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Original Article

Biochemical Responses of Two Species of *Eucalyptus* Exposed to Aluminium Toxicity: Oxidative Stress and Antioxidant Metabolism

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Abstract

In response to oxidative damage resulting from overproduction of reactive oxygen species, plants have developed complex and efficient antioxidant machinery. The aims of this research were to measure composts used as stress indicators, quantifying non-enzymatic compounds and activities of antioxidant enzymes, and to explain probable differences between two species of the gender *Eucalyptus* exposed to low and high aluminium. The experiment employed a factorial that was entirely randomised, with two species (*Eucalyptus platyphylla* and *E. grandis*) combined with aluminium concentrations (and 0.08 and 1.60 mM Al, which are described as low and high Al, respectively). This study revealed that the *E. platyphylla* presented intense modifications on malondialdehyde and electrolyte leakage in leaf and root, being also detected increases to oxidized glutathione, reduced glutathione and total glutathione. In addition, *E. platyphylla* had strong accumulations linked to superoxide and hydrogen peroxide, while *E. grandis* were detected minor alterations to both tissues. In relation to superoxide dismutase, catalase, ascorbate peroxidase and peroxidase were showed similar behaviours, with higher activities in *E. grandis*, if compared to *E. platyphylla*. Therefore, is possible to conclude that *E. grandis* is more tolerant to aluminium due to minor production of reactive oxygen species and decreased alterations on stress indicators. Concomitantly, the antioxidant enzymes effectively contribute to reduce the oxidative stress generated in root and leaf of *E. grandis* exposed to high aluminium.

Keywords: antioxidant enzymes, catalase, element toxic, hydrogen peroxide, reactive oxygen species, tolerance mechanism

Introduction

One of the limiting factors in the growth and development of plants is the soil acidity associated with aluminium (Al) toxicity (Haynes and Mokolobate, 2001). Al is found in trivalent form in acid soils and is soluble and toxic to plants, corroborating the idea that the solubility of this element is highly dependent on the pH (Hoekenga *et al.*, 2003). Under acidic soil conditions at pH < 5.5, the H^+ acts by releasing Al_3^+ ions from minerals that were previously attracted by the negative charges of the clay particles (Delhaize and Ryan 1995; Leite *et al.*, 2011). In other words, the decreased soil pH induces an increase in the Al activity in soil and consequently increases Al solubility, with negative effects linked to oxidative stress in plants (Nolla and Anghinoni, 2004).

Reactive oxygen species (ROS), including superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-), are highly reactive biomolecules and toxic to plants (Blokhina *et al.*, 2003; Edreva, 2005; Barbosa *et al.*, 2014). They are produced in balanced concentrations within the cellular compartments, such as chloroplasts, mitochondria, peroxisomes and apoplast (Moller, 2001; Asada, 2006; Sharma *et al.*, 2012), as a product of the photosynthesis and respiration processes (Asada, 1999; Pitzschke *et al.*, 2006; Miller *et al.*, 2010). The Al toxicity often induces overproduction and accumulation of ROS (Yamamoto *et al.*, 2003; Chen *et al.*, 2010; Li *et al.*, 2011). Excessive ROS generation within cells causes severe oxidative damage, such as lipid peroxidation of the cell membrane, denaturation of proteins and structural damage of DNA (Scandalios, 1993; Mittler, 2002; Meriga, 2004; Achary *et al.*, 2008).

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In addition, the ROS compromise also the functioning of the cell organelles and inhibit respiration and cell growth (Yamamoto *et al*., 2002; Qin *et al*., 2010).

In response to oxidative damage resulting from overproduction of ROS, plants have developed complex and efficient antioxidant machinery (Giannakoula *et al*., 2010; Pereira *et al*., 2014), which is composed of non-enzymatic and enzymatic factors, both used for the elimination of ROS (Mukhopadyay *et al*., 2012; Pereira *et al*., 2015). The ascorbic acid (AsA) and glutathione (GSH) compounds are considered the major non-enzymatic, and are fundamental during oxidative stress (Gill and Tuteja, 2010; Wang *et al*., 2011). Both are responsible to protect cells from oxidative damages and contribute to adequate detoxification process promoted by the antioxidant enzymes (Gratão *et al*., 2005; El-Beltagi and Mohamed, 2013).

