




# Seed Priming of *Handroanthus heptaphyllus* for the Restoration of the Mining Fields

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**Abstract** Seed priming is a low-cost pre-sowing treatment used to improve germination and seedling establishment performance under adverse environmental conditions. The priming using signaling molecules, such as nitric oxide and hydrogen peroxide, act on plant growth and development alleviating the negative influence of abiotic stress by heavy metals. Thus, this work aimed to investigate the influence of priming with NO, H<sub>2</sub>O<sub>2</sub>, or their combination of *Handroanthus heptaphyllus* (Vell.) Mattos seeds on the germination and establishment of seedlings cultivated in iron mining tailings. The priming treatments were as follows: H<sub>2</sub>O, SNP (NO donor), H<sub>2</sub>O<sub>2</sub> as well as SNP + H<sub>2</sub>O<sub>2</sub>. These primed seeds were cultivated

in iron ore tailings or in a reference red oxisoil. The molecules used for the priming technique promoted distinct responses during seedlings' establishment. Seedlings from NO-primed seeds exhibited higher Fe and Mn absorption in the tailing, with increased activities of the antioxidant system. The priming with H<sub>2</sub>O<sub>2</sub> led to an avoidance strategy, with lower absorption of these elements by the seedlings. It can be concluded that NO and H<sub>2</sub>O<sub>2</sub> induced different resistance mechanisms to Fe and Mn in *H. heptaphyllus* seedlings.

**Keywords** Dam's Brumadinho · Hydrogen peroxide · Nitric oxide · Tropical tree species

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## 1 Introduction

The intensification of mining activities as well as its expansion over forests and sensible ecosystems brings a potential negative impact on this sector (Singh, 2005). Apart from the removal of soil and vegetation cover for the establishment of mining industries, their activity generates bypass products and residues that usually exhibit high concentrations of trace elements (Verma et al., 2012). Besides that, there is a high potential of contaminating the mining site and surrounding areas, when considering the possibility of disruption of the accumulated tailings, as could be seen recently in Brazil.

In Brazil, most of the iron ore-producing industries are in Minas Gerais State, in a region known as the Quadrilátero Ferrífero, in areas of the Cerrado and Atlantic Forest, both listed as global biodiversity hotspots (Myers et al., 2000). On January 25, 2019, the most recent event was the rupture of VALE's dam in Córrego do Feijão, in Brumadinho-MG, Brazil, resulting in the leakage of 12 million m<sup>3</sup> of tailings, reaching 46 km of nearby areas (Armada, 2020).

The restoration of the degraded areas urges for efficient, fast, low-cost, and easy management techniques, especially using native tree species considering the reference ecosystem (Aniceto et al., 2021). This way, among the restoration techniques available, direct seeding, which consists of sowing the seeds into the soil of the site to be reforested, has been shown to be effective (Cava et al., 2016; Raupp, et al., 2020; Rodrigues et al., 2020).

In the restoration process, the use of native species such as *Handroanthus heptaphyllus* (Vell.) Matos, popularly known as "ipê-rosa," is extremely important to recover the patterns of biodiversity and ecosystem services as observed in the reference ecosystems, such as *Cerrado* and Atlantic Rainforest. Studies carried out by Gai et al. (2017) show that the *H. heptaphyllus* is a Zn-tolerant species, maintaining its growth even under high concentrations of the metal. Cruz et al. (2020) verified that this species was capable to germinate and establish normal seedlings in iron ore tailings from the Mariana-MG dam rupture and accumulate iron in the aerial part. Moreover, in the recovery of mining areas, the use of tree species, with low growth rates in relation to shrubs and herbs, has to be considered for long-term recovery by facilitating the increment of other species that benefit from the microenvironment created by the trees.

Degraded areas by mining usually have enhanced concentrations of heavy metals in the soil, which could be one of the reasons for the low success rate of revegetation methods, hampering the seedling establishment after germination. Thus, it is essential to apply techniques that improve the seeds and seedlings' tolerance of heavy metals and other adverse effects of mining areas, aiming at greater success in the restoration process.

The seed priming is a low-cost technique that involves exposing the seeds to a conditioner during controlled imbibition, aiming to make them more tolerant to future stress exposure (Beckers &

Conrath, 2007; Borges et al., 2014; Tanou et al., 2012). The priming improves seed germination by synchronizing it and reducing the timing of seeds being exposed to stressful conditions (Borges et al., 2014), which is desirable in mining fields. The priming with signaling molecules, i.e., reactive oxygen species (ROS) and reactive nitrogen species (RNS), might provide greater plant tolerance to abiotic stresses (Sathiyaraj et al., 2014; Teng et al., 2014).

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) plays an important role in the signaling transduction process associated with tolerance to abiotic and biotic stresses (Hossain et al., 2013). The exogenous application of H<sub>2</sub>O<sub>2</sub> can improve and synchronize seed germination (Fontaine et al., 1994; Leung, 2019). Otherwise, exogenous application of nitric oxide (NO) donors, such as sodium nitroprusside (SNP), results in higher seed germination rates and early seedling growth under adverse conditions (Ederli et al., 2009; Zheng et al., 2011; Singh and Bhatla, 2018; Egbichi et al., 2014). Despite the slow growth of trees when compared to shrubs, priming seems to be efficient in stimulating the germination of pioneer native tree species in tropical forests, for the recovery of negatively impacted areas (Rodrigues et al., 2009; Pedrini et al., 2020).

Thus, this work investigated if the priming application in seeds results in physiological, biochemical, and photosynthetic responses during seedling establishment in iron ore tailings. The results shown here will be useful for improving revegetation techniques of areas affected by iron mining activities.

## 2 Material and Methods

### 2.1 Plant Material

The seeds of *Handroanthus heptaphyllus* (Vell.) Matos, Bignoniaceae (Reitz et al., 1983) were collected from the Federal University of Lavras (UFLA) located in Lavras–MG, Brazil, and the exsiccate was deposited at the ESAL Herbarium. The collection of this material took place from matrices cultivated in red oxisoil in a population of at least 10 individuals. Subsequently, they were processed, stored in paper bags, and kept refrigerated at 4 °C until the beginning of tests and experiments.

## 2.2 Seed Characterization for Priming

### 2.2.1 Imbibition and Drying Curve

The seeds' initial fresh mass was measured and then disinfected. The seeds were distributed in 4 replicates with 25 seeds each, placed in Petri dishes on a double layer of filter paper moistened with 2.5 times the weight of the paper with deionized water and kept at room temperature ( $25 \pm 2$  °C). The fresh mass of seeds was determined every hour for 12 h, followed by weighing every 24 h until stabilization (phase II of imbibition). Following the curve of increasing fresh mass, the time of 45 h was defined as representative of imbibition's phase II. Lately, those seeds were kept in an oven at a temperature of 28 °C and had their weight recorded every hour for the first 12 h and then every 2 h for a period of 20 h until reaching the initial fresh weight (seeds were dried slowly to reach the close the same weight of non-primed). Thus, the priming process for *H. heptaphyllus* was defined as a procedure of imbibing seeds at 25 °C for 45 h followed by a drying period at 28 °C for 20 h.

### 2.2.2 Preparation of Priming Solutions

Through a germination test, a dose–response curve was established to determine the concentrations of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and sodium nitroprusside (SNP) as nitric oxide (NO) donors to be used in the preparation and application of priming. The concentrations of  $\text{H}_2\text{O}_2$  at 100, 200, 300, 400, and 500  $\mu\text{M}$  (Hemalatha et al., 2017), and sodium nitroprusside at 50, 100, 150, 200, and 250  $\mu\text{M}$  (Gavassi et al., 2019), were freshly prepared each day of experiments. Intact seeds (cotyledons, axis, and tegument) were sown in these solutions and then dried according to the priming parameters defined previously. After priming application, seeds were placed in a Gerbox containing 150 g of vermiculite substrate, and germination was accompanied daily. The germination experimental design consisted of 4 replicates of 25 seeds for each concentration carried out in a germination chamber at 30 °C and a photoperiod of 12 h (40  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) (Cruz et al., 2020). The germination speed index (GSI) values were evaluated as criteria to define the concentrations of  $\text{H}_2\text{O}_2$  and SNP that would be used in the experiments in greenhouse conditions. Considering this, it was established the concentration

of 100 and 200  $\mu\text{M}$  of  $\text{H}_2\text{O}_2$  and SNP respectively for utilization in priming solutions to *H. heptaphyllus* seeds.

## 2.3 Characterization of the Substrates

The iron mining tailings and the red oxisoil were used as substrates. The mining tailings were collected at Mina do Pico, belonging to the company VALE SA, in the municipality of Itabirito-MG, Brazil. Both substrates were analyzed for chemical composition using the atomic absorption spectrometry method with the Mehlich 1 extractor. Among the elements quantified, both substrates presented elevated concentrations of Fe and Mn, being 61.20  $\text{mg dm}^{-3}$  for Fe in the oxisoil and 33.1  $\text{mg dm}^{-3}$  in the tailings; and 12.0  $\text{mg dm}^{-3}$  for Mn in Oxisoil and 26.0  $\text{mg dm}^{-3}$  in tailings (Supl. Mat.). For oxisoil and tailings, the pH values found were 6.4 and 5.48, respectively.

## 2.4 Experimental Conditions

Two experiments were performed. In the first experiment, the germination of *H. heptaphyllus* seeds in red oxisoil or iron mining tailings was evaluated. In the second experiment, the establishment of *H. heptaphyllus* seedlings in the same conditions as experiment 1 was analyzed. In both experiments, the red oxisoil was mixed in a 2:1 ratio with sand (2 parts of oxisoil and 1 part of sand), and for the iron mining tailings, the mixture was in a 1:1 ratio with sand, to compensate for the more humid aspect of mining tailings and minimize the natural differences in porosity and compaction between the substrates.

