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## **Tolerance of Plants to Toxicity Induced by Micronutrients**

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Allan Klynger da Silva Lobato, Emily Juliane Alvino Lima, Elaine Maria Silva Guedes Lobato, Gabriel Mascarenhas Maciel and Douglas José Marques

Additional information is available at the end of the chapter

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### **Abstract**

Micronutrient elements such as zinc, boron copper, iron, manganese, molybdenum, and chlorine are frequently responsible by the regulatory activity of the cell organelles, being nutrients that are absorbed and found in lower concentrations in plant tissues, they also contribute to supply the nutritional exigency of the plant. Study with *Zea mays* plants exposed to Zn toxicity + Si presented significant increases in stomatal conductance, net photosynthetic rate, transpiration rate, and water use efficiency, respectively, in comparison with treatment only with Zn. In relation to chlorophylls a, b and total and carotenoids presented non-significant increases, when compared to plants exposed to Zn toxicity. This study revealed the positive contribution of the Si on gas exchange and reduction of the negative effects provoked on chlorophylls and carotenoids in maize plants under Zn toxicity. Other results described that prolonged exposure to excessive Cu resulted in serious toxic effects on the rice seedlings. In contrast, Tre pretreatment has been shown to be beneficial in alleviating Cu toxicity, which was mainly attributed to the ability of Tre to restrict Cu uptake and accumulation to maintain Cu homeostasis, and to induce production of antioxidant and Gly enzymes to alleviate excessive Cu-triggered oxidative stress. Stress caused by the excessive supply of micronutrients to plants frequently promotes repercussions on oxidant system, inducing the overproduction of reactive oxygen species. The oxidative damage is a situation characterized by the large ROS accumulation and insufficient detoxification promoted by antioxidant enzymes, such as catalase and glutathione peroxidase. Different mechanisms have been proposed to explain the tolerance of plants to toxicity induced by micronutrients, as uses of other elements and substances, in which it can positively act with specific transporters, metal ion homeostasis and compartmentalization of micronutrients into the vacuole.

**Keywords:** gas exchange, antioxidant metabolism, metals, photosynthetic pigments

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## 1. Introduction

Large amounts of minerals and organic materials can be found in soil [1]; and some of these materials have great significance in the anatomical development and plant morphology. The soil fertility is an important factor of agronomic order. It is related to provision of essential elements in the soil, where the plant will absorb adequate levels of important substances to work with its metabolism [2]. The nutrients are arranged in ionic form, which can interact with the root system of plants via three mechanisms: interception root, diffusion, or mass flow [3].

The plants require basic conditions to develop and supply the essential elements to aid with the metabolism and produce simple and/or complex compounds [4]. These are divided into non-minerals and minerals, which are sub-divided into macro and micronutrients.

## 2. Non-mineral nutrients

The non-mineral nutrients are hydrogen (H), oxygen (O), and carbon (C). These elements help in the formation of tissues. They are responsible for the composition of lipids, carbohydrates, proteins, nucleic acids, and hormones in plants [5]. Hydrogen (H) is the element that contributes to nitrogen fixation and photosynthesis; this is due to their reducing capacity molecules, being a nutrient assimilated from the water. Oxygen (O) is extracted from atmosphere and water, it is used during the process of cell respiration, where sugar transferred by the photosynthetic process is transformed into energy. The plant captures the Carbon (C) in the form of CO<sub>2</sub> from atmosphere. It is an organic nutrient with function to ensure the occurrence of physical, chemical, and biological cycles, ensuring the maintenance of energy reserves throughout vegetative growth.

## 3. Nutrients minerals

These are elements essential for plant to complete its cycle [6]. They are divided into macro and micronutrients [7].

### 3.1. Macronutrients

The macronutrients are composed by nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulphur (S). Large amount of these elements are required for plants to develop and meet its physiological activity. The macronutrients play a vital role in plant structure [8].

### 3.2. Micronutrients

These elements are responsible for the regulatory activity of the cell organelles. These nutrients are absorbed and found in lower concentrations in plant tissues and supply the nutritional exigency of the plant [9].

### 3.2.1. Zinc (Zn)

Zinc is an essential component in thousands of proteins in plants, in which it performs the role of enzymatic activator in various processes related to plant metabolism, such as dehydrogenases, aldolases, isomerases and transphorylases [10]. Its deficiency affects plant development, with stems and leaf expansion directly affected, and also reported to have consequences on chlorophyll levels and possibly necrosis [11].

