



ECOSYSTEMS

Effects of Iron on oxidative stress of *Cecropia hololeuca* and *Carica papaya* plants

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Abstract: Iron is essential to plant development. However, its excess can provoke an increase in reactive oxygen species and oxidative stress in plants. The objective of this work was to verify the effects of high concentrations of iron on the oxidative stress of seeds and young plants of *Cecropia hololeuca* and *Carica papaya*. The species were submitted to concentrations of 0.045, 4 and 8mM of iron in the form of ferrous sulfate and FeEDTA. The experiments of germination and initial growth took place in a growth chamber, with temperature of 25°C and 12h photoperiod. We performed the lipid peroxidation test by extraction and quantification of malonaldehyde and hydrogen peroxide. The application of iron did not cause a significant elevation in the contents of malonaldehyde and hydrogen peroxide in the germination of *C. hololeuca* and *C. papaya*. In the young plants, the hydrogen peroxide did not change in any of the treatments. However, it was possible to observe an expressive increase in malonaldehyde concentration in both species when exposed to FeEDTA 4 to 8mM. The results indicate a sensibility of *C. hololeuca* and *C. papaya* to high iron levels, amplifying the oxidative stress process that can harm their growth and initial development.

Key words: *Carica papaya*, *Cecropia hololeuca*, Malonaldehyde, Peroxide.

INTRODUCTION

The growth of mining activity and processing of Iron ore (Fe), associated with the neglect of the waste from these processes, is considered one of the major factors responsible for increasing Fe levels in the environment (C.O. Rios 2017, unpublished data). This metal presents non-biodegradable characteristics and, when found in its soluble form, is considered one of the primary contaminants of waterways (Punshon et al. 2003, Bai et al. 2009). This situation can generate numerous adverse effects to the aquatic ecosystem because Fe can be readily usable by plants and is toxic when absorbed in excess. Fe toxicity is closely related to damage in several plant communities. The possible damage to the growth and fruiting of cultivated species

and the establishment of riparian vegetation has been gaining increasing interest (Zhang et al. 2010, Barbosa et al. 2014).

Riparian forests stand out as one of the essential maintainers of environmental balance. They contribute to a lower rate of erosion and leaching, greater control of water flow to the soil, and maintenance of the quality of water that reaches water bodies (Castro et al. 2013). However, these forests can also retain a wide range of pollutants, especially Fe, which, due to its persistence in the medium, can accumulate in plant species and increase the environment's vulnerability (Torres et al. 2014). The species *Cecropia hololeuca* Miq. (Cecropiaceae), known as embaúba-prateada, is widely distributed in Brazilian territory and occurs mainly in

riparian forests (Vicentini et al. 2008). Such fact can represent a risk to its development since it can absorb high concentrations of Fe and suffer from harmful effects to the growth and establishment (Sahrawat 2005, Saaltink et al. 2017). This situation was already observed for this species when exposure to elevated amounts of Fe, resulting in damage to the germination process, photosynthetic apparatus and severe growth retardation (Rodrigues Filho et al. 2020b).

Species *Carica papaya* L. (Caricaceae) is an important fruit species of high occurrence in the southeastern Brazilian region, especially in Espírito Santo. It represents one of the economic pillars of the state, with approximately 60 tons of fruit sold annually to the rest of the country and abroad (Ibge 2019). *C. papaya* crops are near regions with high levels of Fe-rich mining waste, so its productivity can be deeply affected by the elevation in the metal levels (C.O. Rios 2017, unpublished data). This crop, as well as other cultivated species, have a long history of harm when looking at contact with toxic amounts of Fe, as examined in plants of Lettuce, Soy, Coffee and Rice (Bataglia & Mascarenhas 1981, Krohling et al. 2016, Laurett et al. 2017, Rodrigues Filho et al. 2020a).

Fe is an essential micronutrient in plant development, acting at low concentrations. However, its excess is related to losses in germination and growth because it causes an increase in the content of reactive oxygen species (ROS) and the oxidative process (Becker & Asch 2005, Kobayashi & Nishizawa 2012). Among the main ROS originating by Fe contents' elevation, hydrogen peroxide (H_2O_2) stands out as one of the most stressful compounds due to its high production and oxidative capacity (Jucosky et al. 2013). Oxidative damage can occur in various parts of plant metabolism, but the occurrence of lipid peroxidation in the cell membrane, estimated based on the

production of malonaldehyde (MDA), the final product of lipid peroxidation, is considered one of the most reliable variables that indicate the intensification of oxidative stress caused by high Fe levels and the beginning of the damage to the plant development (Jucosky et al. 2013, Pinto et al. 2016).

