Response of brachiaria grass to selenium forms applied in a tropical soil

S.J. Ramos[1], F.W. Ávila[2], P.F. Boldrin[2], F.J. Pereira[3], E.M. Castro[3], V. Faquin[2], A.R. Reis[4], L.R.G. Guilherme[2]

[1]*Vale Technological Institute – Mining, Belo Horizonte, Brazil*
[2]*Soil Science Department, Federal University of Lavras, Lavras, Brazil*
[3]*Biology Department, Federal University of Lavras, Lavras, Brazil*
[4]*Department of Civil and Environmental Engineering, Waseda University, Tokyo, Japan*

ABSTRACT

In Brazil the total area of native and cultivated pasture used for livestock is around 180 million hectares, and selenium (Se) is absent from mineral fertilizer formulas. Nutritional supplementation of this element takes place along with provision of mineral salts in the form of sodium selenite. In the present work, the effects of adding selenate and selenite on Se biofortification, antioxidant activity and anatomy alterations in *Brachiaria brizantha* were evaluated. The experiments were disposed in a completely randomized design in a 6 × 2 factorial scheme, by means of five levels of Se (0; 0.5; 1.0; 3.0 and 6.0 mg/kg) applied along with grass plant fertilizer, and two Se forms (sodium selenate and sodium selenite), with six replications. The results of the present study suggest that, in tropical soil conditions, the application of Se as selenate at low doses is more appropriate for *B. brizantha* biofortification than Se as selenite, because it favors a greater shoot Se levels, better activation of the antioxidant system and reduces on lipid peroxidation. Finally, with an increase of Se rates, cellular modifications were observed in internal structures of roots in *B. brizantha*, with aerenchyma appearing.

Keywords: biofortification; forage; antioxidant enzymes; root anatomy; *Brachiaria brizantha*

Selenium (Se) is a naturally occurring trace element that is essential for animal and human nutrition, but the range between dietary requirements and toxic levels is relatively narrow (Khanal and Knight 2010). Selenium deficiency in animals is very common in the world, affecting South America, North America, Africa, Europe, Asia, Australia, and New Zealand (Fordyce 2005). Clinical signs of Se deficiencies in animals include reduced appetite, growth, production, reproductive fertility, and muscular weakness (Khanal and Knight 2010).

Imbalances of Se may be due to low amounts of minerals in soil as well as in forages, which is the main diet of grazing animals (Khan et al. 2010). Although in Brazil there are few studies of this kind, there are indications of low Se intake in production animal diets (Reis et al. 2009), and evidence of Se deficiency in some soils of Brazil were presented by Sillanpää and Jansson (1992).

Brazil is the world's largest exporter of beef, being home to a cattle herd whose size is second only to that of India's (Mathews et al. 2011). It has around 172 million hectares of grasslands that support a cattle herd of approximately 205 million heads. About 60% of the total area of pastures is composed by grasses of the *Brachiaria* genus, and animals fed with this grass characterize a large part of the Brazilian beef cattle production, and Brazilian forage pastures do not provide adequate dietary selenium for livestock (Reis et al. 2009).

Several studies report on the beneficial effects of Se, since it increases antioxidant activity in plants, leading to higher plant yield (Hartikainen et al. 2000, Ramos et al. 2011). However, few studies reported the effects of Se on *Brachiaria brizantha* grown in tropical soils. Thus, due to the importance of Se accumulation in grasses for animal feed, along with the scarcity of information related to

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this issue in tropical regions such as Brazil, the objective of the present work was to evaluate the response of *Brachiaria brizantha* to Se forms applied in a tropical soil.

MATERIAL AND METHODS

Plant growth and treatments. The experiments were carried out in a greenhouse at the Soil Science Department of the Federal University of Lavras, Brazil. Soil samples were taken from the 0–20 cm layer of an Oxisol with low Se level from the 'Cerrado' region. After air drying, the soil was sieved through a 2 mm mesh to perform physical, chemical and mineralogical analyses as stated in Embrapa (1997). The results of soil characterization are shown in Table 1.

Based on chemical soil composition analysis, liming was performed to raise the base saturation to 70% using calcined lime, with 24.9% Ca, 8.4% Mg and total neutralizing power, TNP = 94.5%. After incubation for 30 days with soil moisture close to 60% of total pore volume (TPV), eight grass seeds (*Brachiaria brizantha* Stapf. cv. Xaraés) were sown per pot. Seedlings were thinned to two per pot one week after emergence.