### 3.2.2. Boron (B)

Boron is a nutrient that works with the carbohydrate metabolism and transport of sugars through membranes. This element acts in the formation of the cell wall, sap movement, cell division, development of leaves and inflorescence, and confer resistance to tissues [12]. Its deficiency causes disorder in meristematic activities and reserve mechanisms can be directly affected, with the stem apex, flowers and fruits suffer deformations [13].

### 3.2.3. Copper (Cu)

The copper activity is related to photosynthetic and respiration processes, fixation, and distribution of the nitrogen, all being necessary during plant cycle [14]. When the plant does not absorb adequate amount of this element for their development, it may present negative characteristics, such as changes of colour and structure in leaf [15].

### 3.2.4. Iron (Fe)

Iron is an essential micronutrient used in photosynthesis, cell division, nitrogen fixation, and formation and morphology of plants [16-17]. The excess of this element affects the leaf, and their growth is strongly reduced [18]. The deficiency induces chlorosis [19].

### 3.2.5. Manganese (Mn)

Manganese is a micronutrient of essential character. It plays the metabolic activities of direct or indirect orders, with capacity to activate enzymes responsible for the control of oxidation-reduction processes, such as the production of chlorophylls [20]. The low concentration of this micronutrient causes loss of leaf due to appearance of small spots on leaves and chlorosis, and structural development of the plant normally is compromised [21].

### 3.2.6. Molybdenum (Mo)

Molybdenum is a micronutrient that helps in nitrogen fixation and nitrate assimilation [22]. With the deficiency of this nutrient, plants are affected by chlorotic spots and marginal necrosis, thus the plant yield is negatively affected [23].

### 3.2.7. Chlorine (Cl)

Chlorine acts in the photosynthetic process of plants, aside from interacting with water and during transpiration process [24]. The lack of chlorine causes chlorosis and necrosis, roots

suffer atrophy, affecting the development of the plant [25]. The excess of chlorine in the soil is more common than the deficiency. Indication of its excess is signalled by the burn of the leaf edges [26].

#### 4. Toxicity of micronutrients

Despite micronutrients be required in higher plants, in higher concentrations frequently is toxic and provokes negative effects [27], as reduction in photosynthetic pigments [28], minor integrity and permeability of membranes [29], increase of the oxidative stress related with production and accumulation of reactive oxygen species (ROS), besides to increase the activities of antioxidant enzymes [30], and in levels more extremes to induce cell death [31].

Stress caused by the excessive supply of nutrients to plants promotes repercussion on oxidant system [32-33], inducing the overproduction of reactive oxygen species (ROS) as superoxide radical ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ) [34]. The oxidative damage is a situation characterized by the large ROS accumulation and insufficient detoxification promoted by antioxidant enzymes, such as catalase and glutathione peroxidase [35].

Different mechanisms have been proposed to explain the tolerance of plants to toxicity induced by heavy metals and nutrients. Two specific transporters are metal ion homeostasis and compartmentalization of metals into the vacuole [36-37]. However, responses linked to contribution of Si in plants submitted to Zn excess, more specifically on gas exchanges and photosynthetic pigments, are unknown.

Beneficial repercussions related to Si uses in higher plants are intensively found [38-40]. Isa et al. [41] reported that Si is largely accumulated in leaves. Silva et al. [42] described increases in chlorophylls produced by exogenous Si application. Si also induces higher mechanical resistance from cell wall [43]. Chen et al. [44] found better light reception and increasing net photosynthesis rate and  $CO_2$  capitation after Si treatment.