In relation to antioxidant enzymes, there are the superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) (Coscolin *et al*., 2011). The SOD acts as the first enzyme of the antioxidant machinery, converting the excess O_2^- to H_2O_2 and O_2 (Alscher *et al*., 2002). Similarly, the enzymes CAT, APX and GPX dismutate the H_2O_2 excess, transforming it in H_2O and O_2 (Zhu *et al*., 2010). For this, the CAT performs the dismutation directly, while APX and GPX use AsA and GSH as substrates (Gratão *et al*., 2005; El-Beltagi and Mohamed, 2013).

Our hypothesis is that *Eucalyptus platyphylla* and *Eucalyptus grandis* must present different responses linked to tolerance mechanism under aluminium toxicity, verifying the ROS accumulation and a probable contribution of antioxidant enzymes. Based in this overview, the aims of this research were to measure composts used as stress indicators, quantifying non-enzymatic compounds and activities of antioxidant enzymes, and to explain probable differences between two species of the gender *Eucalyptus* exposed to low and high aluminium.

Materials and Methods

Location and growth conditions

This experiment was performed at the Campus of Paragominas of the Universidade Federal Rural da Amazônia, Paragominas, Brazil (2°55'S and 47°34'W). The study was conducted in a greenhouse without environmental controls, and the minimum, maximum, and median temperatures were 24 °C, 33 °C, and 27.2 °C, respectively. The relative humidity during the experimental period varied between 68% and 87%, and the photoperiod was set to 12 h of light. During the measurement period (12:00 h), the amount of photosynthetically active radiation varied between 572 and 1,564 μmol m^{-2} s^{-1}.

Plants, containers and acclimation

Sixty-day-old seedlings of *Eucalyptus platyphylla* and *Eucalyptus grandis* from DACKO presenting similar aspects and sizes were selected and placed in 1.2-L containers (0.15 m in height and 0.10 m in diameter) filled with substrate mix composed of sand and vermiculite in a 2:1 proportion. For semi-hydroponic cultivation, the previously described containers were equipped with one hole in the bottom and covered with mesh to keep the substrate, and solution absorption by capillarity, being misplaced into the other containers (0.15 m in height and 0.15 m in diameter) containing 500 mL of nutritive Hoagland and Arnon (1950) solution adjusted to the nutritional exigencies of this species. The ionic force started at 25%, and it was modified to 50 and 100% at regular intervals of three days. After these periods, the nutritive solution remained with the total ionic force. Subsequently, the 75-day-old young plants were submitted to low and high aluminium (Al) concentrations.

Experimental design

The experiment employed a factorial that was entirely randomised, with two species (*E. platyphylla* and *E. grandis*) combined with aluminium concentrations (and 0.08 and 1.60 mM Al, which are described as low and high Al, respectively). The experiment was assembled with five replicates for a total of 20 experimental units, with one plant in each unit.

Plant conduction and aluminium treatments

During plant conduction, one young plant was placed in each pot. The treatments received macronutrients and micronutrients from the nutritive solution containing 6 mM KNO_3, 5 mM $Ca(NO_3)_2{\cdot}4H_2O$, 2 mM $NH_4H_2PO_4$, 1 mM $MgSO_4{\cdot}7H_2O$, 62.50 μM KCl, 31.25 μM H_3BO_3, 2.50 μM $MnSO_4{\cdot}H_2O$, 2.50 μM $ZnSO_4{\cdot}7H_2O$, 0.63 μM $CuSO_4{\cdot}5H_2O$, 0.63 μM $NaMoO_4{\cdot}5H_2O$, and 200.0 μM $NaEDTAFe{\cdot}3H_2O$. To simulate low and high Al concentrations, $AlCl_3$ was used at concentrations of 0.08 and 1.60 mM Al, respectively. Two Al concentrations were applied to the young plants for 30 days. During the cultivation, the solutions were changed at 07:00 h over 5-day intervals and their pH values were adjusted to 5.5 and 4.2 to low and high Al concentrations, respectively, by using HCl or NaOH. All plants were physiologically and morphologically measured on the 30th day after the treatments, and the leaf tissue was harvested for nutritional and biochemical analysis.