The experiments were carried out in a greenhouse at an average temperature of  $23 \pm 2$  °C and relative humidity of  $24 \pm 1\%$ . 35 seeds of *H. heptaphyllus* was sown in 0.8 L pots with the following treatments: (1) control (unprimed seeds); (2) primed with  $\text{H}_2\text{O}$ ; (3) primed with 200  $\mu\text{M}$  SNP (NO); (4) primed with 100  $\mu\text{M}$   $\text{H}_2\text{O}_2$ , and (5) primed with 200  $\mu\text{M}$  SNP (NO) + 100  $\mu\text{M}$   $\text{H}_2\text{O}_2$  with two substrates (red oxisoil or tailings). Five replicates of each treatment were used for each type of substrate, totaling 50 experimental plots.

For experiment 1, after 10 days, germination parameters were evaluated, and 25 germinating seeds were collected for biochemical analysis. The seeds were evaluated daily for germination percentage which

criterion was the radicle protrusion, according to Maguire (1962). The  $T_{50}$  was calculated according to Farooq et al., (2005). The remaining seeds were maintained and in experiment 2, after 50 days, ten seedlings of each replicate were sampled for morphophysiological analysis, chlorophyll *a* fluorescence, and biochemical assays.

## 2.5 Biochemical Analysis

The determination of the thermostability of *H. hep-taphyllus* seed membranes was measured by the cell membrane stability index (CMS), using 10 seeds for each replicate, according to Sullivan and Ross (1979).

For biochemical analysis using fresh material, germinated seeds and seedlings were frozen in liquid nitrogen at the sampling and stored at  $-80^{\circ}\text{C}$  until the analysis. For seeds, the integument was removed before the biochemical tests (for reducing non-reacting biological material). A pool of 25 seeds or 5 seedlings per replicate was grounded with liquid nitrogen. For assays requiring dry matter, seeds without teguments and seedlings were dried into a forced circulation oven at  $60^{\circ}\text{C}$  for 72 h.

### 2.5.1 Extraction of Total Soluble Amino Acids

Following the methodology described by Zanandrea et al. (2010), 0.2 g of dry matter (DM) from the seedlings was grounded, and aliquots of the supernatant were collected and used for the analysis of total soluble amino acids. The method described by Yemm and Cocking (1955) was used to quantify the variations in the concentration of total soluble amino acids present in the seedlings and the results were expressed in  $\mu\text{mol.g MS}^{-1}$ .

### 2.5.2 Proline Quantification

The method used was described by (Carillo & Gibbon, 2011) with samples of 0.1 g of fresh matter of seedlings. The absorbances obtained were compared with the standard curve for proline and the results obtained were expressed in micrograms of proline per  $\mu\text{g.mg MS}^{-1}$ .

### 2.5.3 Extraction and Quantification of Total Proteins

The quantification of total soluble proteins followed the extraction protocol described by Gomes and Garcia (2014); the supernatant was collected and used to quantify the protein concentration according to the Bradford method. This extract was also used for the determination of antioxidant enzyme activities (catalase, CAT; superoxide dismutase, SOD; and ascorbate peroxidase, APX).

### 2.5.4 Extraction and Quantification of Hydrogen Peroxide

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) levels were quantified using the method by Velikova et al. (2000) using 0.1 g of seeds or seedlings, and the levels of  $\text{H}_2\text{O}_2$  were quantified using a standard curve. Results were expressed in  $\mu\text{mol H}_2\text{O}_2 \text{ g}^{-1}$ .

### 2.5.5 Enzymes of the Antioxidant System

Quantification of the enzyme catalase (CAT) was performed as described by Anderson et al. (1995). Results were expressed as CAT activity ( $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$ ). Ascorbate peroxidase (APX) activity was measured following Nakano and Asada (1981) and the results were expressed as APX activity ( $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$ ). While superoxide dismutase (SOD) had its activity quantified as described by Giannopolitis & Ries (1977), one SOD unit was defined as the amount of enzyme needed to inhibit the reduction of NBT by 50% and the results were expressed as SOD activity ( $\text{U min}^{-1} \text{ g protein}^{-1}$ ).

### 2.5.6 Pigments

The method of Lichtenthaler and Buschmann (2001) was followed for the quantification of chlorophylls (*a*, *b*, total, and carotenoids) in the seedlings collected in Experiment II.

## 2.6 Fluorescence of Chlorophyll *a*

The fluorescence of chlorophyll *a* measurements was performed for the second experiment after 50 days on fully expanded leaves in the morning with the portable Mini-PAM fluorometer (Heinz Walz GmbH,

Effeltrich, Germany). Leaf tissues were previously dark acclimated for 40 min to obtain initial fluorescence (F0) and maximum fluorescence (Fm). The maximum quantum efficiency of PSII (Fv/Fm), the effective quantum yield of PSII, and the non-photochemical quenching (NPQ) was calculated according to Bilger and Bjoörkman (1990) and the electron transport rate (ETR), according to Baker (2008).

## 2.7 Tolerance Parameter Analysis

Seedlings from experiment II were collected after 50 days for analysis of seedling length and dry mass measurements. The abiotic stress tolerance index was calculated according to Rahman et al. (2013).

## 2.8 Metal Accumulation Analysis

The concentration of Fe and Mn metals in the seedlings was quantified by atomic absorption spectrophotometry after nitric-perchloric digestion, using 0.1 g of dry mass. The roots, to quantify the Fe and Mn contents, were additionally washed in a solution consisting of 0.03 M sodium citrate and 0.125 M sodium bicarbonate with 0.06 M sodium dithionite for 3 h and rinsed in deionized water to remove the Fe deposits in the root surface (Taylor and Crowder, 1983). The transfer factor (FT) of each metal was determined by the calculation according to Lubben and Sauerbeck (1991), and the removal efficiency (E%) of each metal was determined by the equation proposed by Jorge et al. (2010).

## 2.9 Experimental Design and Statistical Analysis

A completely randomized design was adopted, with 5 replicates containing 35 seeds or 10 seedlings in a 2×5 factorial scheme, using two types of substrates: red oxisoil and iron mining tailings and five priming treatments. The normality of the data and the homogeneity of the variances were verified using the Shapiro–Wilk and Levene tests, respectively, and submitted to factorial analysis of variance, with two factors (significance level 5% by the Tukey test), using free software Rbio©. The graphics were produced in SigmaPlot© software.

## 3 Results

### 3.1 Effects of Priming on Germination of *H. heptaphyllus* Seeds in the Soil (Red Oxisoil) and Iron Mining Tailings

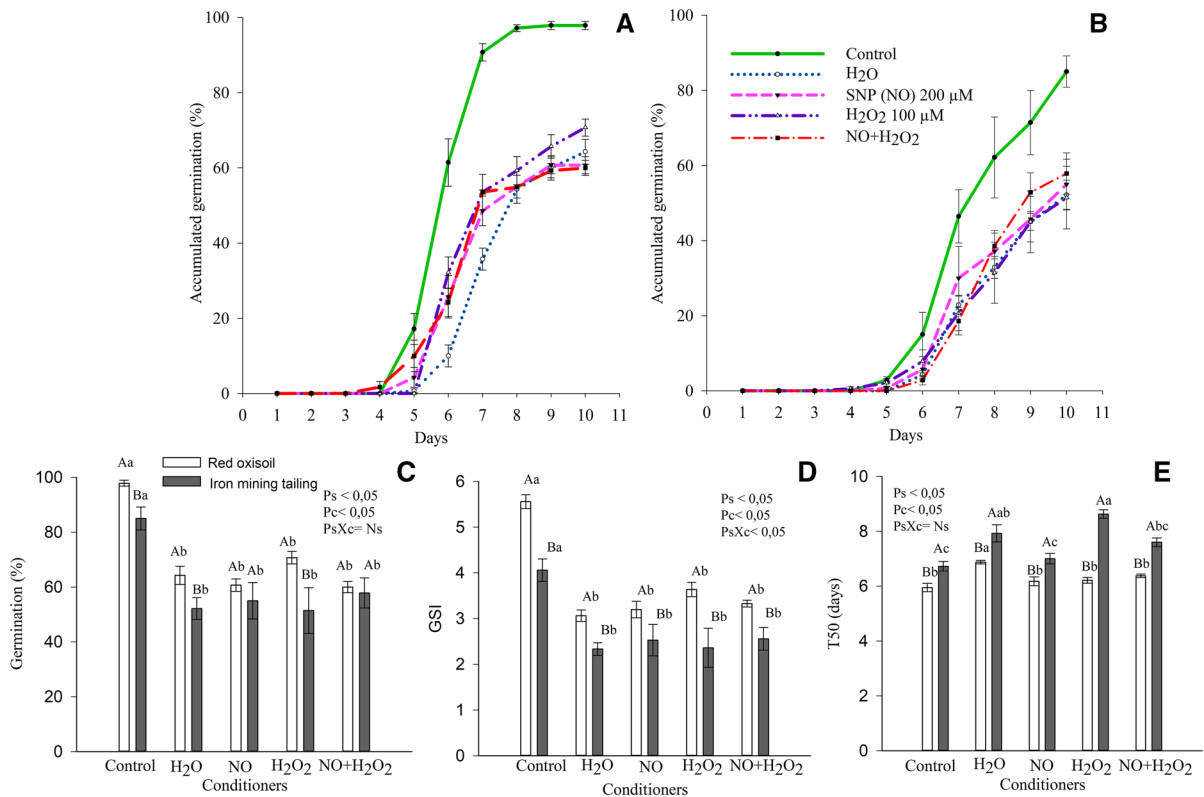
Seed germination percentage differed between the substrate of cultivation. The seeds from the control treatment in the red oxisoil had higher germination and GSI, and lower  $T_{50}$ , while in the priming treatments, germination was around 70%. In the iron mining tailings, the seeds of control showed final germination of approximately 80% while the seeds treated with the conditioning agents showed about 60% of germination (Fig. 1A–E).