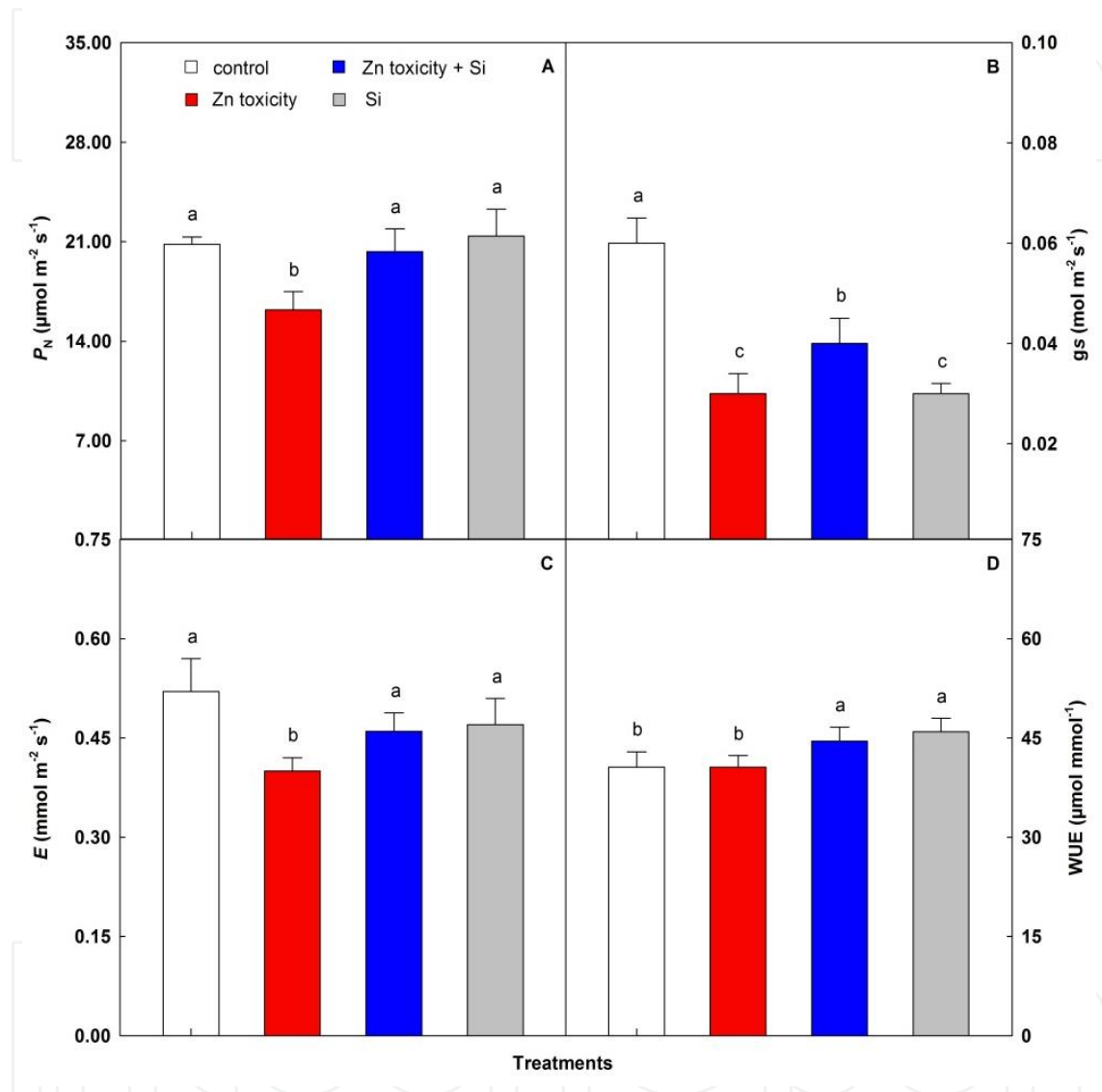
#### 5. Objectives

This chapter aim to: (i) define what nutrient toxicity is; (ii) present the modifications produced in the biochemical and physiological levels; (iii) explain the consequences to plant induced by the nutrient toxicity.

#### 6. Effects of zinc toxicity in gas exchange

Study conducted by Paula et al. [45] with *Zea mays* plants under Zn toxicity and treated with Si (silicon) evaluating the gas exchanges and photosynthetic pigments was shown in Figure 1. Zinc toxicity produced a negative interaction promoting stomatal closing, and consequently,

reduction in stomatal conductance. This effect can be attributed to Zn excess, in which it will induce a minor density and size of these structures, with structural differences in adaxial and abaxial sides, besides minor stomatal aperture sizes [46]. Similar results were obtained by Pavlíková et al. [47] in *Nicotiana tabacum* plants submitted to stress by Zn.