Thus, this work aimed to investigate the possible oxidative effects of high concentrations of Fe on seeds and young plants of *C. hololeuca* and *C. papaya*, that can compromise its development.

MATERIALS AND METHODS

Storage and the germination of the seeds

C. hololeuca and *C. papaya* seeds were stored in the Seeds and Forest Ecophysiology Laboratory of the Federal University of Espírito Santo in Vitória-ES in a cold chamber (5°C) until the beginning of the experiment. For the analysis of possible oxidative effects on germination, seeds of both species were previously disinfected with 2% sodium hypochlorite for 2 minutes and placed in Petri plates lined with two sheets of filter paper and moistened with Fe solutions. It was used concentrations of 0.045 (control), 4mM and 8mM applied as ferrous sulfate and FeEDTA. We set up the experiment in a B.O.D. germination chamber under a constant temperature of 25 °C and photoperiod 12h/12h for ten days (Ferreira & Borguetti 2005).

Production of the young plants

Seeds of both species were sown in 0.5L polyethylene pots with unfertilized substrate and washed sand in a 1:1 ratio to verify the oxidative stress on young plants. We set up ten pots with one plant each, with ten experimental units per treatment. Twenty days after germination, we subjected seedlings to the respective treatments using concentrations of 0.045 (control), 4mM and

8mM applied as ferrous sulfate and FeEDTA via soil. The cultivation took place in a growth room at 25° C, photoperiod 12h/12h, for fifty days. We kept all plants in Hoagland solution at half ionic strength and pH 5.0 (Hoagland & Arnon 1950).

The oxidative test through the quantification of H₂O₂ and MDA

It was verified the oxidation degree and cell damage through H₂O₂ and MDA contents for the seeds and young plants (Cakmak & Horst 1991). At the end of each experiment, we macerated 300 mg of seeds and young plant tissue samples using liquid nitrogen in 2.0 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 xg, 4° C for 15 min, collecting the supernatant. 1mL aliquots of the supernatant were used added to 1mL of 0.5% (w/v) thiobarbituric acid (TBA) solution prepared in 20% (w/v) TCA for MDA content. We incubated samples at 90 °C for 35 minutes and then rapidly cooled them on ice. We took the readings at 532 nm and 600 nm. The MDA content was calculated from the extinction coefficient of 155 mM cm⁻¹ applying the formula: MDA content (ηM) = [(A₅₃₂-A₆₀₀)/1.56] x 105 (Buege & Aust 1978). We based H₂O₂ content on the method of Alexieva et al. (2001). The reaction medium consisted of 0.5 mL of the supernatant added to 0.5 mL of 100 mM potassium phosphate buffer (pH 7.0) and 2 mL of 1 M potassium iodide (KI). We carried out the reaction in the dark for one hour, and the absorbance reading was at 390 nm. We calculated the H₂O₂ concentration using a standard curve.

Statistical analyses

We performed both experiments in an entirely randomized design. Results data were submitted to variance analysis (ANOVA) and later to Tukey's test at a 5% significance level. We did

all statistical analyses using the program Sisvar version 5.6 (Ferreira 2011).

RESULTS AND DISCUSSION

Fe stress on the germination

The results showed that exposure to the two Fe sources at concentrations of 4 and 8 mM did not cause an increase in MDA and H₂O₂ levels in the germination of *C. hololeuca* and *C. papaya* seeds (Figure 1).

High concentrations of heavy metals can profoundly affect seed germination, as already demonstrated in several works that verified the interaction of these compounds and the germination process (Akinci & Akinci 2010, Nasr 2013, Wan et al. 2013, Prodanovic et al. 2016). Other authors have also found deleterious effects when using high concentrations of Fe in wheat, bean, and Vigna genus species (El Rasafi et al. 2016, Verma & Pandey 2017). More specifically, in the germination of *C. hololeuca* and *C. papaya*, it was possible to observe a significant drop in the germination percentage of these two species when exposed to Fe (Rodrigues Filho et al. 2020a, b).