The endogenous levels of  $H_2O_2$  (Fig. 2A) were higher in seeds cultivated in the red oxisoil and primed with  $NO + H_2O_2$ . For seeds germinated in iron mining tailings, the priming with  $H_2O_2$  resulted in the highest value of hydrogen peroxide levels. The thermostability of the membrane showed values lower than control ( $100 \text{ dS} \cdot \text{m}^{-1}$ ) in all conditions tested (Fig. 2B).

Among the specific activities of the enzymes of the antioxidant system, it was observed that the catalase (CAT) activity (Fig. 2C) in seeds cultivated in the red oxisoil was higher for the control treatment and the priming with  $H_2O_2$  compared to the other conditioners. However, lower CAT activities were found in seeds treated with  $NO + H_2O_2$  on both substrates and seeds primed with  $H_2O$  on iron mining tailing (Fig. 2C). Seeds cultivated in the red oxisoil, in general, presented the highest activity of APX (Fig. 2D) compared to those cultivated in iron mining tailings, except when primed with  $NO + H_2O_2$ . For the specific activity of SOD (Fig. 2E), the seeds cultivated in the red oxisoil presented higher values when primed with  $H_2O$ . In the iron mining tailings, the priming with  $H_2O$ ,  $NO$ ,  $H_2O_2$ , and  $NO + H_2O_2$  presented a higher SOD activity when compared to the control (Fig. 2E).

### 3.2 Effects of Priming on the Establishment of *H. heptaphyllus* Seedlings Cultivated in Red Oxisoil and Iron Mining Tailings

Iron (Fig. 3A) and manganese (Fig. 3B) levels were higher in *H. heptaphyllus* seedlings grown in iron mining tailings in which the previous seeds were primed with  $NO$ . Seedlings from  $H_2O_2$  conditioners



**Fig. 1** Cumulative germination of seeds in the red oxisoil (A) and in the tailings (B). Germination percentage (C); germination speed index, GSI (D); and T<sub>50</sub> (E) of preconditioned *Handroanthus heptaphyllus* seeds (Control, H<sub>2</sub>O, 200 μM SNP (NO), 100 μM H<sub>2</sub>O<sub>2</sub> and 200 μM SNP (NO)+100 μM H<sub>2</sub>O<sub>2</sub>). Bars are means ± standard error. Capital letters compare differ-

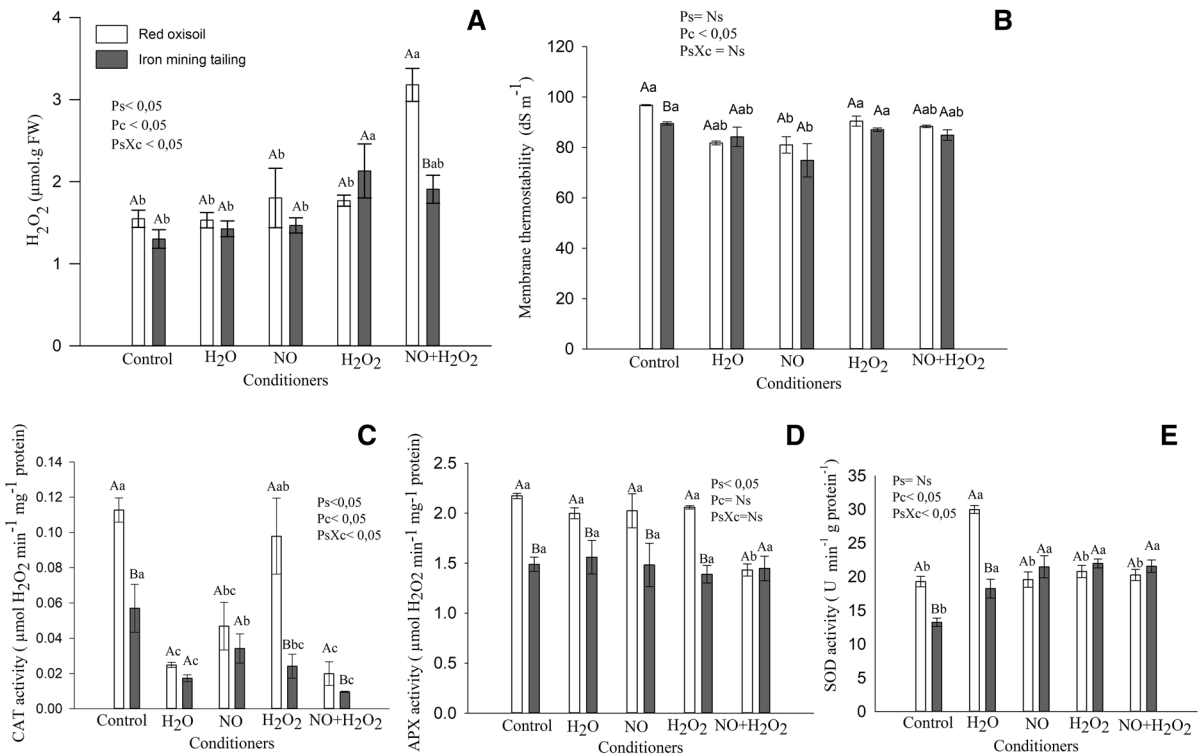
ences between substrates within the same conditioner and lowercase letters compare conditions within the same substrate. The same letters or Ns (not significant) do not show any significant difference by Tukey's test at 5% significance. P<sub>s</sub>, p-value between substrates; P<sub>c</sub>, p conditioners; and P<sub>sXc</sub>, interaction substrates and conditioners

cultivated in iron mining tailings were the ones that accumulated these elements. When cultivated in red oxisoil, the treatments with priming did not show a significant difference with the control for both analyzed nutrients.

There was no difference between the priming treatments for the transfer factor (FT) and removal efficiency (E%) variables for seedlings cultivated in the red oxisoil (Table 1). Compared to seedlings grown in iron mining tailings, there was a higher tolerance index (TI) for seedlings cultivated in the red oxisoil. In addition, in the iron mining tailings, seedlings whose seeds were treated with NO and H<sub>2</sub>O<sub>2</sub> were classified as moderate and high tolerant respectively. In general, seedlings in the red oxisoil had a greater total length compared to those grown in the iron tailings. Among the treatments, seedlings grown in the

iron mining tailings that came from seeds treated with the H<sub>2</sub>O<sub>2</sub> conditioner had the highest total biomass when compared to the other treatments in the iron mining tailings (Table 1).

Higher values of chlorophyll *a* (Fig. 4A), chlorophyll *b* (Fig. 4B), and total chlorophylls (Fig. 4C) were observed when seedlings coming from seeds treated with H<sub>2</sub>O<sub>2</sub> were cultivated in the red oxisoil. These values were higher when compared to other treatments and when compared to the seedlings cultivated in the iron mining tailings. When grown in the iron mining tailings, the seedlings of control had a higher concentration of chlorophyll *a* and chlorophyll *b* than the seedlings of the other treatments except for chlorophyll *a* of the seedlings from treatment with H<sub>2</sub>O<sub>2</sub>. The levels of total chlorophylls were higher in the control, H<sub>2</sub>O<sub>2</sub>, and NO



**Fig. 2** Endogenous hydrogen peroxide levels (A); membrane thermostability (B); specific catalase activities, CAT (C); ascorbate peroxidase-APX (D), and superoxide dismutase-SOD (E) in newly germinated seeds of preconditioned *Handroanthus heptaphyllus* cultivated in red oxisoil and iron mining tailings. Bars are means ± standard error. Capital let-

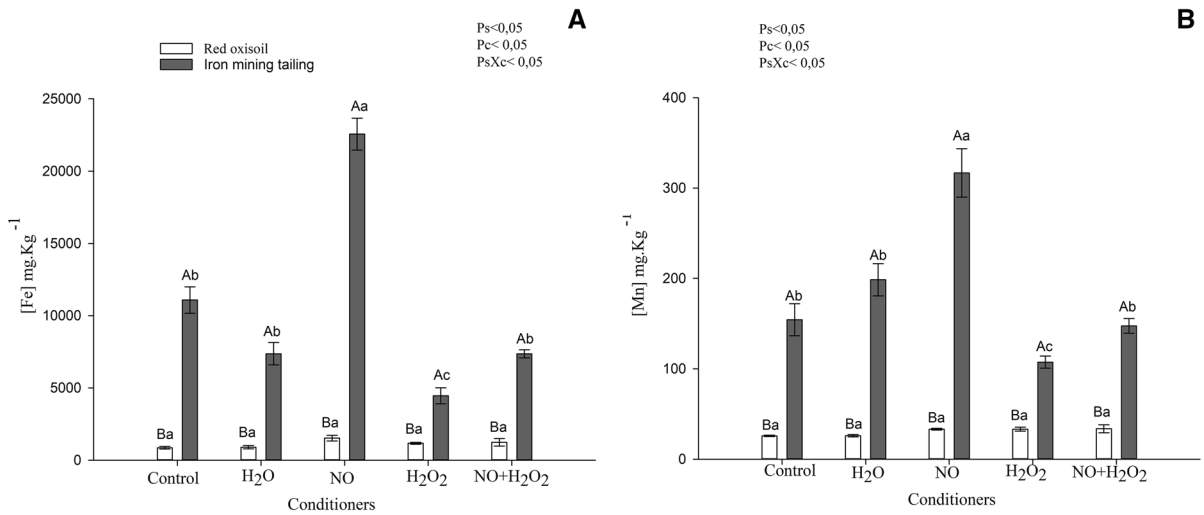
ters compare differences between substrates within the same conditioner, and lowercase letters compare conditions within the same substrate. The same letters or Ns (not significant) do not show any significant difference by Tukey’s test at 5% significance. P<sub>s</sub>, p-value between substrates; P<sub>c</sub>, conditioners; and P<sub>sxc</sub>, interaction substrates and conditioners

treatments compared to the other conditioners when cultivated in the iron mining tailings. Higher values of carotenoids (Fig. 4D) were verified in seedlings of treatments with H<sub>2</sub>O<sub>2</sub> cultivated in the iron mining tailings when compared to those in the red oxisoil.