**Figure 1.** Stomatal Conductance (A), Photosynthesis Rate (B), Transpiration Rate (C), and Water Use Efficiency (D) in *Zea mays* plants subjected to silicon and zinc toxicity. Different letters to treatments indicate significant differences from the Skott-Knott test ( $P < 0.05$ ). Columns represent the mean values from four repetitions, and bars represent the standard deviations [45].

Zn induced a reduction in net photosynthetic rate, as explained by the stomatal limitation, arising of minor stomatal conductance, and consequent decrease of the CO<sub>2</sub> assimilation to photosynthetic process [48-49]. Similar results were found by Shi and Cai [50] working with *Arachis hypogaea* plants submitted to Zn stress, corroborating with results obtained in this research.

The reduction of the transpiration in plants under exogenous application of Zn was possibly attributed to decrease in stomatal conductance. This stomatal limitation reduces the transpiration rate, promoting minor water loss from plant to atmosphere, and consequently limited nutrients reposition, in form of adsorbed ions into substrate with water, using the via root system [51]. In other words, the transpiration is responsible with the dynamic of nutrient transport from substrate in direction root and leaf [52], thus avoid the cavitation in xylem [53]. Fernández et al. It was also described that there is a significant reduction in transpiration rate in *Populus deltoides* plants submitted to high Zn concentrations [54].

The exogenous application of Si promoted an increase in water use efficiency (WUE), this result can be explained by the increase in net photosynthetic rate ( $PN$ ) and maintenance in transpiration rate ( $E$ ). The ratio between photosynthesis and transpiration will result in WUE [55], being a physiological parameter that describes quantitatively the behavior momentaneous of the gas exchanges in leaf, it also reveals the efficiency that the plant utilizes the water resource [56]. Our results are corroborated by Moussa [57] working with *Zea mays* seedlings under exogenous application of Si.

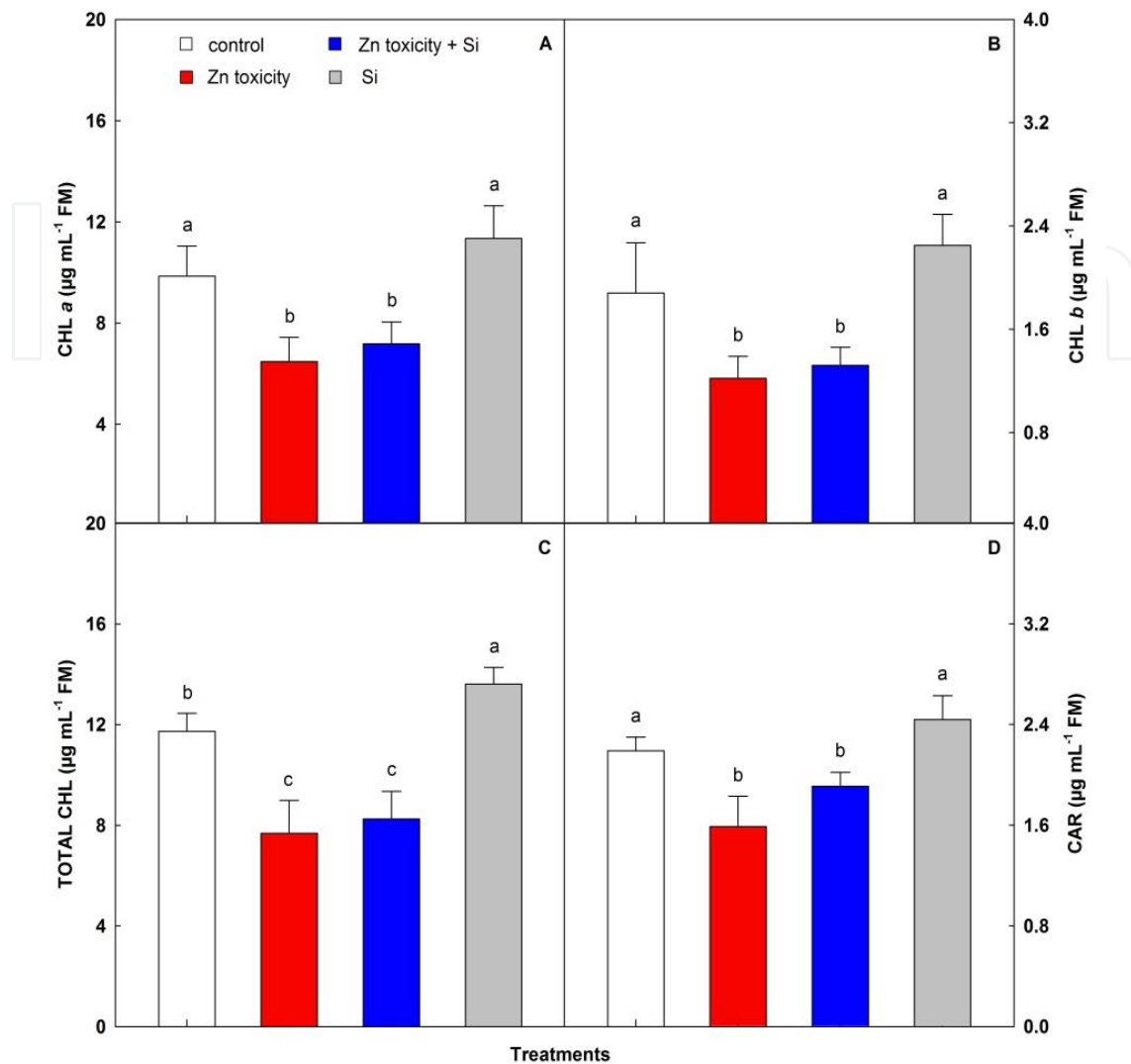
## 7. Zinc toxicity and silicon benefits on photosynthetic pigments