Grass plants were grown in pots containing 5 kg of soil and each pot received macronutrient fertilizer containing 100 mg N, 200 mg P, 100 mg K, and 40 mg S per kg of soil. Micronutrient application consisted of 3.6 mg Mn, 1.5 mg Cu, 5 mg Zn, 0.8 mg B and 0.15 mg Mo per kg of soil. For covering fertilizer of grass plants, 150 mg N and K per kg of soil was applied, splitted into three applications. While the experiment was being conducted, the soil moisture was rigorously controlled by daily weighing of the pot-soil-plant set, replacing the lost volume with distilled water.

Three completely expanded leaves were collected during blooming phase. They were immediately frozen in liquid nitrogen and stored at –80°C for antioxidants enzymes analyses. For anatomical analyses, roots were collected from each replication and prepared with FAA 70 (formaldehyde + acetic acid + 70% ethyl alcohol) during 72 h. Samples were preserved in 70% ethanol until light microscopy analyses. The plants were then harvested and dried in a forced-air drying oven at 55–60°C until constant mass.

The experiments were disposed in completely randomized design in a 6 × 2 factorial scheme, with five Se rates (0; 0.5; 1.0; 3.0 and 6.0 mg/kg) applied along with *B. brizantha* fertilizer, and two Se forms (sodium selenate and sodium selenite, both purchased from Sigma-Aldrich, Saint Louis, USA), with six replicates.

Activity of antioxidant enzymes. To estimate superoxide dismutase (SOD) and catalase enzyme activity, frozen tissues were homogenized in a cooled 0.1 mol/L Tris-HCl buffer at pH 7.8 containing 1 mmol/L EDTA, 1 mmol/L dithiothreitol and 5 mL of 4% polyvinyl pyrrolidone per gram of fresh weight. The homogenate was filtered through a nylon mesh and centrifuged at 14 000 rpm for 30 min at 4°C. The supernatant was used to measure enzyme activity.

Superoxide dismutase (EC 1.15.1.1) activity was assayed by monitoring photochemical inhibition of nitroblue tetrazolium (NBT) reduction (Beyer and Fridovich 1987). A 5 mL reaction mixture, containing 50 mmol/L Na_2CO_3 (pH 10.0), 13 mmol/L methionine, 0.025% (v/v) Triton X-100, 63 µmol/L NBT, 1.3 mmol/L riboflavin, and an appropriate quantity of enzyme extract was used. The reaction mixtures were illuminated for 15 min at photosynthetic photon flux density (PPFD) of 380 µmol/m^2/s. Non-illuminated mixtures were used to correct for background absorbance. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT reduction as monitored at 560 nm.

Catalase (EC 1.11.1.6) activity was tested by observing H_2O_2 consumption at 240 nm for 5 min (Rao et al. 1997). The reaction mixture (3 mL total volume) contained 25 mmol/L Tris-acetate buffer (pH 7.0), 0.8 mmol/L EDTA-Na, 20 mmol/L H_2O_2, and enzyme assay was carried out at 25°C.

Determination of lipid peroxidation. For the malondialdehyde (MDA) assay, 0.5 g of *B. bri-*

Table 1. Chemical and physical properties of soil studied

$pH_{(H2O)}$	OC (%)	CEC ($mmol_{(+)}$/kg)	P	B	Cu	Fe	Mn	Zn	Se	Si[a]	Fe[a]	Al[a]	Ti[a]	Clay	Silt	Sand
			(mg/kg)							(g/kg)				(%)		
5.4	3.48	370	0.86	0.09	0.65	26.2	0.36	0.42	0.06	40.4	24.1	89.4	5.1	23	17	60

OC – organic carbon; CEC – cation exchange capacity; [a]sulfuric acid digestion; P and micronutrients extracted with Mehlich 1; Se determined as described by Cartes et al. (2005)

zantha frozen tissues was homogenized in 5 mL of 50 mmol/L buffer solution (containing 0.07% $NaH_2PO_4 \cdot 2\,H_2O$ and 1.6% $Na_2HPO_4 \cdot 12\,H_2O$), ground with a cooled mortar and pestle, and centrifuged at 14 000 rpm for 30 min (4°C). MDA concentration was calculated using the extinction coefficient of 155 mmol/L/cm (Heath and Packer 1968).