The multiple adverse effects caused by Fe can be attributed to biochemical disorders and decreased uptake of water and various ions in the soaking phase (Kobayashi & Nishizawa 2012). Another mechanism by which Fe can generate toxicity is by elevating H₂O₂ and other ROS levels (Becker & Asch 2005). In excess, these oxidizing compounds will accumulate in plant tissues and cause increased membrane lipid peroxidation and MDA contents (Siddiqui et al. 2011, Gall & Rajakaruna 2013, Seneviratne et al. 2019).

However, we did not verify this in the germination phase of *C. hololeuca* and *C. papaya* seeds since the concentrations of MDA and H₂O₂ did not show a significant difference between treatments, which seems not to indicate the

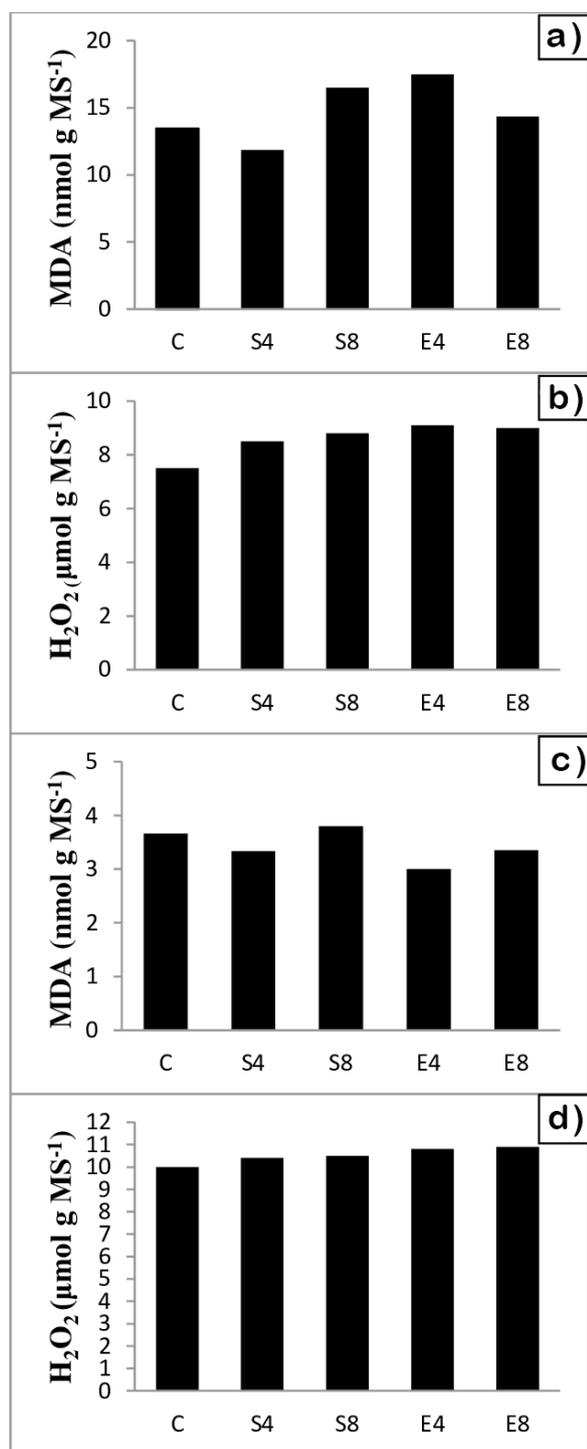


Figure 1. MDA and H₂O₂ contents in germination of *C. hololeuca* a) and b) and *C. papaya* c) and d) seeds submitted to high Fe contents. (Tukey test, $p < 0.05$). C: Control; S4: Ferrous sulfate 4mM; S8: Ferrous sulfate 8mM; E4: FeEDTA 4mM; E8: FeEDTA 8mM.

presence of oxidative stress in the germination of both species. This can be explained by the nature of the integumentary layer, which can act as a physical block to metal contamination by limiting its absorption. Another strategy is seen through the increase of the antioxidant activity of the plant, reducing the levels of peroxidation and consequently the MDA contents, a fact already confirmed in some species exposed to high concentrations of Copper, Cadmium, and Fe (E.G. Pereira 2006, unpublished data, Banu Doğanlar 2013). Other protection systems can compartmentalize Fe in the cell vacuole by complexation to peptides and binding proteins, which prevents its activity (Souza et al. 2011, Fryzova et al. 2017).