Figure 2 shows the same study conducted by Paula et al. [45] on photosynthetic pigments in *Zea mays* plants under Zn toxicity and Si. The decrease in CHL  $a$  occurred due to Zn toxicity, being probably related to minor biosynthesis rate of CHL  $a$  [58]. The Zn excess negatively interferes in NADPH availability into chloroplasts [59], because the NADPH is one of the substrates of the divinyl chlorophyllide an 8-vinyl-reductase enzyme, which is responsible to CHL  $a$  synthesis [60].

The chlorophylls are responsible to the photochemical and biochemical reactions during light capitation [61], while carotenoids present an important role related to photoprotection against excessive sunlight [62], given that both pigments work simultaneously into photosynthetic machinery [63]. However, the excessive B supply represents a problem to photosynthetic pigments, with consequent decrease in chlorophylls [64-65].

The Zn excess promoted a decrease in CHL  $b$  level, and this result can be explained by the oxidative stress induced by the overproduction of reactive species oxygen (ROS), such as  $H_2O_2$  [66-67]. The  $H_2O_2$  aside from being toxic in chloroplasts, is considered an inhibitor of the carbon metabolism, [68], resulting in acceleration of leaf senescence through of the lipid peroxidation and oxidative damages [69]. Similar results were reported by Bettaieb [70] evaluating *Solanum tuberosum* cultivars.

The CHL total levels were reduced after Zn toxicity, which is related to magnesium (Mg) substitution in molecule of chlorophyll by the Zn. It will result to the inadequate work of the light-harvesting complex (LHCII), and consequently the photosynthesis limitation [71-73]. Our results on reduction in CHL total were corroborated by Bassi and Sarma [74] in *Triticum aestivum* seedlings.