Anatomical modifications analysis. For anatomy analyses, cross sections were taken from roots, 3 ± 0.5 cm from the top, using a manual desk microtome. Samples were clarified in 5% sodium hypochlorite for 5 min and washed three times with distilled water. The material was stained using Safrablau (1% saphranine and 0.1% astrablau at a 7:3 ratio) as described by Kraus and Arduin (1997). Then, samples were mounted on semi-permanent slides with 50% glycerin and slides lutation was carried out with nitrocellulose resin. The slides were observed using a Ken-a-vision TT18 light microscope (Ken-a-vision Mfg. Co., Inc., Kansas, USA) and microphotographed using a Canon Power Shot A620 digital camera (Canon Inc., Tokyo, Japan).

Analysis of total Se and micronutrient levels. Total Se and micronutrient contents in the samples were determined in a PerkinElmer Analyst 800 atomic absorption spectrophotometer (PerkinElmer Inc., San Jose, USA) with electrothermal atomization by (pyrolytic) graphite furnace essentially as described previously (Ramos et al. 2010). Briefly, dried tissues (approximately 500 mg) were weighed and acid digested in 10 mL HNO_3 in Teflon PTFE flasks (Corporation, Matthews, USA) and submitted to 0.76 MPa for 10 min in a microwave oven (CEM, model Mars 5 CEM Corporation, Matthews, USA). After cooling to room temperature, the extract was filtered (Whatman No. 40 filter) and diluted by adding 5 mL of bi-distilled water. Certified reference material (tomato leaves, NIST 1573a, National Institute of Standards and Technology (NIST), Gaithersburg, USA) were included for quality control. Blank and certified reference samples were analyzed along with every batch of digestion.

RESULTS AND DISCUSSION

To evaluate the effect of Se on *B. brizantha* growth, the plants were grown in soil without or with different rates of Na_2SeO_4 or Na_2SeO_3 and their biomasses were measured. Depending on the Se form applied in the soil, different responses in terms of root and shoot growth were observed ($P < 0.05$, Figure 1). Selenate caused a significant

