghum bicolor* plants (cv. BR-700) exposed to 0, 50, 100 e 150 µM of aluminum. The bars represent the mean standard errors.

over effect of aluminum toxicity, and this nutrient in excess provokes decrease in total soluble amino acids of both specie. Decrease in proline level suggests that anti-stress mechanism of this species is not efficient to control changes induced by aluminum. Proline is an amino acid that is normally associated to protection mechanism of proteins, enzymes, and membrane structures against reactive oxygen specie (ROS) (Sharma and Dubey, 2005) in stress conditions induced by heavy metals, salts, and water deficiency. Giannokoula et al. (2007) studying two contrasting hybrids of *Zea mays* to aluminum obtained that proline reduced in leaf and root of sensitive hybrid, compared to tolerant plants. Plants treated with aluminum presented decrease in total soluble proteins, and these results showed are connected to fact of nitrate

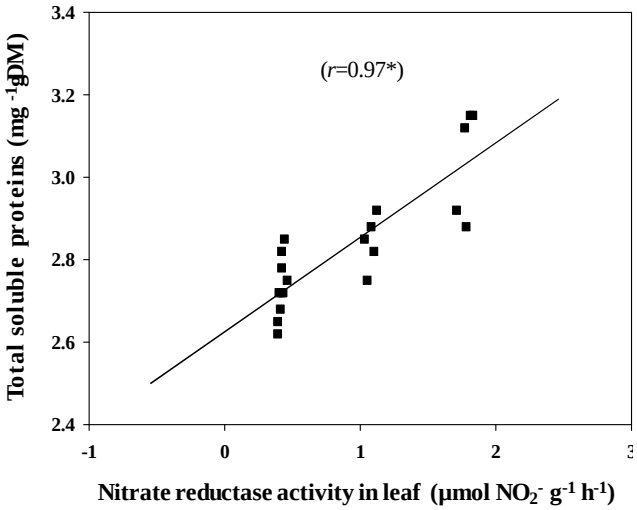

Fig 3. Linear relationship between nitrate reductase activity in leaf and total soluble proteins of *Sorghum bicolor* plants (cv. BR-700) exposed to 0, 50, 100 e 150 µM of aluminum. Asterisk (*) indicates significance at 0.05 probability level.

reductase activity in leaf to be inhibited. During stress induced by aluminum this element acts as limiting factor in nitrogen assimilation, because it reduces the nitrate reductase activity, that is first enzyme linked to nitrogen metabolism, and with lower nitrogen supply will have as consequence decrease in protein synthesis.

Morphological modifications induced by aluminum

Significant decreases were obtained in shoot dry matter (Figure 4 A), and root dry matter (Figure 4 B), with both parameters presenting linear equation. In relation to leaf area also was showed significant reduction ($P < 0.05$) promoted by aluminum, as well as equation more adequate was quadratic (Figure 4 C). In correlation analysis was found a positive and linear relationship (Figure 5) between total soluble proteins and shoot dry matter (r=0.95; $P < 0.05$). Reduction in shoot dry matter was promoted by lower protein production, as well as this explanation is supported by correlation analysis. This result reveals the fundamental role of proteins in plant metabolism of *Sorghum bicolor*. Decrease in root dry matter was probably provoked by phytotoxic effect of Al^{+3}, because aluminum inhibits cell division due to aluminum ligation to DNA during cell transcription, which impossibilities your synthesis and, consequently, decrease in root growth (Matsumoto, 1991). Similar results are described by Justino et al. (2006) showed reductions in shoot dry matter and root dry matter of *Oryza sativa* plants exposed to aluminum. Leaf area also suffered decrease, and this change is probably linked to lower nitrogen supply showed in nitrate and ammonium in leaf, that are normally two nitrogen forms absorbed in this culture. Therefore, aluminum induces lower nitrogen assimilation and this reduction promotes reduction in leaf area. In selection of *Sorghum bicolor* plants aiming silage production and consequently maximum leaf area, nitrogen metabolism exercises direct influence in growth and development of plants, and this fact suggests other studies