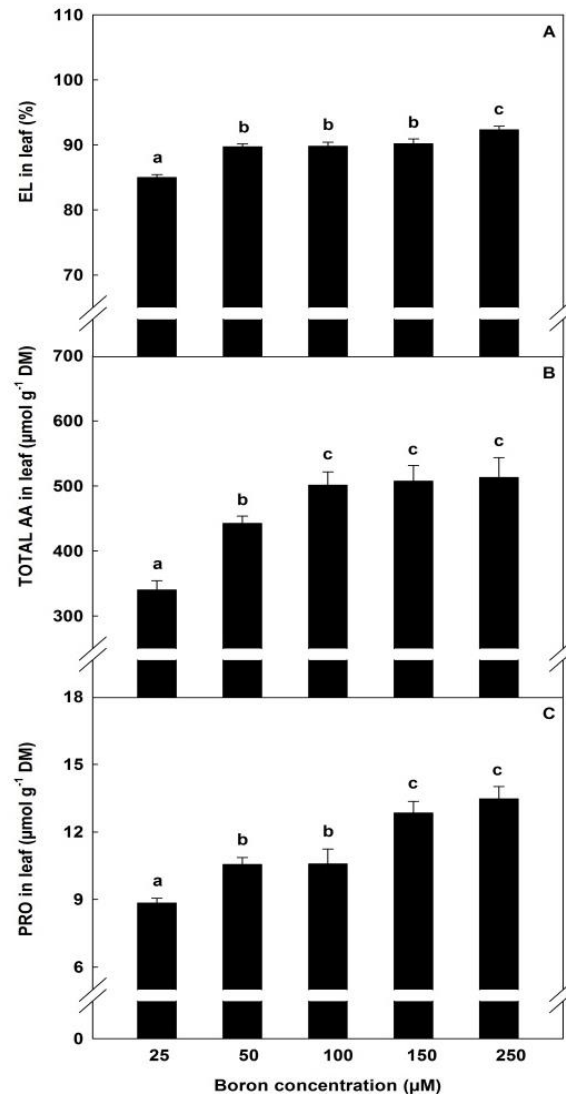


**Figure 2.** Chlorophyll *a* (A), Chlorophyll *b* (B), Total Chlorophyll (C) and Carotenoids (D) in *Zea mays* plants subjected to silicon and zinc toxicity. Different letters to treatments indicate significant differences from the Skott-Knott test ( $P < 0.05$ ). Columns represent the mean values from four repetitions, and bars represent the standard deviations [45].

## 8. Boron toxicity induces modifications in nitrogen metabolism

Silva et al. [75] working with *Schizolobium parahyba* plants exposed to progressive boron levels determined amino acids total and proline is depicted in Figure 2. The total amino acids accumulated in leaf must be associated to proteases enzyme activity and concomitantly to inhibition of the protein biosynthesis rate, due to the high B levels. According to Paula et al. [76], the increases in proteases activities promote the breakdown and consequent decrease of proteins, increasing the total AA amount, which will be used in synthesis of other AA, like as PRO. Cervilla et al. [77] evaluating the effects of the B toxicity in *Solanum lycopersicum* plants found similar results.





**Figure 3.** Electrolyte Leakage (A), Total Amino Acids (B), Proline (C) of young *Schizolobium parahyba* var. *amazonicum* plants subjected to boron toxicity. Different letters for boron levels indicate significant differences from the Skott-Knott test ( $P < 0.05$ ). Columns represent the mean values from 5 repetitions, and bars represent the standard deviations [75].

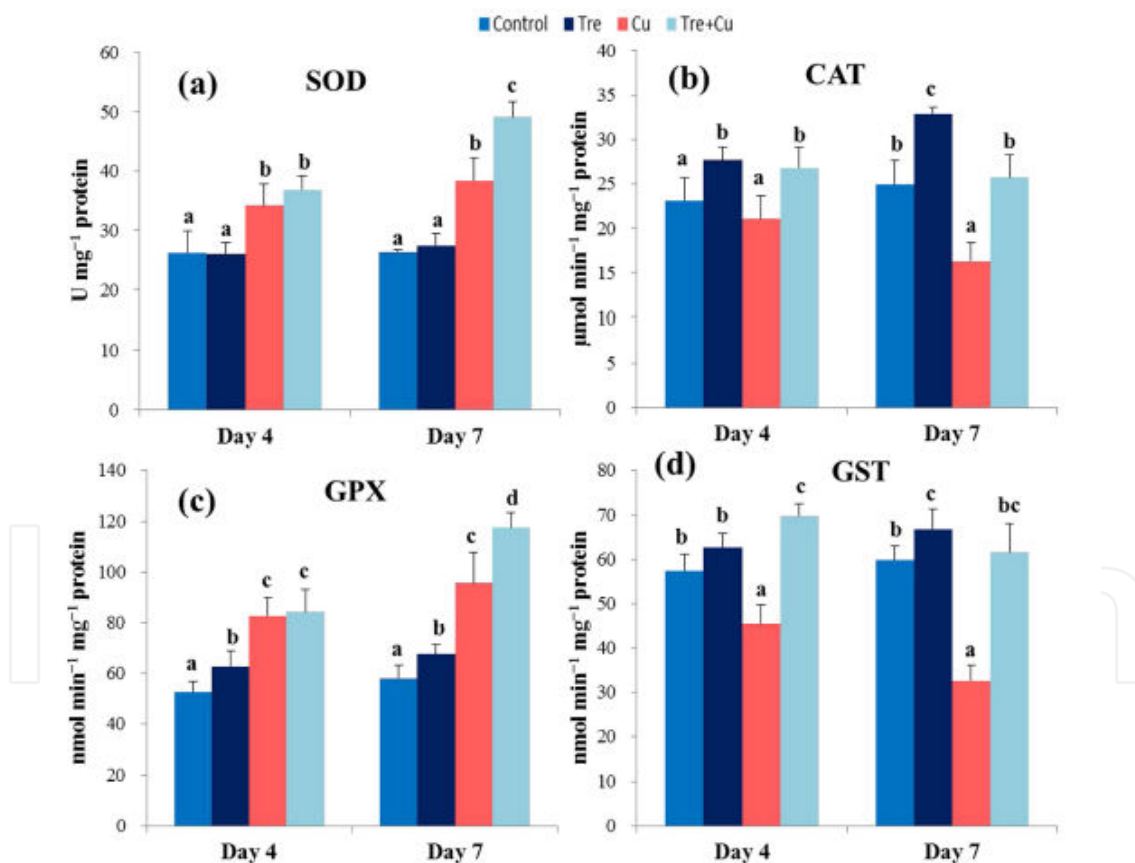
The proline concentration was maximized and this result is related to the amino acid acting in detoxification process of ROS and membrane protection against lipid peroxidation [78-79]; associated with ROS antagonist, it aims to attenuate the oxidative stress and to avoid the cell death. In other activities, the PRO protects the protein structure against denaturation and it will stabilize the cell membranes during interaction with phospholipids [80]. Contreras et al. [81] evaluating the B and NaCl effects in *Solanum lycopersicum* plants verified similar results of this research.