Extracting and determining of Al in leaves

Samples containing 100 mg of dry leaf matter were pre-digested with 2 mL of concentrated HNO_3 (sub-boiling) for 48 h in 50 mL conic tubes (BD, model Falcon). Afterwards, 4 mL of H_2O_2 (30% v/v, Fluka) + 4 mL of ultra-pure water (Milli-Q) were added and the mixture was transferred to a PFA digestion vessel and digested in a microwave system (Milestone, model ETHOS 1600) according to the following heating regimen: i) 100 °C during 20 min; ii) 150 °C during 20 min; iii) 230 °C for 10 min; and iv) left to cool. The volume was then filled up to 50 mL with ultra-pure water and rhodium was added as an internal standard (10 μg L^{-1}). The analyses were performed by using an inductively coupled plasma mass spectrometer (ICP-MS Perkin Elmer, model ELAN DRC II).

Electrolyte leakage

Electrolyte leakage was measured according to the method described by Gong *et al*. (1998) with minor modifications. Fresh leaves and roots (200 mg) were cut into pieces with a length of 1 cm and were placed in containers containing 8 mL of distilled deionised water. The containers were incubated in a water bath at 40 °C for 30 min, and the initial electrical conductivity of the medium (EC_1) was measured. The samples were boiled at 95 °C for 20 min to release the electrolytes. After the samples were cooled, the final electrical conductivity (EC_2) was measured (Gong *et al*., 1998). The percentage of electrolyte leakage was calculated using the formula EL (%) = (EC_1 / EC_2) x 100.

Extraction of non-enzymatic compounds

Non-enzymatic compounds (H_2O_2, MDA, GSSG and total GSH) were extracted as described by Wu *et al.* (2006). Briefly, an extraction mixture was prepared by homogenising 500 mg of fresh leaf matter in 5 mL of 5% (w/v) trichloroacetic acid. Subsequently, the samples were centrifuged at 15,000 x g for 15 min at 3°C, and the supernatant was collected.

Hydrogen peroxide determination

For H_2O_2 detection, 200 μL of supernatant and 1800 μL of reaction mixture (2.5 mM potassium phosphate buffer [pH 7.0] and 500 mM potassium iodide) were mixed, and the absorbance was measured at 390 nm (Velikova *et al.*, 2000).

Malondialdehyde quantification

MDA was determined by mixing 500 μL of supernatant with 1,000 μL of the reaction mixture, which contained 0.5% (w/v) thiobarbituric acid in 20% trichloroacetic acid. The mixture was incubated in boiling water at 95 °C for 20 min, and the reaction was terminated by placing the reaction container in an ice bath. The samples were centrifuged at 10,000 × *g* for 10 min, and the absorbance was measured at 532 nm. The amount of non-specific absorption at 600 nm was subtracted from the absorbance data. The amount of MDA–TBA complex (red pigment) was calculated based on the method of Cakmak and Horst (1991) with minor modifications, using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Content glutathione estimations

For total GSH detection, 200 μL of supernatant, 1,800 μL of reaction mixture (containing 100 mM phosphate buffer [pH 7.6] and 0.60 mM 2-nitrobenzoic acid) were combined, and the absorbance was measured at 412 nm (Wu *et al.*, 2006).

To GSSG determination, 195 μL of neutralized extract was incubated with 2.6 μL of 2-vinylpyridine for 1 h at 25 °C. Subsequently, were added 0.25 mM NADPH, 0.42 mM DTNB [pH 7.6], and 1 unit of GR, being the absorbance was measured at 412 nm (Griffith, 1980). The concentration of reduced glutathione (GSH) was calculated as the difference between total GSH and GSSG.

Extraction of antioxidant enzymes and soluble proteins

Superoxide, soluble proteins and antioxidant enzymes (SOD, CAT, APX, and GPX) and were extracted from leaf and root tissues as per the method of Badawi *et al.* (2004). The extraction mixture was prepared by homogenizing 500 mg of fresh matter in 5 ml of extraction buffer, consisting of 50 mM phosphate buffer (pH 7.6), 1.0 mM ascorbate and 1.0 mM EDTA. Samples were centrifuged at 14,000 × *g* for 4 min at 3 °C, and the supernatant was collected. Quantification of the total soluble proteins was performed using the method described by Bradford (1976). The absorbance was measured at 595 nm, using bovine albumin as standard.

Superoxide determination

The O_2^- determination, 1 ml of extract was incubated with 30 mM phosphate buffer [pH 7.6] and 0.51 mM hydroxylamine hydrochloride for 20 min at 25 °C. Subsequently, were added 17 mM sulphanilamide and 7 mM α-naphthylamine to incubation mixture for 20 min at 25 °C. After reaction, ethyl ether in the same volume was added and centrifuged at 3,000 × *g* for 5 min. The absorbance was measured at 530 nm (Elstner and Heupel, 1976).