The maximum PSII quantum yield (Fv/Fm) (Fig. 5A) was lower in the seedlings grown in the iron mining tailings when compared with those grown in the red oxisoil, showing no statistical difference with the conditions in both substrates. As for the effective quantum yield of PSII (ΦPSII) (Fig. 5B), it was noted that in the iron mining tailings, seedlings treated with NO had significantly higher ΦPSII values compared to those grown in red oxisoil. It was also observed that in the iron mining tailings, among all the conditioning factors, H<sub>2</sub>O<sub>2</sub> was the one that promoted the lowest value of ΦPSII.

The seedlings from conditioning with H<sub>2</sub>O<sub>2</sub> showed higher values of non-photochemical quenching (NPQ) (Fig. 5C) when grown in red oxisoil compared to iron mining tailings. In the iron mining tailings, the seedlings of the conditioner with H<sub>2</sub>O presented a higher value than the other treatments, followed by the seedlings that came from the conditioners NO and H<sub>2</sub>O<sub>2</sub> (Fig. 5C). Regarding ETR (Fig. 5D), differences between substrates were only noticeable in seedlings from H<sub>2</sub>O and H<sub>2</sub>O<sub>2</sub> conditions.

Endogenous levels of hydrogen peroxide (Fig. 6A) were higher in seedlings from the control and NO+H<sub>2</sub>O<sub>2</sub> treatments when grown in the red oxisoil. The seedlings from the NO conditioner presented higher levels of H<sub>2</sub>O<sub>2</sub> when grown in the iron mining tailings than in the red oxisoil. The cultivation in red oxisoil resulted in greater activity of CAT (Fig. 6B) in the control seedlings compared



**Fig. 3** Concentration of iron (A) and manganese (B) in pre-conditioned *Handroanthus heptaphyllus* seedlings and cultivated in red oxisoil and iron mining tailings. Bars represent means  $\pm$  standard error. Capital letters compare differences between substrates within the same conditioner, and lowercase

letters compare conditions within the same substrate. Means followed by the same letters or Ns (not significant) do not show any significant difference by Tukey’s test at 5% significance.  $P_s$ ,  $p$ -value between substrates;  $P_c$ , conditioners; and  $P_{sXc}$ , interaction substrates and conditioners

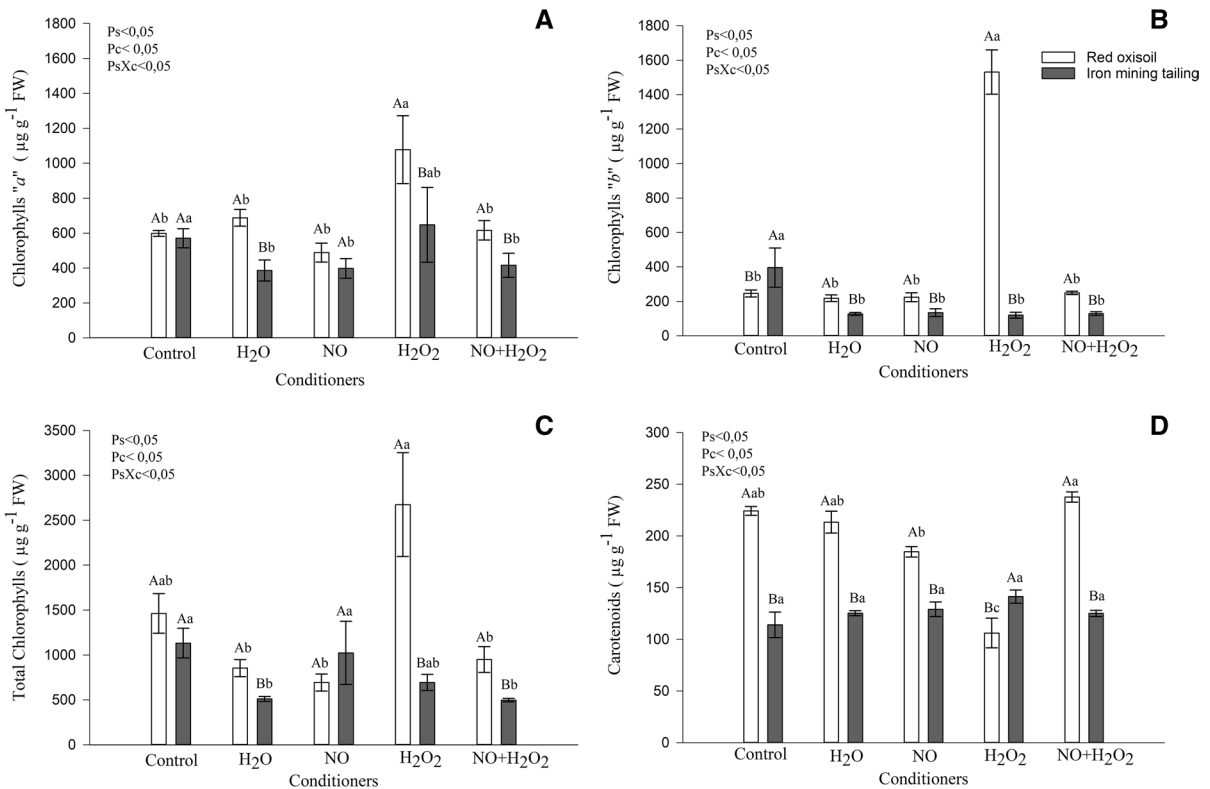
**Table 1** Tolerance index (TI), transfer factor (FT), and removal efficiency (E%)\* for iron and manganese, seedling length, and total biomass in preconditioned *Handroanthus heptaphyllus* seedlings (with 50 days) seeded in red oxisoil and iron mining tailings. Data are means  $\pm$  standard error; capital letters compare differences between substrates within the same

condition, and lowercase letters compare conditions within the same substrate. Same letters or Ns (not significant) do not show any significant difference by Tukey’s test at 5% significance.  $P_s$ , substrates;  $P_c$ , conditioners; and  $P_{sXc}$ , interaction substrates and conditioners

	Conditioners	TI	Fe		Mn		Seedling length (cm)	Total biomass (g)
			FT (mg kg <sup>-1</sup> )	E (%)	FT (mg kg <sup>-1</sup> )	E (%)		
Red oxisoil	Control	1.0	0.2 $\pm$ 0.01 <sup>Ba</sup>	4.0 $\pm$ 0.23 <sup>Ba</sup>	0.03 $\pm$ 0 <sup>Aa</sup>	0.71 $\pm$ 0 <sup>Ba</sup>	15.73 $\pm$ 0.36 <sup>Ab</sup>	0.46 $\pm$ 0.02 <sup>Aa</sup>
	H <sub>2</sub> O	1.16	0.051 $\pm$ 0.05 <sup>Ba</sup>	5.37 $\pm$ 1.0 <sup>Ba</sup>	0.04 $\pm$ 0 <sup>Aa</sup>	0.86 $\pm$ 0 <sup>Aa</sup>	17.82 $\pm$ 1.07 <sup>Aa</sup>	0.53 $\pm$ 0.03 <sup>Aa</sup>
	NO	1.05	0.39 $\pm$ 0.02 <sup>Ba</sup>	7.84 $\pm$ 1.50 <sup>Ba</sup>	0.05 $\pm$ 0 <sup>Ba</sup>	1.05 $\pm$ 0 <sup>Ba</sup>	15.37 $\pm$ 0.29 <sup>Ab</sup>	0.48 $\pm$ 0.01 <sup>Aa</sup>
	H <sub>2</sub> O <sub>2</sub>	1.10	0.36 $\pm$ 0.02 <sup>Ba</sup>	7.38 $\pm$ 0.48 <sup>Ba</sup>	0.05 $\pm$ 0 <sup>Aa</sup>	1.05 $\pm$ 0 <sup>Aa</sup>	17.19 $\pm$ 0.37 <sup>Aa</sup>	0.50 $\pm$ 0.01 <sup>Aa</sup>
	NO + H <sub>2</sub> O <sub>2</sub>	1.18	0.44 $\pm$ 0.01 <sup>Ba</sup>	8.97 $\pm$ 2.31 <sup>Ba</sup>	0.05 $\pm$ 0 <sup>Aa</sup>	1.11 $\pm$ 0 <sup>Aa</sup>	17.83 $\pm$ 0.69 <sup>Aa</sup>	0.54 $\pm$ 0.02 <sup>Aa</sup>
Iron mining tailing	Control	0.59	1.83 $\pm$ 0.074 <sup>Ab</sup>	36.75 $\pm$ 1.52 <sup>Ab</sup>	0.04 $\pm$ 0 <sup>Ab</sup>	0.97 $\pm$ 0 <sup>Ab</sup>	13.24 $\pm$ 0.77 <sup>Ba</sup>	0.27 $\pm$ 0.02 <sup>Bb</sup>
	H <sub>2</sub> O	0.60	1.65 $\pm$ 0.142 <sup>Ab</sup>	33.10 $\pm$ 0.20 <sup>Ab</sup>	0.04 $\pm$ 0 <sup>Ab</sup>	0.89 $\pm$ 0 <sup>Ab</sup>	14.18 $\pm$ 0.16 <sup>Ba</sup>	0.27 $\pm$ 0.02 <sup>Bb</sup>
	NO	0.46	3.33 $\pm$ 0.28 <sup>Aa</sup>	66.74 $\pm$ 5.74 <sup>Aa</sup>	0.07 $\pm$ 0 <sup>Aa</sup>	1.51 $\pm$ 0 <sup>Aa</sup>	14.02 $\pm$ 0.36 <sup>Ba</sup>	0.25 $\pm$ 0.03 <sup>Bb</sup>
	H <sub>2</sub> O <sub>2</sub>	0.80	1.21 $\pm$ 0.11 <sup>Ac</sup>	24.29 $\pm$ 2.38 <sup>Ac</sup>	0.03 $\pm$ 0 <sup>Ac</sup>	1.04 $\pm$ 0 <sup>Ac</sup>	15.53 $\pm$ 0.27 <sup>Ba</sup>	0.37 $\pm$ 0.03 <sup>Ba</sup>
	NO + H <sub>2</sub> O <sub>2</sub>	0.69	1.73 $\pm$ 0.28 <sup>Ab</sup>	34.60 $\pm$ 5.60 <sup>Ab</sup>	0.04 $\pm$ 0 <sup>Ac</sup>	1.03 $\pm$ 0 <sup>Ab</sup>	13.88 $\pm$ 0.35 <sup>Ba</sup>	0.32 $\pm$ 0.01 <sup>Bb</sup>
	$P_s$		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
	$P_c$		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
	$P_{sXc}$		<0.05	<0.05	<0.05	<0.05	Ns	Ns