Proline is an amino acid synthesized into nitrogen metabolism with functions related to osmoprotection [82] and cellular homeostasis [83], which can contribute to improve the plant tolerance under situations of abiotic stress, as B toxicity.

## 9. Effects of the copper toxicity and exogenous trehalose on antioxidant enzymes

In agreement with Mostofa et al. [84], the regulating Cu homeostasis is crucial in maintaining the intracellular Cu level to avoid toxicity. Plants have developed various mechanisms to restrict Cu toxicity, such as inhibition of Cu uptake by binding with root exudates like organic acids, intracellular sequestration by strong ligands like cysteine-rich compounds and phytochelatins, and exclusion of excessive Cu from the cells by sugar alcohols like trehalose (Tre) [26–28]. Tre, a non-reducing disaccharide of glucose, protects plant cells against long-term desiccation by stabilizing enzymes, proteins, and biological membranes under dehydration [29].

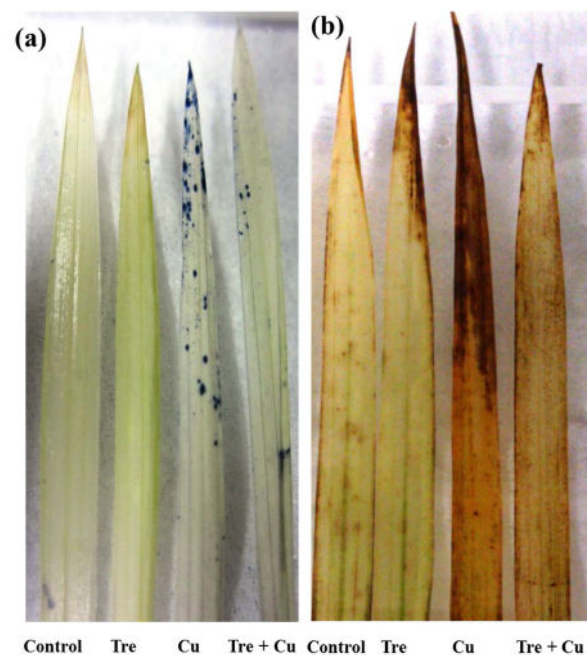
In relation to antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GST), the pretreatment with non-reducing disaccharide Tre induces increases in enzyme activities when compared with control or Cu stress treatments (Figure 4).