Superoxide dismutase assay

For SOD assay (EC 1.15.1.1), 2.8 ml of reaction mixture containing 50 mM phosphate buffer (pH 7.6),0.1 mM EDTA, 13 mM methionine (pH 7.6), 75 μM NBT, 4 μM riboflavin were mixed with 0.2 ml of supernatant. The absorbance was then measured at 560 nm (Giannopolitis and Ries, 1977).

Catalase assay

For CAT assay (EC 1.11.1.6), 0.2 ml of supernatant and 1.8 ml of reaction mixture containing 50 mM phosphate buffer (pH 7.0) and 12.5 mM hydrogen peroxide were mixed, and the absorbance was measured at 240 nm (Havir and McHale, 1987).

Ascorbate peroxidase assay

For APX assay (EC 1.11.1.11), 1.8 ml of reaction mixture containing 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM EDTA, and 1.0 mM hydrogen peroxide were mixed with 0.2 ml of supernatant, and the absorbance was measured at 290 nm (Nakano and Asada 1981).

Peroxidase assay

For GPX assay (EC 1.11.1.7), 1.78 ml of reaction mixture containing 50 mM phosphate buffer (pH 7.0) and 0.05% guaiacol was mixed with 0.2 ml of supernatant, followed by addition of 20 μL of 10 mM hydrogen peroxide. The absorbance was then measured at 470 nm (Cakmak and Marschner 1992).

Data analysis

The data were subjected to an analysis of variance, and significant differences between the means were determined by Scott-Knott test at a probability level of 5% (Steel *et al.*, 2006). Standard deviations were calculated for each treatment. The statistical analyses were performed with Assistat software.

Results

Al concentration in leaf and modifications linked to O_2^-, SOD and H_2O_2

Treating with high Al produced significant increases in the Al concentrations of the leaves in both species. The *E. platyphylla* and *E. grandis* exhibited increases of 104 and 29% (Table 1), respectively, when compared with plants of the same species under low Al.

The O_2^- levels in leaf and root showed significant increases of 88.2 and 275% in *E. platyphylla* under high Al (Fig. 1 A and B),

Table 1. Concentrations of Al in leaf (dry matter) of *E. platyphylla* and *E. grandis* exposed to low and high Al concentrations

Specie	Aluminium concentration	Aluminium concentration in leaf (μg g^{-1} DM)		
E. platyphylla	Low	8.9	±0.4	Ba
E. platyphylla	High	18.3	±0.7	Aa
E. grandis	Low	8.0	±0.3	Bb
E. grandis	High	10.4	±0.6	Ab

Columns with different uppercase letter into species (*E. platyphylla* and *E. grandis*) and lowercase letter into Al concentrations (low and high) indicate significant differences from the Scott-Knott test ($P < 0.05$). Values described corresponding to means from four repetitions and standard deviations.