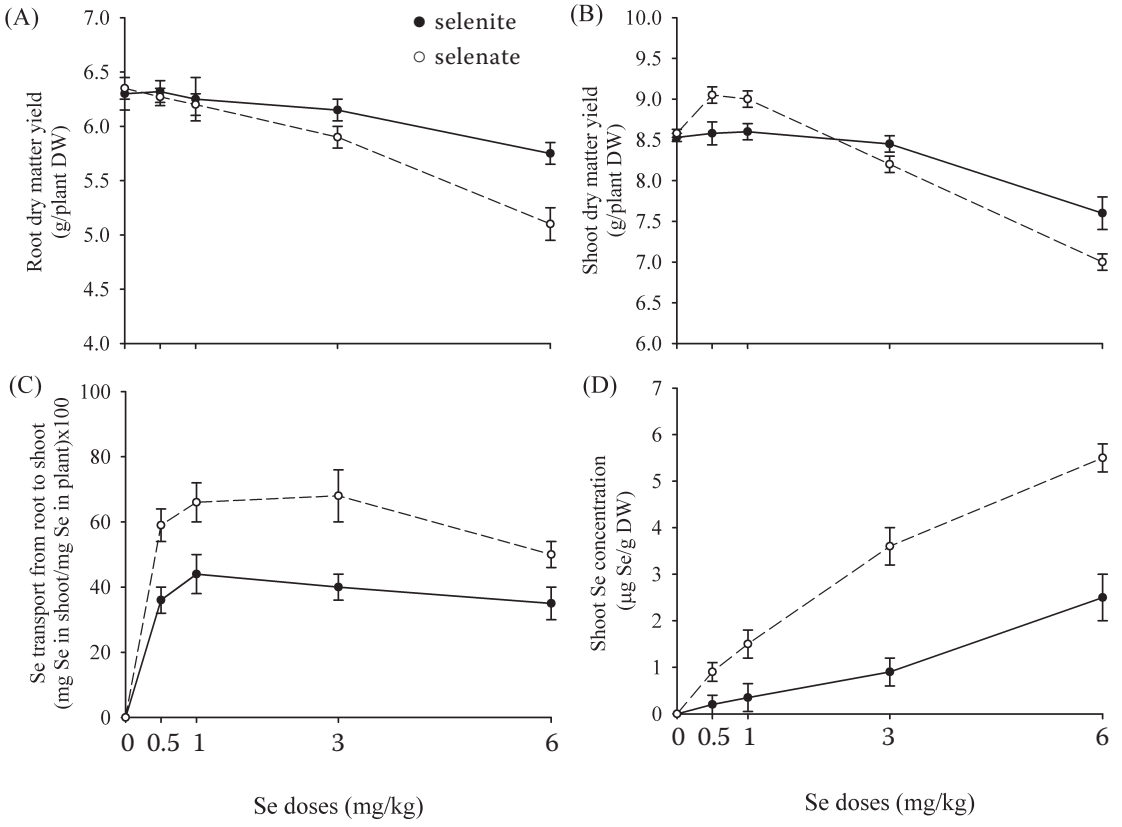

Figure 1. Root (A) and shoot (B) yield, Se transport (C) and shoot Se concentration (D) in *Brachiaria brizantha* treated with doses and forms of Se

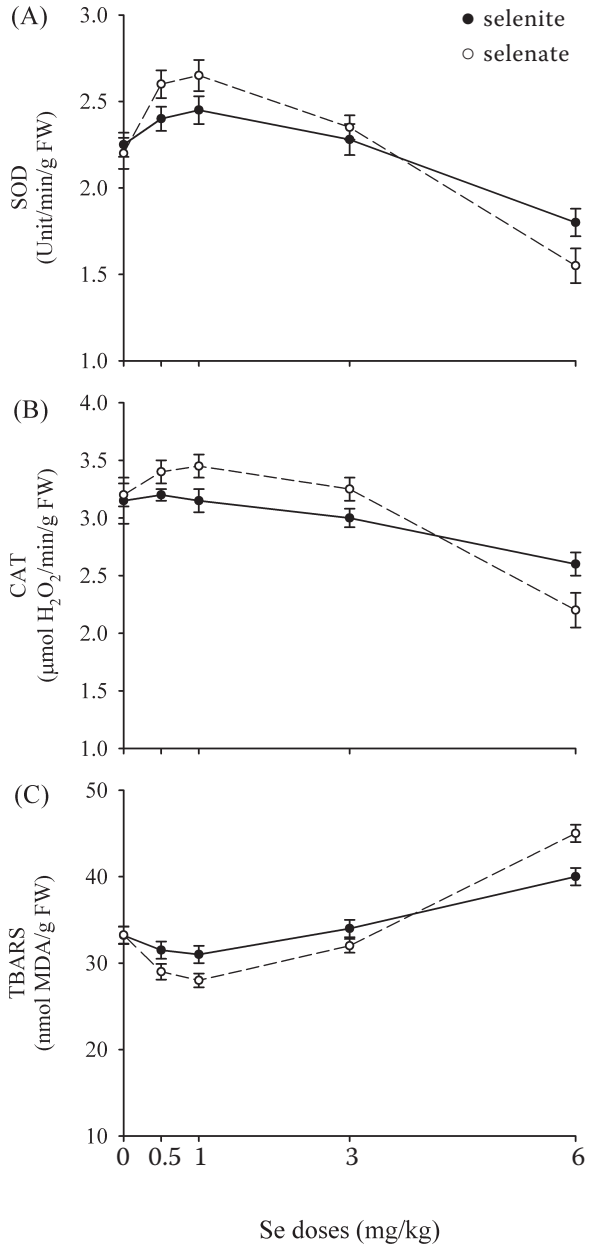

Figure 2. Activity of superoxide dismutase (SOD) (A), catalase (CAT) (B) and lipid peroxidation (TBARS) (C) in *Brachiaria brizantha* treated with doses and forms of Se; MDA – malondialdehyde