\*TI, BMT/BMC, BMT accumulated biomass in each treatment, BMC accumulated biomass in the control (Rahman et al., 2013); FT, QPA/QR, QPA, amount of metal, QR, amount of the metal extracted from the soil (Lubben & Sauerbeck, 1991); E(%), FT\*100/N; N, number of replicates (Jorge et al., 2010)





**Fig. 4** Contents of chlorophylls a (A), chlorophylls b (B), total chlorophylls (C), and carotenoids (D) in preconditioned *Handroanthus heptaphyllus* leaves cultivated in red oxisoil and iron mining tailings. Bars are means ± standard error. Capital letters compare differences between substrates within the same

conditioner, and lowercase letters compare conditions within the same substrate. The same letters or Ns (not significant) do not show any significant difference by Tukey’s test at 5% significance. P<sub>s</sub>, p-value between substrates; P<sub>c</sub>, conditioners; and P<sub>sxc</sub>, interaction substrates and conditioners

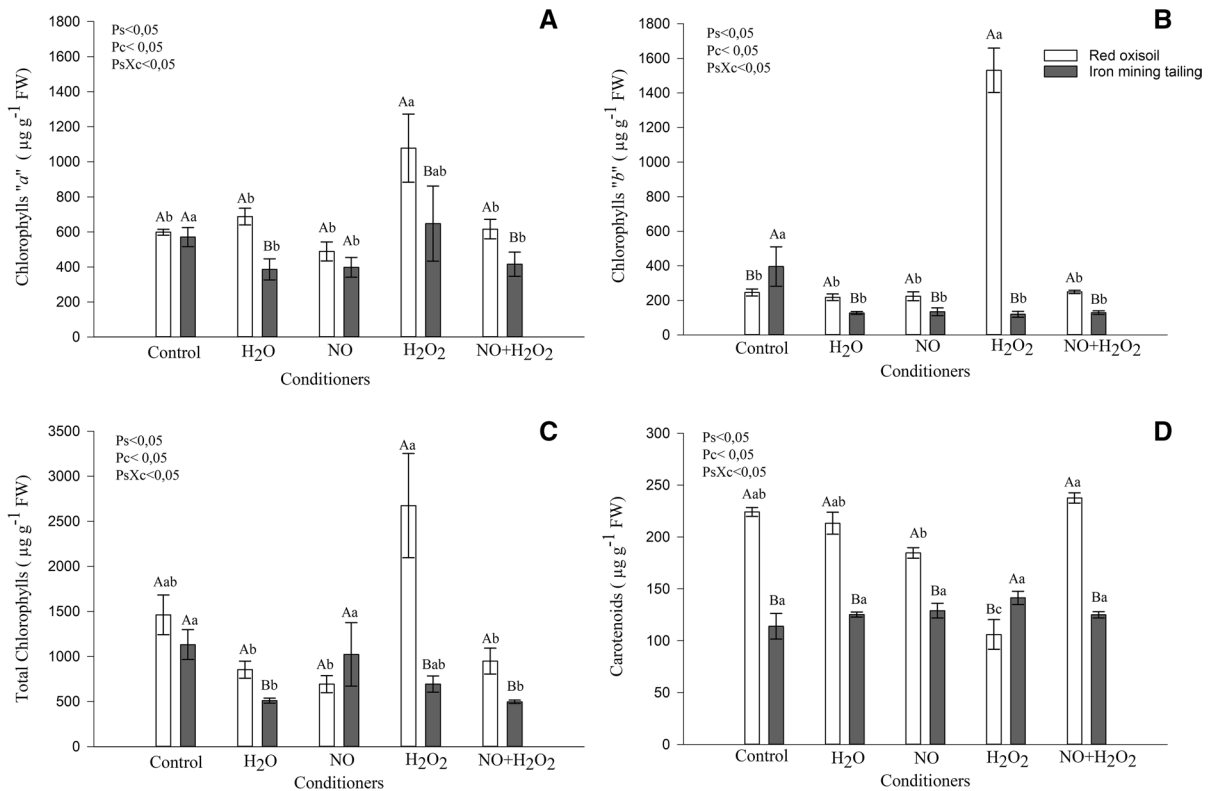
to the other treatments. In seedlings cultivated in the iron mining tailings, greater CAT activity was observed compared to the red oxisoil in the NO, H<sub>2</sub>O<sub>2</sub>, and NO+H<sub>2</sub>O<sub>2</sub> conditions, with the treatment NO being the one with the highest activity among the conditions in the iron mining tailings. As for the APX activity (Fig. 6C), the seedlings cultivated in the iron mining tailings presented higher levels compared to the red oxisoil seedlings, with higher values in the conditions H<sub>2</sub>O<sub>2</sub> and NO+H<sub>2</sub>O<sub>2</sub>. Greater activities of SOD (Fig. 6D) were observed in seedlings in the treatments in the iron mining tailings compared to the red oxisoil, with the primed with NO in the tailings providing the highest activity value.

There is a decrease in the total soluble protein content in seedlings grown in the iron mining tailings when

compared to the red oxisoil (Fig. 7A). In the red oxisoil, the highest protein concentrations were related to the seedlings of the control and H<sub>2</sub>O treatments. In seedlings grown in the red oxisoil, the highest concentrations of total amino acids (Fig. 7B) were present in the control and the NO+H<sub>2</sub>O<sub>2</sub> conditioner, and in the iron mining tailings, the highest concentrations were observed in seedlings from the H<sub>2</sub>O, NO, and H<sub>2</sub>O<sub>2</sub> conditions at the red oxisoil. The levels of proline in the seedlings (Fig. 7C) showed a lower value in the NO conditioning in both substrates when compared to the other treatments.

#### 4 Discussion

This work showed that the conditioner used for seed priming totally influenced the antioxidant metabolism



**Fig. 5** Maximum quantum yield, Fv/Fm (A); the quantum yield of electron flow through PSII,  $\Phi$ PSII (B); non-photochemical quenching, NPQ (C); and electron transport rate, ETR (D) in preconditioned *Handroanthus heptaphyllus* leaves cultivated in red oxisoil and iron mining tailings. Bars are means  $\pm$  standard error. Capital letters compare differences

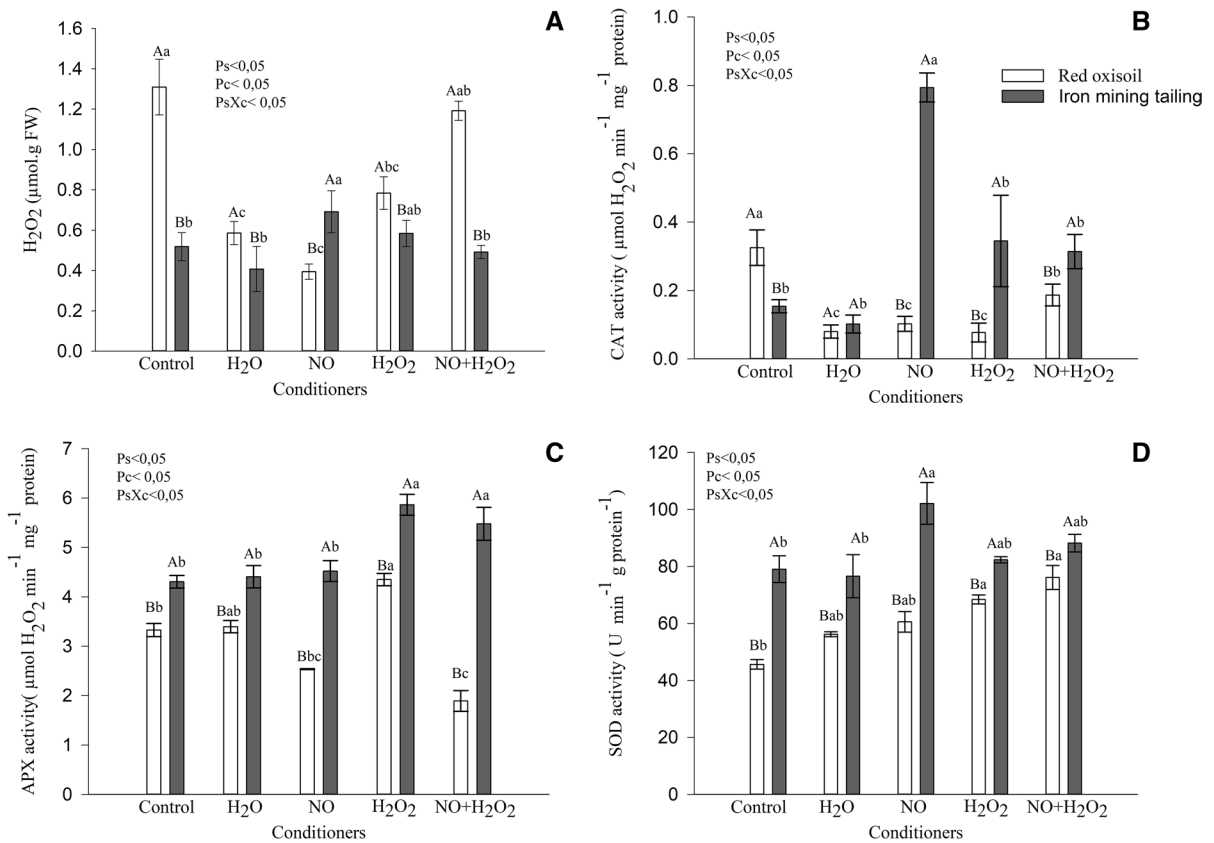
between substrates within the same conditioner and lowercase letters compare conditions within the same substrate. The same letters or Ns (not significant) do not show any significant difference by Tukey's test at 5% significance.  $P_s$ ,  $p$ -value between substrates;  $P_c$ , conditioners; and  $P_{sxc}$ , interaction substrates and conditioners

and nutrient absorption by seedlings of *H. heptaphyllus* during the first stages of development. Priming induced biochemical and physiological changes in seeds (Pawar & Laware, 2018) leading to germination synchronization and improvement in the seedling establishment (Lechowska et al., 2019). In this work, seeds grown in iron mining tailings resulted in a delay in germination independently of priming treatments when compared to the seeds sowed in the red oxisoil.