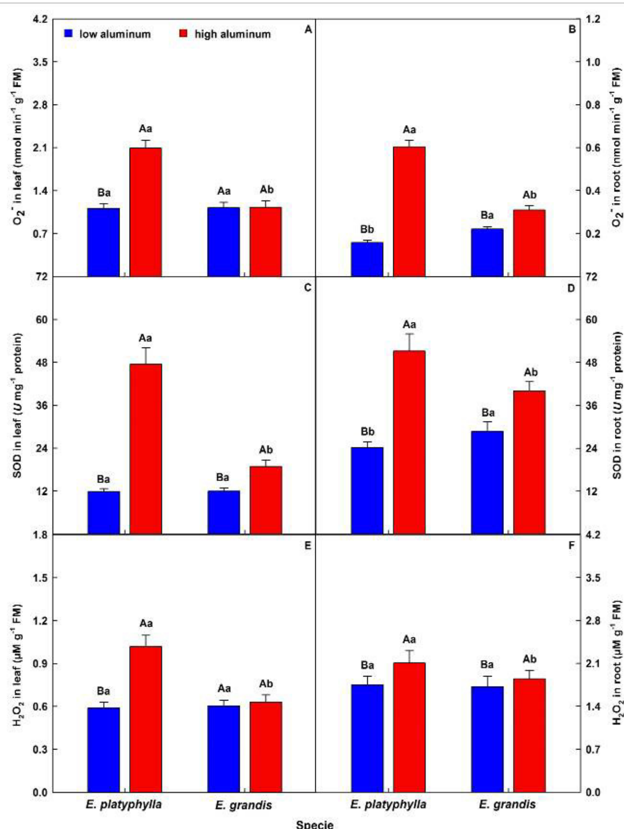

Fig. 1. Superoxide (A and B), superoxide dismutase (C and D) and hydrogen peroxide (E and F) in *E. platyphylla* and *E. grandis* exposed to low and high Al concentrations. Different uppercase letter into species (*E. platyphylla* and *E. grandis*) and lowercase letter to Al concentrations (low and high) indicate significant differences from the Scott-Knott test ($P < 0.05$). Columns represent the mean values from five repetitions, and bars represent the standard deviations

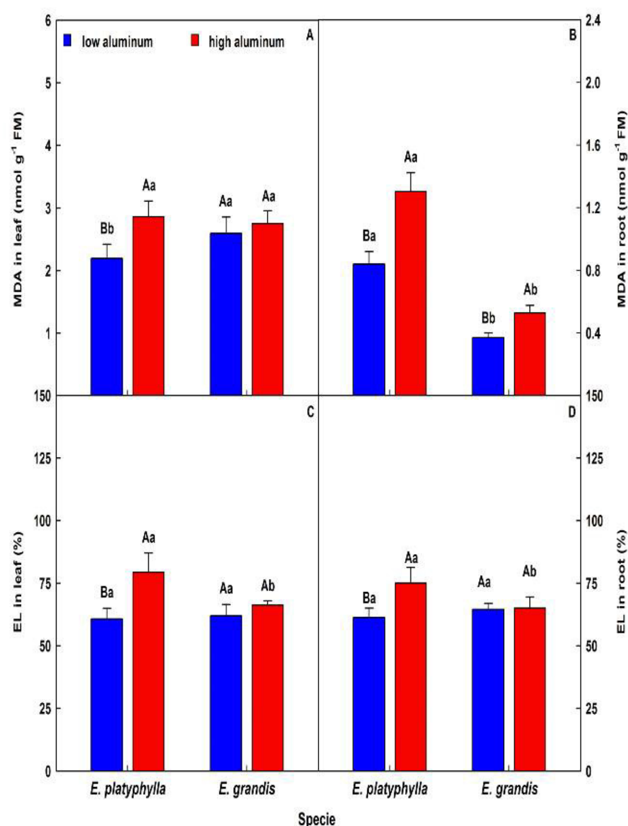

Fig. 2. Malondialdehyde (A and B) and electrolyte leakage (C and D) in *E. platyphylla* and *E. grandis* exposed to low and high Al concentrations. Different uppercase letter into species (*E. platyphylla* and *E. grandis*) and lowercase letter to Al concentrations (low and high) indicate significant differences from the Scott-Knott test ($P < 0.05$). Columns represent the mean values from five repetitions, and bars represent the standard deviations

when compared to specie under low Al concentration. The SOD activities showed significant increases in leaf and root of both species being observed in leaf increases of 299 and 57% for *E. platyphylla* and *E. grandis* (Fig. 1 C and D), compared to plants of the same specie with low Al. For the H_2O_2, significant increase was observed on leaf only in *E. platyphylla* (Fig. 1 E), while the root, increases were significant in both species being observed increases of 20.5 and 7.5% for *E. platyphylla* and *E. grandis*, respectively (Fig. 1 F), compared with the same plants exposed to low Al concentrations.

Cell damages in contrasting species after Al treatment

The MDA level in leaf of *E. platyphylla* presented a significant increase of 30.1%, while that *E. grandis* had only 5.2% (Fig. 2 A), if compared to the same specie with low Al concentration. In root, *E. platyphylla* and *E. grandis* had significant increases of 55.2 and 41.6% (Fig. 2 B), comparison made in the same specie with low Al application. To EL, the plants treated with high Al concentrations suffered significantly increased in leaf and root tissues only for the *E. platyphylla* (Fig. 2 C and D).