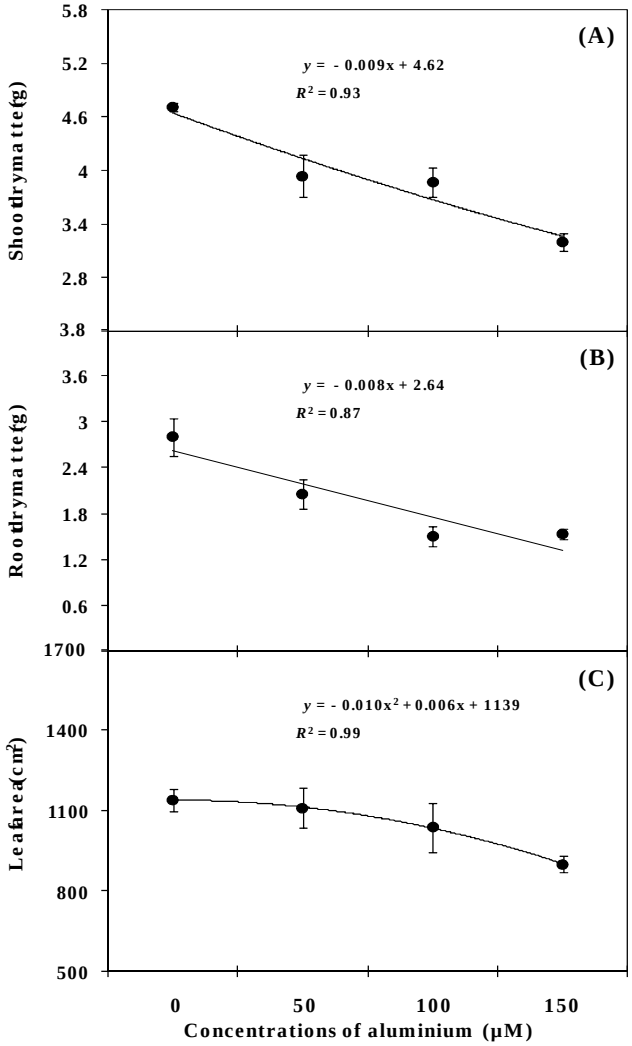

Fig 4. Shoot dry matter (A), root dry matter (B), and leaf area (C) of *Sorghum bicolor* plants (cv. BR-700) exposed to 0, 50, 100 e 150 µM of aluminum. The bars represent the mean standard errors.

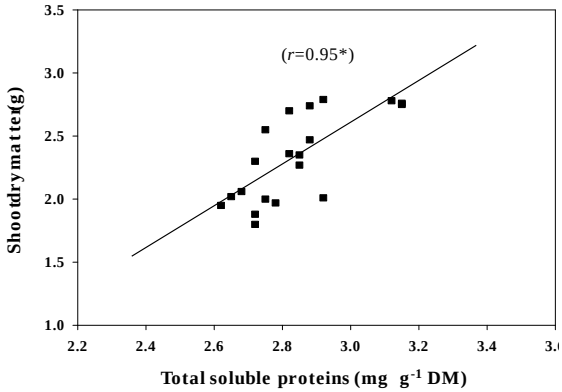

Fig 5. Linear relationship between total soluble proteins and shoot dry matter of *Sorghum bicolor* plants (cv. BR-700) exposed to 0, 50, 100 e 150 µM of aluminum. Asterisk (*) indicates significance at 0.05 probability level.

linked to interaction between nitrogen and cultivar. Aluminum promotes reduction in nitrate reductase activities
of leaf and root, and this nutrient contributed indirectly to decrease total soluble amino acids, proline, and total soluble proteins in leaf of *Sorghum bicolor* plants (cv. BR-700). These reductions in nitrogen compounds are explained by the limited nitrogen assimilation, because there is a linear relationship between nitrate reductase activity in leaf and total soluble proteins. Results linked to morphological parameters such as shoot dry matter, root dry matter, and leaf area also suffered significant decreases, and was found relationship between total soluble proteins and shoot dry matter. Therefore, this study reveals that this cultivar is sensitive to mineral stress simulated by aluminum.

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