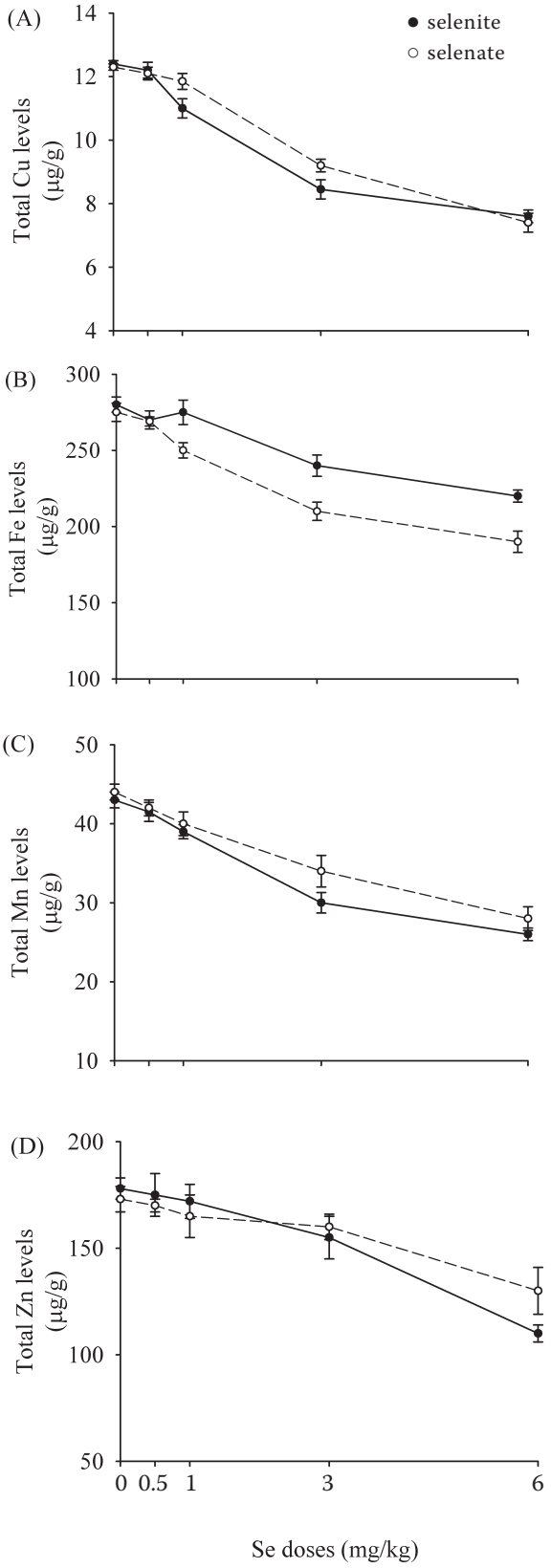

Figure 3. Total levels of Cu (A), Fe (B), Mn (C) and Zn (D) in leaves of *Brachiaria brizantha* treated with doses and forms of Se

increase in shoot biomass yield at lower rates and decreased both root and shoot biomass yield at the highest levels (Figure 1B), while for selenite the reduction in shoot yield followed an almost linear trend from the dose of 3 mg/kg (Figure 1A). At the highest Se level supplied (6 mg/kg) there was observed a reduction in shoot yield of 9 and 15%, when Se was supplied as selenite and selenate, respectively (7 and 14% for root yield). Our results are in agreement with Fargašová (2003) who observed an inhibitory effect of Se on the mustard growth. The diverse effect of selenate and selenite on the growth of brachiaria plants could be caused by distinct mechanisms of metabolism of different Se forms (Sors et al. 2005).