Interferences on GSSG, GSH and total GSH during Al toxicity

The GSSG values were increased in both tissues of *E. platyphylla* and *E. grandis* subjected to high Al level (Fig. 3 A and B). The increase in leaf of the *E. platyphylla* was 498.7%, while for the *E. grandis* was 122.5%, if compared the same specie cultivated with low Al level. In relation to GSH, only the *E. platyphylla* showed an increase in leaf and root, under high Al concentration (Fig. 3 C and D). The increases were 39.7 and 96.8% in the leaf and root, respectively, to *E. platyphylla*. For total GSH, the increase presented on leaf to *E. platyphylla* was 75%, while *E. grandis* reached around 14% (Fig. 3 E), when compared with plants grown under low Al. To the root, the increase for the *E. platyphylla* was 162%, and the *E. grandis* arrived at 45.5% (Fig. 3 F).

Contribution of the antioxidant enzymes to attenuate the Al stress

Plants under high Al application showed a significant increase in CAT activity, both in the leaves and in the roots, within two species. In *E. grandis* increases were 86.5 and 168% in the leaf and root, respectively (Fig. 4 A and B). In APX activity were obtained increases to root of 242.1% and 580.4% in *E. platyphylla* and *E. grandis* (Fig. 4 D), respectively, when compared with plants of the same specie that received low Al concentration. Plants under application high Al showed a significant increase in GPX activity, than leaf that to root, in both species (Fig. 4 E and F).

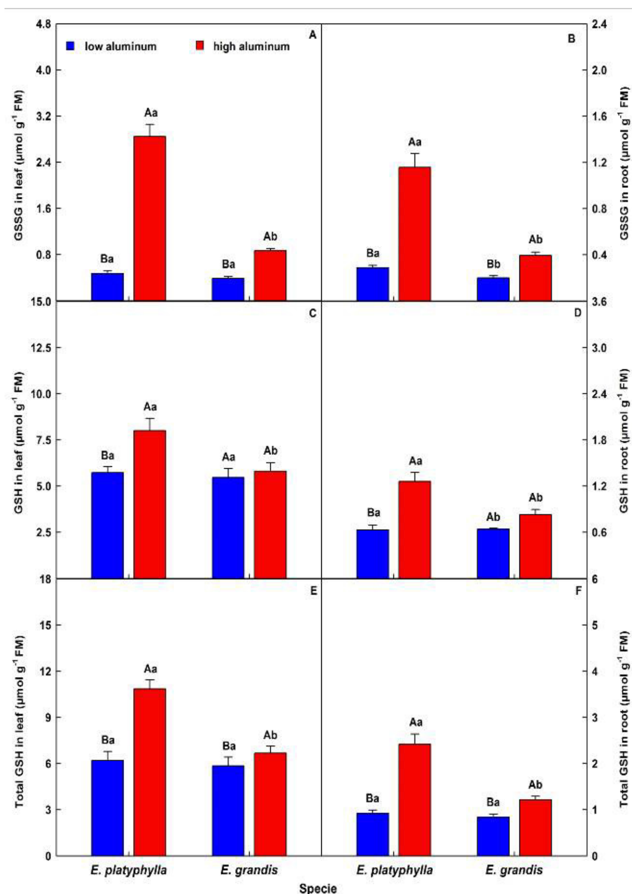

Fig. 3. Oxidized glutathione (A and B), reduced glutathione (C and D) and total glutathione (E and F) in *E. platyphylla* and *E. grandis* exposed to low and high Al concentrations. Different uppercase letter into species (*E. platyphylla* and *E. grandis*) and lowercase letter to Al concentrations (low and high) indicate significant differences from the Scott-Knott test ($P < 0.05$). Columns represent the mean values from five repetitions, and bars represent the standard deviations.