The increase of H<sub>2</sub>O<sub>2</sub> and lower activities of CAT and APX in seeds primed with NO + H<sub>2</sub>O<sub>2</sub> cultivated in red oxisoil did not result in loss or membrane damage, as shown by the thermostability results. Thus, we can suggest these internal levels of H<sub>2</sub>O<sub>2</sub> found in seeds of *H. heptaphyllus* in this work are related to signaling, within an "oxidative window" as described by Bailly et al. (2008).

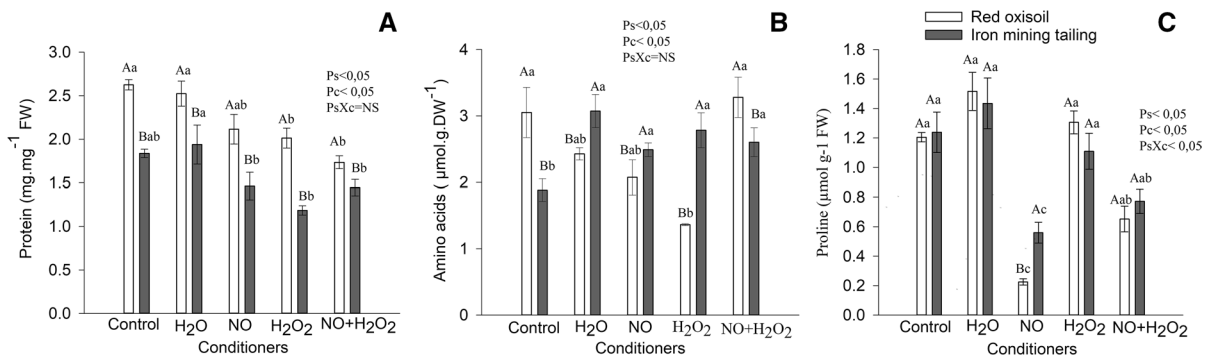
Changes due to priming application in seeds were more prominent in the establishment of seedlings, especially regarding the accumulation of Fe and Mn. On both substrates, the lower pH makes these two elements more available for absorption (Andresen et al., 2018). The higher concentration of available Fe in the red oxisoils is related to its origin and composition (Fernandes et al., 2004); however, it did not result in greater absorption of Fe by the seedlings.

*H. heptaphyllus* seedlings whose seeds were primed with NO, when cultivated in the iron mining tailings, accumulated a concentration of Fe above the toxicity limit in the plants, which is 500 mg Kg<sup>-1</sup> (Marschner, 2012). For Mn, the concentration in *H. heptaphyllus* seedlings was between the toxicity ranges of several species, according to Marschner (2012), but the level of Mn toxicity for tree species is



**Fig. 6** Endogenous hydrogen peroxide levels (A); specific catalase activities, CAT (B); ascorbate peroxidase, APX (C); and superoxide dismutase, SOD (D) in preconditioned *Handroanthus heptaphyllus* and cultivated in red oxisoil and iron mining tailings. Bars are means  $\pm$  standard error. Capital letters compare differences between substrates within the same con-

ditioner and lowercase letters compare conditions within the same substrate. The same letters or Ns (not significant) do not show any significant difference by Tukey’s test at 5% significance.  $P_s$ ,  $p$ -value between substrates;  $P_c$ , conditioners; and  $P_{sXc}$ , interaction substrates and conditioners



**Fig. 7** Total soluble proteins (A), total amino acids (B), and proline (C) in preconditioned *Handroanthus heptaphyllus* seedling and cultivated in soil (red oxisoil) and iron mining tailings. Bars are means  $\pm$  standard error. Capital letters compare differences between substrates within the same con-

ditioner, and lowercase letters compare conditions within the same substrate. The same letters or Ns (not significant) do not show any significant difference by Tukey’s test at 5% significance.  $P_s$ ,  $p$ -value between substrates;  $P_c$ , conditioners; and  $P_{sXc}$ , interaction substrates and conditioners

not known. Fe is an essential micronutrient for plants, but in high concentrations, this metal can cause oxidative damage, leading to inhibition of the activity of antioxidant enzymes, growth, and damage to the photosynthetic apparatus (Andresen et al., 2018; Küpper, 2017; Tewari et al., 2013). However, these damages were not observed throughout the experiment.

The interactions of NO with Fe, regulating Fe homeostasis, and the application of NO in promoting greater bioavailability of this metal has already been reported in the literature (Graziano & Lamatina, 2005; Ramirez et al., 2010). However, it was not known until now how seed priming affects the uptake of Fe by plants. Furthermore, this is the first study to describe the interaction between priming with NO and increased Fe absorption. It is estimated that Mn plays a role in the likely mechanisms of interaction between NO and Fe. The influx of  $Mn^{2+}$  can be mediated by the protein IRT1 (Iron-regulated transporter 1) which is responsible for the absorption of  $Fe^{2+}$  (Korshunova et al., 1999). Thus, IRT1 is capable of transporting both Fe and Mn (Pedas et al., 2008). However, the mechanism by which priming with NO promoted greater Fe absorption by the seedlings studied here is still uncertain.

According to Ci et al. (2010), the tolerance index (TI) to abiotic stresses ranges from 0 to 1.0 being (i) 0 to 0.2 showing that the plant is more sensitive to the contaminant; (ii) 0.2 to 0.4 is classified as sensitive; (iii) 0.4 to 0.6 is classified as moderately tolerant; (iv) 0.6 to 0.8 being classified as tolerant; and (v) 0.8 to 1.0 is considered highly tolerant. In this work, it was possible to note that the priming with NO induced moderate tolerance in the seedlings cultivated in iron mining tailings. Even though these seedlings accumulated more Fe and Mn, they showed a higher transfer factor and removal efficiency. On the other hand, when the seeds were treated with  $H_2O_2$  and cultivated in the iron mining tailings, the seedlings presented a higher tolerance index and increased seedling biomass. These observations were associated with lower absorption of Fe and Mn, suggesting that this treatment ( $H_2O_2$  priming) promoted an avoidance mechanism of these metals in the seedlings (Gupta et al., 2019).

The *H. heptaphyllus* seedlings from priming with NO cultivated in the iron mining tailings did not show reduced growth compared to other treatments, even accumulating potentially phytotoxic concentrations

of Fe and Mn. This would be due to the low levels of nutrients and organic matter present in many iron mining tailings, as also discussed by Cruz et al. (2020), and to the compaction of these tailings, also mentioned by Clark et al. (2003).

The highest content of chlorophylls in seedlings treated with  $H_2O_2$  in the soil in this work was also reported in seedlings of *Annona muricata* whose seeds were soaked in  $H_2O_2$  (Veloso et al., 2020). However, it is still unclear how this molecule influences the content of photosynthetic pigments. On the other hand, in seedlings from primed seeds grown in the iron mining tailings, with excess Fe, a decrease in chlorophylls and carotenoids and consequently a reduction in the photosynthetic process has already been reported (Li et al., 2019 and Xue et al., 2018). These discrepancies may be related to the levels of Fe and Mn absorbed and the inherent tolerance of plants shown in each treatment. In this work, the reduction of these pigments did not significantly affect photosynthesis, as verified by the values of Fv/Fm,  $\Phi PSII$ , ETR, and NPQ. However, according to Peña-Olmos & Casierra-Posada, (2013), ETR and Fv/Fm can decrease under severe Fe and Mn toxicity. Damage to PSII may occur due to the inability to transfer excitation energy from the antenna to the reaction center (Baker & Rosenqvist, 2004) coupled with limited non-photochemical dissipation due to the oxidative damage caused by excess Fe (Pinto et al., 2016). In this case, however, we observe that the increase in NPQ in the  $H_2O_2$  conditioning occurs due to the increase in chlorophylls associated with the antenna complex, promoting a photoprotection, and dissipating the excess of energy.

For enzymes of the antioxidant system, a similar response was observed regarding APX in seeds treated with  $NO+H_2O_2$  and cultivated in the red oxisoil as described by Corpas et al. (2005 and 2017). However, for this same condition, seedlings, when cultivated in iron mining tailings, present an opposite response from what was described by these same authors. The conditioning with NO in the iron mining tailings showed high activity of SOD and CAT in seedlings, which can be explained as a compensatory response to the accumulation of metals observed (Cargnelutti et al., 2006). Studies show that sodium nitroprusside, as a NO donor, has a protective effect by increasing the activity of SOD and CAT (Kumari et al., 2010), ensuring an effective action of these enzymes, and preventing further formation of  $H_2O_2$ .