Fe stress on young plants

Despite not promoting oxidative stress during the germination phase, high doses of Fe caused a significant increase in the MDA content in both species when exposed to FeEDTA in the initial growth and compared to the control (Figure 2). The concentration of this metabolite reached, respectively, a 30% and 140% increase in *C. papaya* and *C. hololeuca* plants when using the 8mM EDTA treatment. There was no difference in H₂O₂ content at this stage.

It was verified the oxidative effects of Fe under young plants of *C. hololeuca* and *C. papaya* for both concentrations of FeEDTA used. In the chelated version, Fe presents high absorptive capacity, triggering more significant accumulation in root tissue, the primary organ of absorption, and the first part of coming into contact with the metal, as well as in the stem and leaves of plants (Zhao et al. 2010, Onaga et al. 2016). The absorbed Fe entailed higher ROS production in young plants, which caused the peroxidation of membrane lipids, a fact proven by the significant increase in MDA contents of plants treated with 4 and 8mM FeEDTA. The increase

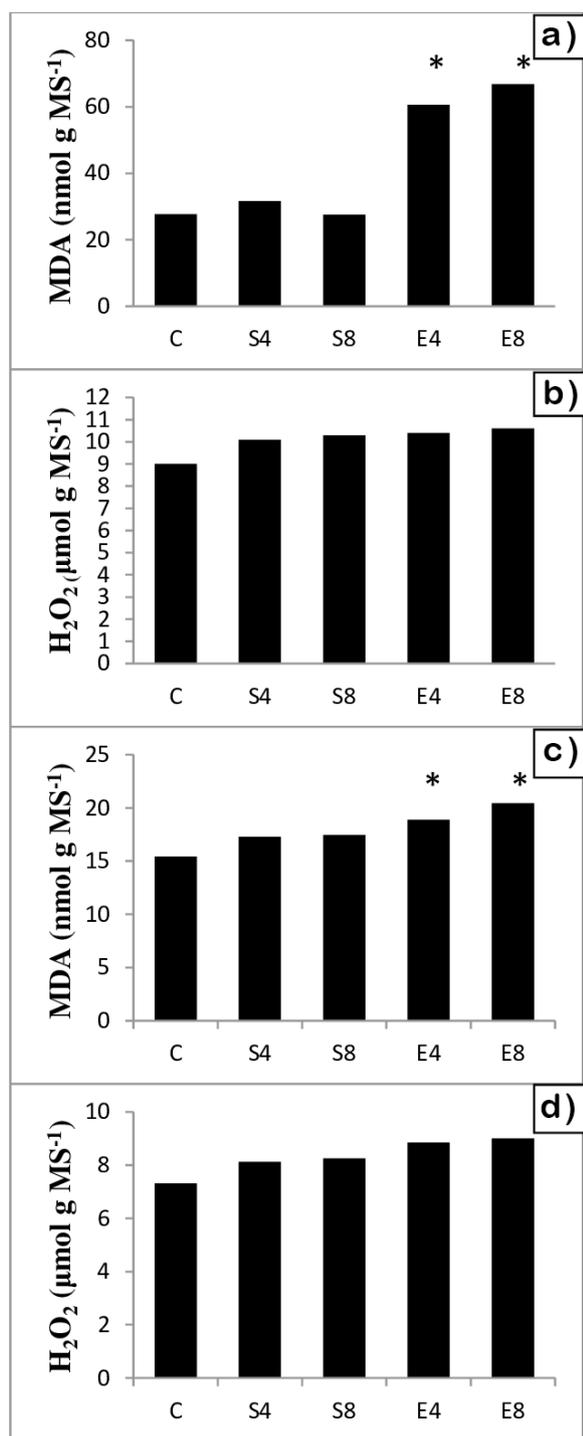


Figure 2. MDA and H₂O₂ contents in young plants of *C. hololeuca* a) and b) and *C. papaya* c) and d) submitted to high Fe contents. The asterisk indicates significant difference compared to control (Tukey's test, $p < 0.05$). C: Control; S4: Ferrous sulfate 4mM; S8: Ferrous sulfate 8mM; E4: FeEDTA 4mM; E8: FeEDTA 8mM.