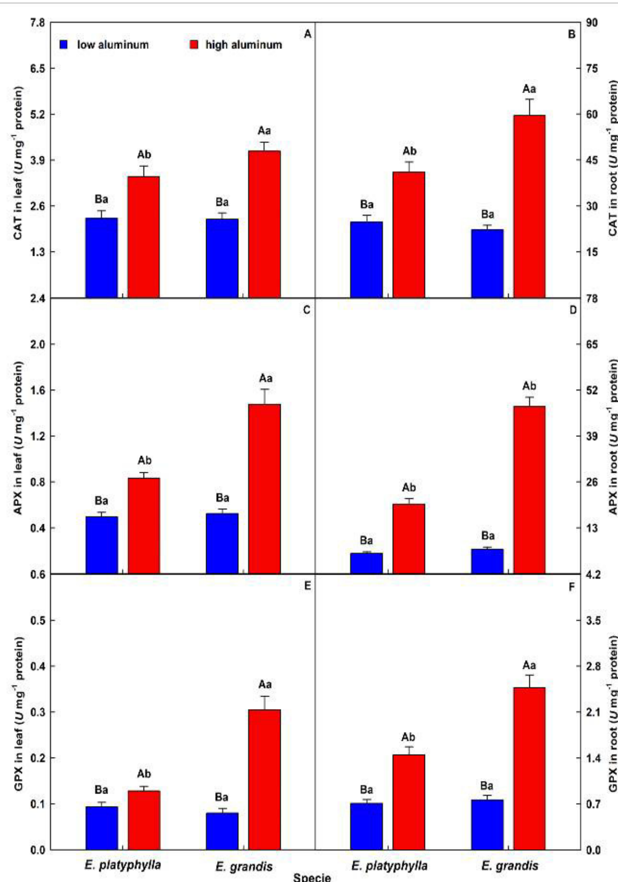

Fig. 4. Activities of catalase (A and B), ascorbate peroxidase (C and D), and peroxidase (E and F) in *E. platyphylla* and *E. grandis* exposed to low and high Al concentrations. Different uppercase letter into species (*E. platyphylla* and *E. grandis*) and lowercase letter to Al concentrations (low and high) indicate significant differences from the Scott-Knott test ($P < 0.05$). Columns represent the mean values from five repetitions, and bars represent the standard deviations.

Discussion

The increased Al contents in plants treated with high Al concentrations demonstrate that this element was absorbed and transported to leaves. Additionally, *E. grandis* presented a slight increase. Similar results in terms of minor Al accumulation in tolerant plants were verified by Noya *et al.* (2014).

Superoxide (O_2^-) levels suffered increase in species exposed Al, being it is a result of oxidative stress induced by the toxicity of this element, which promotes the reduction of O_2 to O_2^- and depending of the stress intensity may generate a subsequent generation of other ROS (Qin *et al.*, 2010). O_2^- is first ROS formed in cell, in this case the process was catalyzed by Al, initiating a cascade of reactions to generate ROS secondary, such as H_2O_2 (Sharma *et al.*, 2012). Wang and Yang (2005) also observed increase in O_2^- levels of *Cassia tora* plants under Al application, corroborating with results of this study.

Increase of the SOD activity in plants treated with Al indicates O_2^- overproduction, resulting from stress induced by the Al (Boscolo *et al.*, 2003). The SOD acts as the first line of defense of the plants against oxidative stress, catalyzing the conversion of O_2^- to H_2O_2 and O_2 (El-Beltagi and Mohamed, 2013). Nogueirol *et al.* (2015), working with plants of the *Solanum* genus exposed to the Al application, found similar results to activity of this enzyme.

H_2O_2 levels were increased in two species exposed Al, being related to the increase in SOD activity. SOD is an enzyme present in mitochondria and chloroplast membranes, the increase in your activity often results in overproduction of H_2O_2 (Schneider and Oliveira, 2004; Radic *et al*, 2010). Similar results on the increase in H_2O_2 levels were also reported by Yamamoto *et al.* (2015) evaluating *Solanum lycopersicum* plants exposed to Al.

The higher MDA values observed in two species exposed Al indicate increase in peroxidation of membrane lipids, being it explained by overproduction of reactive radicals, such as O_2^-, which compromises the structure cell due to the stress oxidative (Guo *et al.*, 2004). The O_2^-, as well as other ROS, cause damages

to membrane structures due to lipid peroxidation and increasing the MDA content, which is considered the final product resulting of the membrane peroxidation (Guo *et al*., 2004; Tabaldi *et al*., 2007; Wu *et al*., 2014). Reyes-Diaz *et al*. (2010) studying *Vaccinium corymbosum* plants sensitive and exposed to Al, reported similar results.

The increase in EL is related to oxidative damage caused by Al. In conditions of stress, the increase in the ROS amounts cause structural changes in the membrane, decreasing the ion exchange capacity and increasing ions release, resulting in the rupture of the membrane (Gill and Tuteja, 2010; Dawood *et al*., 2012). The EL is usually the last step observed due to the oxidative stress, also considered as a form to evaluate the state of permeability and membrane integrity and intensity of the cellular damages (Dawood *et al*., 2012; Langaro *et al*., 2014). Pereira *et al*. (2010) working with plant *Cucumis sativus* stressed with different Al concentrations described similar results.