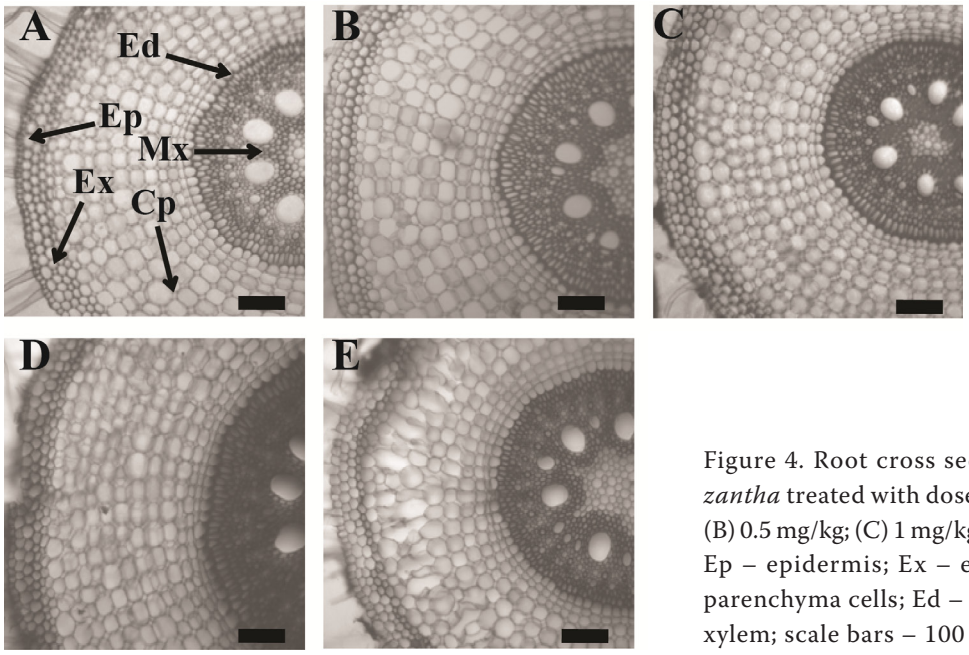

Figure 4. Root cross sections of *Brachiaria brizantha* treated with doses of selenate (A) 0 mg/kg; (B) 0.5 mg/kg; (C) 1 mg/kg; (D) 3 mg/kg; (E) 6 mg/kg. Ep – epidermis; Ex – exodermis; Cp – cortical parenchyma cells; Ed – endodermis; Mx – metaxylem; scale bars – 100 μm

The increase in shoot yield at lower rates of selenate may also result in the protective effect of Se by increasing SOD and CAT activities and reduction of lipid peroxidation, mainly when applied in lower rates of Se (Figures 2A–C). In addition, the activities of these enzymes varied depending on Se forms, but both enzymes showed increased activities in the presence of selenate at lower rates (0.50 and 1.0 mg/kg of Se) (Figures 2A–B). We also found smaller lipid peroxidation under low doses of selenate, and significant increases in lipid peroxidation of *B. brizantha* plants exposed to high Se rates application (Figure 2C). The role of Se as an antioxidant in ryegrass suggests that Se addition to the soil may improve the forage quality, by diminishing senescence and improving persistence of the Se-deficient pastures (Cartes et al. 2005).

In the present study, despite the utilization of a soil of medium texture (23% clay) and low levels of Fe and Al in sulfuric acid digestion (Table 1), the total Se levels in shoots of *B. brizantha* supplied with selenite were remarkably lower than that with selenate (Figure 1D). According to Barrow et al. (2005), differences between selenate and selenite in soil are expected because of the differences in affinity of these compounds with minerals in soil, which consequently affect solubility and availability to plants. For Brazilian Se fortification