At high concentrations of Fe and other metals, the soluble protein content has been reported to decrease while the free amino acid content increases (Mehraban et al., 2008; Seneviratne, et al., 2017). In this work, the accumulation of proline present in *H. heptaphyllus* seedlings did not differ between substrates. However, in the treatment with NO, there is a reduction of this amino acid in both substrates, and so far, there is no evidence of a direct interaction pathway between NO and proline, since proline is an osmoprotective molecule and, in response to stress, it can be accumulated or reduced (Verbruggen & Hermans, 2008).

## 5 Conclusion and Perspectives

This work demonstrated that the priming technique in native seeds can be used with different purposes in programs for the recovery of degraded environments, depending on the condition used. The main effects of the priming were observed on seedlings, improving their capacity of establishment in mining fields with high control against oxidative damage even increasing absorption of Fe and Mg. Seedlings coming from priming with H<sub>2</sub>O<sub>2</sub> grown in iron mining tailings probably developed a mechanism of avoidance of trace elements stress. Seedlings that came from seeds primed with SNP as NO donor cultivated in iron mining tailings accumulated Fe and Mn, greater removal efficiency, greater activity of the antioxidant enzyme system, and no damage to the photosynthetic system and probably developed a tolerance mechanism. With further studies, the priming technique can be used in restoration practices of vegetation cover in places degraded by mining activity even with the purpose of phytoextraction and phytoremediation.

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**Data Availability** The authors declare that all other data supporting the findings of this study are available within the article.

### Declarations

**Conflict of Interest** The authors declare no competing interests.

## References

- Anderson, M. D., Prasad, T. K., & Stewart, C. R. (1995). Changes in isozyme profiles of catalase, peroxidase, and glutathione reductase during acclimation to chilling in mesocotyls of maize seedlings. *Plant Physiology*, *109*(4), 1247–1257.
- Andresen, E., Peiter, E., & Küpper, H. (2018). Trace metal metabolism in plants. *Journal Of Experimental Botany*, [S.L.], *69*(5), 909–954. 13 fev.
- Aniceto, A. F. B., Ikeda-Castrillon, S. K., Fernandez, J. R. C., Martins, B. A. A., Duarte, Í. S., & Morais, F. F. (2021). Evaluation of no-tillage and litter transposition techniques for the emergence and establishment of Manduvi (*Sterculia apetala* (jacq). Karts.) in ecological restoration work in an area of degraded springs in the PantanalMato-grossense. *Research, Society And Development*, [S.L.], *10*(2), 1–16. 3 fev.
- Armada, C. A. S. (2020). The environmental disasters of Mariana and Brumadinho in the face of the Brazilian socio-environmental state. *Territorium*, *28*, 13–22.
- Bailly, C., El-Maarouf-Bouteau, H., & Corbineau, F. (2008). From intracellular signaling networks to cell death: The dual role of reactive oxygen species in seed physiology. *Comptes Rendus Biologies*, [s.l.], *331*(10), 806–814, out.
- Baker, N. R. (2008). Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annual review of plant biology*, *59*, 89.
- Baker, N. R., & Rosenqvist, E. (2004). Applications of chlorophyll fluorescence can improve crop production strategies: An examination of future possibilities. *Journal of Experimental Botany*, *55*(403), 1607–1621.
- Beckers, G. J. M., & Conrath, U. (2007). Priming for stress resistance: From the lab to the field. *Current Opinion in Plant Biology*, *10*, 1–7. <https://doi.org/10.1016/j.pbi.2007.06.002>
- Bilger, W., & Björkman, O. (1990). Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosynthesis research*, *25*(3), 173–185.
- Borges, A. A., Jiménez-Arias, D., Expósito-Rodríguez, M., Sandalio, L. M., & Pérez, J. A. (2014). Priming crops against biotic and abiotic stresses: MSB as a tool for studying mechanisms. *Frontiers in Plant Science*, *5*, 642. <https://doi.org/10.3389/fpls.2014.00642>
- Cargnelutti, D., Tabaldi, L. A., Spanevello, R. M., Jucoski, G. O., Battisti, V., Redin, M., Linares, C. E. B., Dressler, V. L., Flores, E. M. M., Nicoloso, F. T., Morsch, V. M., & Schetinger, M. R. C. (2006). Mercury toxicity induces oxidative stress in growing cucumber seedlings. *Chemosphere*, *65*, 999–1006.
- Carillo, P., & Gibbon, Y. (2011). Protocol: Extraction and determination of proline. Protocols in ecological & environmental plant physiology.
- Cava, M. G. B., Isernhagen, I., Mendonça, A. H., & Durigan, G. (2016). Comparison of techniques for restoration of woody Cerrado vegetation in abandoned pastures. *Hoehnea*, *43*(2), 301–315. <https://doi.org/10.1590/2236-8906-18.2016>

- Ci, D., Jiang, D., Dai, T., Jing, Q., & Cao, W. (2010). Variation in cadmium tolerance and accumulation and their relationship in wheat recombinant inbred lines at seedling stage. *Biological Trace Element Research*, 142(3), 807–818.
- Clark, L.J., Whalley, W. R., Barraclough, P.B. (2003). How do roots penetrate strong soil? In: Abe, J. (Ed.), *Roots: The dynamic interface between plants and the earth. Developments in Plant and Soil Sciences*. Springer, Dordrecht, pp. 93–104
- Corpas, F. J., Gupta, D. K., & Palma, J. M. (2005). Production sites of reactive oxygen species (ROS) in organelles from plant cells. In D. K. Gupta, J. M. Palma, & F. J. Corpas (Eds.), *Reactive oxygen species and oxidative damage in plants under stress* (pp. 1–22). Springer.
- Corpas, F. J., Barroso, J. B., Palma, J. M., & Rodríguez-Ruiz, M. (2017). Plantperoxisomes: A nitro-oxidativecocktail. *Redox Biology*, 11, 535–542.
- Cruz, F. V., Da S., Gomes, M. P., Bicalho, E. M., Della Torre, F., & Garcia, Q. S. (2020). Does Samarco's spilled mud impair the growth of native trees of the Atlantic Rainforest? *Ecotoxicology and Environmental Safety*, 189
- Ederli, L., Reale, L., Madeo, L., Ferranti, F., Gehring, C., Fornaciari, M., & Pasqualini, S. (2009). NO release by nitric oxide donors in vitro and in planta. *Plant Physiology and Biochemistry*, 47(1), 42–48. <https://doi.org/10.1016/j.plaphy.2008.09.008>
- Egbichi, I., Keyster, M., & Ludidi, N. (2014). Effect of exogenous application of nitric oxide on salt stress responses of soybean. *South African Journal of Botany*, 90, 131–136. <https://doi.org/10.1016/j.sajb.2013.11.002>
- Farooq, M., et al. (2005). Thermal hardening: A new seed vigor enhancement tool in rice. *Journal of Integrative Plant Biology*, 47(2), 187–193.
- Fernandes, R., Barrón, V., Torrent, J., & Fontes, M. (2004). Quantification of iron oxides in Brazilian Oxi soils by diffuse reflectance. *Revista Brasileira De Ciência Do Solo*, 28, 245–257.
- Fontaine, O., Huault, C., Pavis, N., & Billard, J. P. (1994). Dormancy breakage of *Hordeumvulgare* seeds: Effect of hydrogen peroxide and stratification on glutathione level and glutathione reductase activity. *Plant Physiology and Biochemistry*, 32, 677–683.
- Gai, A. P. C., Dos Santos, D. S., & Vieira, E. A. (2017). Effects of zinc excess on antioxidant metabolism, mineral content and initial growth of *Handroanthus impetiginosus* (Mart. ex DC.) Mattos and *Tabebuia roseoalba* (Ridl.) Sandwith. *Environmental and Experimental Botany*, 144, 88–99.
- Gavassi, M. A., Gaion, L. A., Monteiro, C. C., Santos, J. C., & Carvalho, R. F. (2019). Seed priming with sodium nitroprusside attenuates the effects of water deficit on soybean seedlings. *Comunicata Scientiae*, 10(1), 176–184.
- Giannopolitis, C. N., & Ries, S. K. (1977). Superoxide dismutases: I. Occurrence in higher plants. *Plant physiology*, 59(2), 309–314.
- Gomes, M. P., & Garcia, Q. S. (2014). Reactive oxygen species and seed germination. *Biologia (poland)*, 68(3), 351–357.
- Graziano, M., & Lamattina, L. (2005). Nitric oxide and iron in plants: An emerging and converging story. *Trends in Plant Science*, 10(1), 4–8.
- Gupta, D. K., Palma, J. M., Corpas, F. J. (Eds.). (2019). Nitric oxide and hydrogen peroxide signaling in higher plants. <https://doi.org/10.1007/978-3-030-11129-8>
- Hemalatha, G., Renugadevi, J., & Eevera, T. (2017). Studies on seed priming with hydrogen peroxide for mitigating salt stress in rice. *International Journal Of Current Microbiology And Applied Sciences*, [S.L.], 6(6), 691–695.
- Hossain, M. A., & Fujita, M. (2013). Hydrogen peroxide priming stimulates drought tolerance in mustard (*Brassica juncea* L.). *Plant Gene Trait*, 4, 109–123.
- Hossain, M. A., Bhattacharjee, S., Armin, S. M., Qian, P., Xin, W., Li, H. Y., ... & Tran, L. S. P. (2015). Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. *Frontiers in plant science*, 6, 420.
- Jorge, R. A. B., Abreu, C. A., Andrade, C. A., & Camargo, O. A. (2010). Filter cake and peat as amendments of contaminated soil with residue of scrap rich in boron. *Bragantia*, 69, 467–476.
- Korshunova, Y. O., Eide, D., Clark, W. G., Guerinot, M. L., & Pakrasi, H. B. (1999). The IRT1 protein from *Arabidopsis thaliana* is a metal transporter with a broad substrate range. *Plant Molecular Biology*, 40, 37–44.
- Kumari, A., Sheokand, S., & Swaraj, K. (2010). Nitric oxide induced alleviation of toxic effects of short term and long term Cd stress on growth, oxidative metabolism and Cd accumulation in Chickpea. *Brazilian Journal Of Plant Physiology*, 22(4), 271–284.
- Küpper, H. (2017). Lead toxicity in plants. In: Sigel A, Sigel H, Sigel RKO, eds. *Lead: Its effects on environment and health*. Berlin: Walter de Gruyter, GmbH, 491–500
- Lechowska, K., Kubala, S., Wojtyła, Ł., Nowaczyk, G., Quinet, M., Lutts, S., & Garnczarska, M. (2019). New insight on water status in germinating *Brassica napus* seeds in relation to priming-improved germination. *International Journal of Molecular Sciences*, 20(3), 540.
- Leung, D. W. M. (2019). Participation of hydrogen peroxide and nitric oxide in improvement of seed germination performance under unfavourable conditions. *Nitric Oxide and Hydrogen Peroxide Signaling in Higher Plants*, 145–156. [https://doi.org/10.1007/978-3-030-11129-8\\_7](https://doi.org/10.1007/978-3-030-11129-8_7)
- Li, B., Sun, L., Huang, J., Göschl, C., Shi, W., Chory, J., et al. (2019). GSNOR provides plant tolerance to Fe toxicity via preventing iron-dependent nitrosative and oxidative cytotoxicity. *Nature Communications*, 10(1), 1–13.
- Lichtenthaler, H. K., & Buschmann, C. (2001). Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Current Protocols in Food Analytical Chemistry*, 1(1), F4.3.1-F4.3.8. <https://doi.org/10.1002/0471142913.faf0403s01>
- Lubben, S., & Sauerbeck, D. (1991). The uptake and distribution of heavy metals by spring wheat. *Water, Air and Soil Pollution*, 57–58, 239–247.
- Maguire, J. D. (1962). Speed of germination aid in selection and evaluation for seedling emergence and vigor. *Crop Science, Madison*, 2(2), 176–177.
- Marschner, H. (2012). *Marschner's mineral nutrition of higher plants* (p. 2173). Academic Press.
- Mehraban, P., Zadeh, A. A., & Sadeghipour, H. R. (2008). Iron toxicity in rice (*Oryza sativa* L.), under different