The increase in GSSG is related to H_2O_2 accumulation in tissue caused by Al. During oxidative stress, the antioxidant defense mechanism, composed by enzymatic and non-enzymatic components, is driven aiming the detoxification process. One of the products formed in this process is the GSSG, considered an indicator of the physiological activity of intracellular defense against ROS, obtained from the GPX activity that uses H_2O_2 as substrate for the reaction (Apel and Hirt, 2004; Gill and Tuteja, 2010). Nguyen *et al*. (2005) working with *Eucalyptus camaldulensis* plants treated with Al reported similar results.

Increase in GSH levels in plants exposed to Al is related to increase of the activity of glutathione reductase (GR) (Sharma and Dubey, 2007). This enzyme plays a crucial role against oxidative stress, maintaining the GSH levels in adequate concentrations (Balakhinina and Borkowska, 2013). To produce GSH, the GR enzyme reduces GSSG due NADPH consumption (Foyer and Noctor, 2000). Similar results on increase of this antioxidant compound were found by Xu *et al*. (2012) in *Triticum aestivum* plants cultivated in presence of Al.

Total GSH was increased after Al treatment, and it is considered as the combined effect of the accumulation observed in GSH and GSSG levels, also called non-enzymatic components of the redox glutathione cycle. This increase is related to the intensification of redox reactions of this cycle on stress conditions, aiming to reduce the production and accumulation of ROS. These reactions are mediated by the GR and GPX enzymes, which are catalytic agents of the reactions in the glutathione cycle (Noctor *et al*., 2002; Gill and Tuteja, 2010). Studies conducted by Yang *et al*. (2007) reported increases in total GSH values to *Oryza sativa* plants under Al application.

The increase in CAT activity in plants submitted to Al is related to higher concentrations of H_2O_2. The CAT is one of the enzymes linked to antioxidant machinery responsible to catalyse the H_2O_2 to H_2O and O_2. This action prevents the diffusion and accumulation of H_2O_2 in other cellular compartments, and making that this group remains in balanced concentrations within cells (Scandalios, 2005). Aftab *et al*. (2010) studying *Artemisia annua* plants exposed Al found similar results related to increase in activity of this enzyme.

The activity of APX enzyme increased in plants under Al exposition, and this response is related to overproduction of ascorbate (AsA). The AsA is an of the mains antioxidant compounds, acting on protection plants against oxidative damage under stress conditions (El-Beltagi and Mohamed, 2013). During the dismutation reaction of the H_2O_2 until H_2O and O_2, the APX uses the AsA as substrate to oxidize the monodehydroascorbate (MDHA) in end of the process (Apel and Hirt, 2004). *Cucurbita pepo* plants treated with Al also showed higher APX activity (Dipierro *et al*., 2005).

The increase in the GPX activity in plants after high Al is related due to intensification of oxidative stress generated by the concentration of this toxic metal, that it increase the ROS levels inside the cells. GPX is one of the main enzymes involved in the homeostatic control of the levels of ROS in the plant metabolism, and an increase in the activity of this enzyme corroborates the hypothesis linked to an efficient antioxidant defense system (Singh *et al*., 2012; Ribeiro *et al*., 2012) in *E. grandis*. Similar results were found by Prakash and Kumar (2014) evaluating *Sorghun bicolor* plants under influence of Al, agreeing with our results.

Conclusion

This study revealed that the *E. platyphylla* presented intense modifications on malondialdehyde and electrolyte leakage in leaf and root, being also detected increases to oxidized glutathione, reduced glutathione and total glutathione, In addition, *E. platyphylla* had strong accumulations linked to superoxide and hydrogen peroxide, while *E. grandis* were detected minor alterations to both tissues. In relation to superoxide dismutase, catalase, ascorbate peroxidase and peroxidase were showed similar behaviours, with higher activities in *E. grandis*, if compared to *E. platyphylla*. Therefore, is possible to conclude that *E. grandis* is more tolerant to aluminium due to minor production of reactive oxygen species and decreased alterations on stress indicators. Concomitantly, the antioxidant enzymes effectively contribute to reduce the oxidative stress generated in root and leaf of *E. grandis* exposed to high aluminium.

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