in the lipid peroxidation process associated with Fe is well known, entails severe effects on growth, and has been demonstrated in different forest and cultivated species (Sinhá et al. 1997, Kuki et al. 2008, Stein et al. 2008, Jucosky et al. 2013). The cell membrane is considered the first and primary site of the attack by ROS generated by exposure to high Fe contents. This attack, in turn, triggers the oxidation of lipids, which stimulates the increment of malonic aldehyde (MDA) content, a byproduct of the breakdown of membrane fatty acids and a probative factor of the presence of excess free radicals (Wu et al. 1998, Pinto et al. 2016).

However, initially, we cannot attribute the increased lipid peroxidation observed in this study to elevated H₂O₂ production. H₂O₂ is considered one of the primary and most common oxidizing agents produced by plants when exposed to high Fe concentrations, with the ability to easily cross membranes and degrade lipids and enzymes through the oxidation of their thiol groups (Gadjev et al. 2008, Karuppanapandian et al. 2011, Jucosky et al. 2013). We did not observe elevation in the content of this metabolite in *C. hololeuca* and *C. papaya* species when subjected to the two Fe sources at concentrations of 4 and 8mM. Similar results were found for *Oryza sativa* L. plants exposed to concentrations of up to 10mM of Fe, with an elevation of MDA without alteration in the H₂O₂ content (Fang et al. 2001). This fact may be justified by the antioxidant defense mechanisms present in plants, capable of neutralizing the toxicity of the various ROS produced, especially H₂O₂. Catalase and Peroxidases (POX) act effectively in the detoxification process, converting H₂O₂ to H₂O and molecular oxygen. This reaction prevents the elevation in the concentration of H₂O₂ and consequently the oxidative damage caused by this compound, a fact already verified in

studies that evaluated the antioxidant capacity against high Fe contents (Jucosky et al. 2013). Moreover, when Rodrigues Filho et al. (2020a, b) worked with the species *C. hololeuca* and *C. papaya* subjected to Fe stress at concentrations of 8mM, they found an increase in POX enzyme activity when plants were exposed to FeEDTA, indicating a pathway of control of H₂O₂ levels and mitigation for this stress.

Also, the increase in MDA levels and lipid peroxidation in plants of both species exposed to FeEDTA seems to indicate other oxidizing compounds acting on these plants. Fe at high concentrations can also intensify the production of other ROS, such as the singlet oxygen (¹O₂) and the hydroxyl radical (HO[•]). Recent studies have shown that ¹O₂ can diffuse through all parts of the plant, and its action can primarily cause lipid peroxidation (Triantaphylidès & Havaux 2009, Barbosa et al. 2014). The hydroxyl radical (HO[•]) can be formed directly by the action of Fe, or through the Fenton reaction, in which the H₂O₂ accumulated in tissues reacts with Fe giving rise to new HO[•] molecules (Nikolic & Pavlovic 2018), a fact that can also explain the stability of H₂O₂ contents in plants. HO[•], in turn, is considered one of the most oxidizing ROS. Its excess causes changes in proteins through the oxidation of sulfhydryl groups, in nitrogenous bases, and damage to carbohydrate and fatty acid molecules, that can finally lead to lipid peroxidation (Barbosa et al. 2014, Pinto et al. 2016).

CONCLUSIONS

Seeds of *C. hololeuca* and *C. papaya* show no evidence of oxidative stress when exposed to 4 and 8mM concentrations of Fe during the germination period. However, in its EDTA form, Fe can generate oxidative damage in young plants of both species, promoting lipid peroxidation

and consequently damage to metabolism and growth. Among the compounds responsible for this process, we discarded H₂O₂ and possibly the singlet oxygen and the hydroxyl radical stand out. Thus, high concentrations of Fe in the environment may be a limiting factor for riparian and cultivated species establishment.

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JRF, VBC and ITALP conceived and designed the study. JRF, RHW and CRS performed the analyses. JRF, VBC and JFNF interpreted the results. JRF, VBC, ITALP and JFNF participate in the writing of the manuscript. Moreover, JRF proceeded with the corrections and all of the other authors read and approved the final paper.