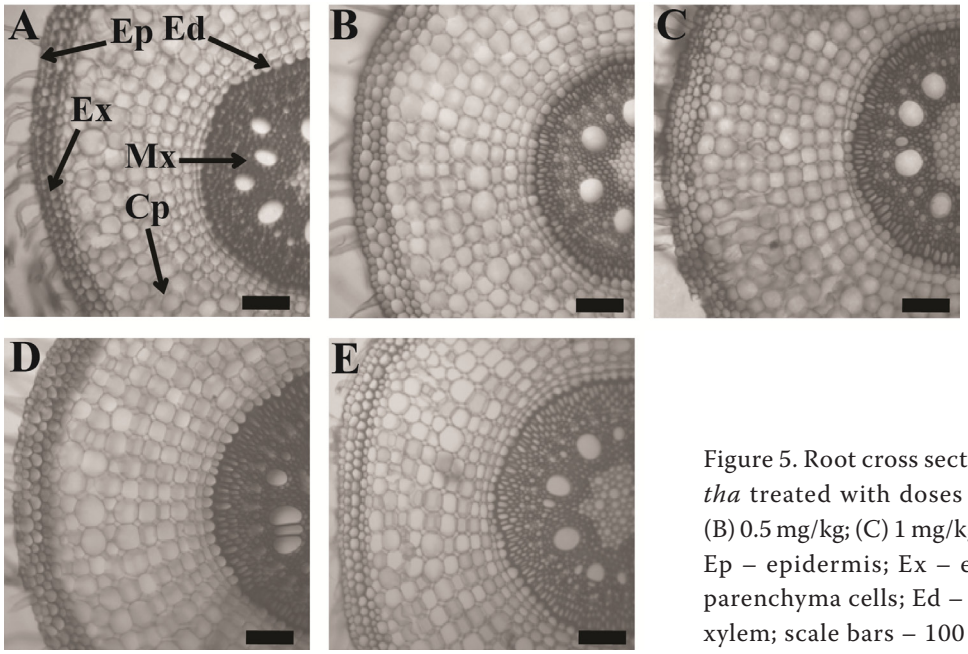

Figure 5. Root cross sections of *Brachiaria brizantha* treated with doses of selenite. (A) 0 mg/kg; (B) 0.5 mg/kg; (C) 1 mg/kg; (D) 3 mg/kg; (E) 6 mg/kg; Ep – epidermis; Ex – exodermis; Cp – cortical parenchyma cells; Ed – endodermis; Mx – metaxylem; scale bars – 100 μm

programs, low selenate doses may be the choice since selenite can be specifically absorbed with oxides of Fe and Al, which are present in large quantities in weathered tropical soils in Brazil (Rovira et al. 2008).

Former studies indicate that selenate and selenite provide distinct responses in Se translocation (Ramos et al. 2010). In this study, lower Se treatments (0.5 and 1.0 mg/kg), around 65% of the element applied as selenate and 40% as selenite was found in leaves of *B. brizantha* (Figure 1C). These results fairly agree with those obtained in former studies (Cartes et al. 2005), which reported that selenate is superior to selenite in translocation by higher plants. This happens when selenite is taken up by roots, as it is rapidly assimilated into organic forms that are not highly mobile in the xylem, while selenate is not easily converted to organic forms in roots and is therefore readily transported to leaves (Li et al. 2008).

The effects of sources and Se application rates on micronutrients accumulation in *B. brizantha* leaves are shown in Figure 3. The application of more than 1 mg/kg selenite or selenate form decreased dramatically the micronutrients content in brachiaria leaves. One of the effects of the extensive absorption of Se by higher plants is the uptake disturbance of the indispensable micronutrients in plants. The levels of Cu^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+} is reported to be inhibited by an increasing level of Se treatment in several plants (Pazurkiewicz-Kocot et al. 2003, Ramos et al. 2011).

Histochemical assay showed modifications in roots of *B. brizantha* plants due to rates and sources of Se applied (Figures 4 and 5). Both forms of Se over than 1 mg/kg increased apoplastic barriers, with an increase in exoderm and endoderm, however, this increase was non-significant. Cortical parenchyma cells did not change with selenite rates supply (Figure 5), however, in high levels of selenate, aerenchyma development in root of *B. brizantha* plants was observed (Figure 4E). An increase in apoplastic barriers could be associated with blockage of contaminant flow from the soil solution to vascular cylinder (Pereira et al. 2008).

Our study showed that aerenchyma was observed only at 6 mg/kg of selenate (Figure 4E). Aerenchyma is an adaptation that allows storage and distribution of gases along the root plants, aiding in respiration and ATP production. Plants that received selenate can develop aerenchyma in cortical tissues and this adaptation could be related to development of a volatilization system for Se excess (Azaizeh et al. 2003).

The results of the present study suggest that, in tropical soil conditions, the application of selenate is more appropriate for Se increase levels in *B. brizantha* than selenite, since in lower rates, selenate application showed greater translocation of Se to shoot, and therefore higher levels of shoot Se in this plant part. Moreover, at lower rates of selenate there was better activation of the antioxidant system and reduced lipid peroxidation in brachiaria plants. Histochemical analysis showed the formation of aerenchyma in internal structures of roots at high levels of selenate supply.

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Corresponding author:

Dr. Sílvio Júnio Ramos, Vale Technological Institute – Mining, Belo Horizonte-MG, 30112-010, Brazil
phone: + 55 35 9869 2909, phone/fax: + 55 31 3254 9790, e-mail: silvio.ramos@vale.com