- potassium nutrition. *Asian Journal of Plant Science*, 7(3), 251–259.
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403(6772), 853–858. <https://doi.org/10.1038/35002501>
- Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology*, 22(5), 867–880.
- Nicolás, J., Chiari, M., Crespo, J., Orellana, I. G., Lucarelli, F., & Nava, S. (2008). Quantification of Saharan and local dust impact in an arid Mediterranean area by the positive matrix factorization (PMF) technique. *Atmospheric Environment*, 42(39), 8872–8882.
- Pawar, V. A., & Laware, S. L. (2018). Seed priming a critical review. *International Journal Of Scientific Research In Biological Sciences*, 5(5), 94–101. 31 out.
- Pedas, P., Ytting, C. K., Fuglsang, A. T., Jahn, T. P., Schjoerring, J. K., & Husted, S. (2008). Manganese efficiency in barley: identification and characterization of the metal ion transporter HvIRT1. *Plant physiology*, 148(1), 455–466.
- Pedrini, S., Gibson-Roy, P., Trivedi, C., Gálvez-Ramírez, C., Hardwick, K., Shaw, N., & Dixon, K. (2020). Collection and production of native seeds for ecological restoration. *Restoration Ecology*, 28, S228–S238.
- Peña-Olmos, J. E., & Casierra-Posada, F. (2013). Photochemical efficiency of photosystem II (PSII) in broccoli plants (*Brassica oleracea* var Italica) affected by excess iron. *Orinoquia*, 17(1), 15–22.
- Pinto, S. D. S., Souza, A. E. D., Oliva, M. A., & Pereira, E. G. (2016). Oxidative damage and photosynthetic impairment in tropical rice cultivars upon exposure to excess iron. *Scientia Agricola*, 73(3), 217–226.
- Rahman, M. M., Azirun, S. M., & Boyce, A. N. (2013). Enhanced accumulation of copper and lead in amaranth (*Amaranthus paniculatus*), Indian mustard (*Brassica juncea*) and sunflower (*Helianthus annuus*). *PLOS ONE*, 8, e62941.
- Ramirez, L., Zabaleta, E. J., Lamattina, L. (2010). Nitric oxide and frataxin: two players contributing to maintain cellular iron homeostasis. *Ann Bot*
- Raupp, P. P., Ferreira, M. C., Alves, M., Campos-Filho, E. M., Sartorelli, P. A. R., Consolaro, H. N., & Vieira, D. L. M. (2020). Direct seeding reduces the costs of tree planting for forest and savana restoration. *Ecological Engineering*, 148, 105788.
- Reitz, R., Klein, R. M., & Reis, A. (1983). Rio Grande do Sul wood project. *Sellowia, Itajaí*, 34(e35), 1–525.
- Rodrigues, R. R., Lima, R. A. F., Gandolfi, S., & Nave, A. G. (2009). On the restoration of high diversity forests: 30 years of experience in the Brazilian Atlantic Forest. *Biological Conservation*, 142, 1242–1251.
- Rodrigues, A. B. M., Giuliatti, N. M., & Júnior, A. P. (2020). Application of methodologies for the recovery of degraded areas in Brazilian biomes. *Brazilian Applied Science Review*, 4(1), 333–369.
- Sathiyaraj, G., Srinivasan, S., Kim, Y. J., Lee, O. R., Balusamy, S. D. R., & Khorolaragchaa, A. (2014). Acclimation of hydrogen peroxide enhances salt tolerance by activating defense-related proteins in *Panax ginseng* CA Meyer. *Molecular Biology Reports*, 41, 3761–3771. <https://doi.org/10.1007/s11033-014-3241-3>
- Seneviratne, M., Rajakaruna, N., Rizwan, M., Madawala, H. M. S. P., Ok, Y. S., & Vithanage, M. (2017). Heavy metal-induced oxidative stress on seed germination and seedling development: A critical review. *Environmental Geochemistry and Health*. <https://doi.org/10.1007/s10653-017-0005-8>
- Singh, O. P. (2005). Problems and remedies. In O. P. Singh (Ed.), *Mining Environment* (pp. 278–279). Regency Publications.
- Singh, N., & Bhatla, S. C. (2018). Nitric oxide regulates lateral root formation through modulation of ACC oxidase activity in sunflower seedlings under salt stress. *Plant Signaling & Behavior*, 25, 1–7.
- Sullivan, Y., & Ross, M. (1979). Selecting for drought and heat resistance in grain sorghum. In: Mussell H. & Staples R., C. (Eds.). *Stress physiology in crop plants*. Wiley, New York, pp 263–281.
- Tanou, G., Filippou, P., Belghazi, M., Diamantidis, G., Fotopoulos, V., & Molassiotis, A. (2012). Oxidative and nitrosative-based signaling and associated post-translational modifications orchestrate the acclimation of citrus plants to salinity stress. *The Plant Journal*, 72, 585–599. <https://doi.org/10.1111/j.1365-313X.2012.05100.x>
- Taylor, G. T., & Crowder, A. A. (1983). Uptake and accumulation of copper, nickel and iron by *Thyphalatifolia* grown in solution culture. *Canadian Journal of Botany*, 61, 1825–1830.
- Teng, K., Li, J., Liu, L., Han, Y., Dum, Y., & Zhang, J. (2014). Exogenous ABA induces drought tolerance in upland rice: The role of chloroplast and ABA biosynthesis-related gene expression on photosystem II during PEG stress. *Acta Physiologiae Plantarum*, 36, 2219–2227. <https://doi.org/10.1007/s11738-014-1599-4>
- Tewari, R. K., Hadacek, F., Sassmann, S., & Lang, I. (2013). Iron deprivation-induced reactive oxygen species generation leads to non-autolytic PCD in *Brassica napus* leaves. *Environmental and Experimental Botany*, 91, 74–83.
- Velikova, V., Yordanov, I., & Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Science*, 151(1), 59–66.
- Veloso, L. L. De S. A., Capitulino, J. D., Soares De Lima, G., Vieira De Azevedo, C. A., Rodrigues Da Silva, A. A., & Gheyi, H. R. (2020). Methods of applying hydrogen peroxide to soursop seedlings irrigated with saline water. *Comunicata Scientiae*, 12
- Verbruggen, N., & Hermans, C. (2008). Proline accumulation in plants: A review. *Amino Acids*, 35, 753–759.
- Verma, S. R., Chaudhari, P. R., & Satyanarayanan, S. (2012). Impact of leaching from iron ore mines on terrestrial and aquatic environment. *International Journal of Environmental Sciences*, 2, 2378–2386.
- Xue, S. G., Wang, J., Wu, C., Li, S., Hartley, W., Wu, H., Zhu, F., & Cui, M. Q. (2018). Physiological response of *Polygonum perfoliatum* L. following exposure to elevated manganese concentrations. *Environmental Science and Pollution Research*, 25, 132–140.
- Yemm E. W., & Cocking F., C. (1955). The determination of amino acids with ninhydrin. *Analyst*, 80, 208–213.

- Zanandrea, I., Alves, J. D., Deuner, S., de FP Goulart, P., Henrique, P. D. C., & Silveira, N. M. (2010). Tolerance of *Sesbania virgata* plants to flooding. *Australian Journal of Botany*, 57(8), 661–669.
- Zheng, Y., Shen, L., Yu, M., Fan, B., Zhao, D., Liu, L., & Sheng, J. (2011). Nitric oxide synthase as a postharvest response in pathogen resistance of tomato fruit. *Post Harvest Biology and Technology*, 60, 38–46.

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