**Figure 4.** Effect of exogenous trehalose on the activities of antioxidant enzymes in rice seedlings with or without Cu stress. (a) Superoxide Dismutase (SOD), (b) Catalase (CAT), (c) Glutathione Peroxidase (GPX), and (d) Glutathione Reductase (GST). Control, Tre, Cu, and Tre + Cu correspond to control, 10 mM trehalose, 100 μM CuSO<sub>4</sub>, and 10 mM trehalose + 100 μM CuSO<sub>4</sub>, respectively. Bars represent standard deviation (SD) of the mean (n = 3). Different letters indicate significant differences among treatments at p < 0.05, according to Duncan's multiple range test [84].

NBT staining indicated an increased amount of  $O_2^-$  as scattered dark blue spots in the leaf plate of the Cu-stressed seedlings compared with the non-treated control (Figure 5a). Similarly, DAB staining confirmed a marked increase in brown polymerization products, which indicated the over-accumulation of  $H_2O_2$  in the leaves of the Cu-stressed seedlings relative to control (Figure 5b) [84].

Results described by Mostofa et al. [84] indicate that prolonged exposure to excessive Cu resulted in serious toxic effects on the rice seedlings. In contrast, Tre pretreatment has been shown to be beneficial in alleviating Cu toxicity, which was mainly attributed to the ability of Tre (i) to restrict Cu uptake and accumulation to maintain Cu homeostasis, and (ii) to induce production of antioxidant and Gly enzymes to alleviate excessive Cu-triggered oxidative stress [84].



**Figure 5.** Effect of exogenous trehalose on ROS accumulation in leaves of rice seedlings with or without Cu stress. (a) Superoxide ( $O_2^-$ ) and (b) Hydrogen Peroxide ( $H_2O_2$ ) production in rice leaves were detected using nitro-blue tetrazolium (NBT) solution and 3,3'-diaminobenzidine (DAB), respectively, at day 7 of Cu stress. Control, Tre, Cu, and Tre + Cu correspond to control, 10 mM trehalose, 100  $\mu$ M  $CuSO_4$ , and 10 mM trehalose + 100  $\mu$ M  $CuSO_4$ , respectively [84].

## 10. Final considerations

Micronutrient elements such as zinc, boron copper, iron, manganese, molybdenum, and chlorine are responsible to the regulatory activity of the cell organelles. These nutrients are absorbed and found in lower concentrations in plant tissues, and contribute to supply the nutritional exigency of the plant.

Study with *Zea mays* plants exposed to Zn toxicity + Si presented more significant increases in stomatal conductance, net photosynthetic rate, transpiration rate, and water use efficiency

compared with the treatment only with Zn. On the contrary, chlorophylls a, b and total and carotenoids presented non-significant increases when compared with plants exposed to Zn toxicity.

This study also revealed the positive contribution of the Si on gas exchange and reduction of the negative effects provoked on chlorophylls and carotenoids in *Zea mays* plants under Zn toxicity. Other result described that prolonged exposure to excessive Cu resulted in serious toxic effects on the rice seedlings. In contrast, Tre pretreatment has been shown to be beneficial in alleviating Cu toxicity, which was mainly attributed to the ability of Tre to restrict Cu uptake and accumulation to maintain Cu homeostasis. It induced production of antioxidant and Gly enzymes to alleviate excessive Cu-triggered oxidative stress.

Stress caused by the excessive supply of micronutrients to plants promotes repercussion on oxidant system, inducing the overproduction of reactive oxygen species. The oxidative damage is a situation characterized by the large ROS accumulation and insufficient detoxification promoted by antioxidant enzymes, such as catalase and glutathione peroxidase. Different mechanisms have been proposed to explain the tolerance of plants to toxicity induced by micronutrients, as uses of other elements and substances, in which it can positively act with specific transporters, metal ion homeostasis and compartmentalization of micronutrients into the vacuole.

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## Author details

Allan Klynger da Silva Lobato<sup>1\*</sup>, Emily Juliane Alvino Lima<sup>1</sup>, Elaine Maria Silva Guedes Lobato<sup>1</sup>, Gabriel Mascarenhas Maciel<sup>2</sup> and Douglas José Marques<sup>3</sup>

\*Address all correspondence to: [allanllobato@yahoo.com.br](mailto:allanllobato@yahoo.com.br)

1 Núcleo de Pesquisa Vegetal Básica e Aplicada, Universidade Federal Rural da Amazônia, Paragominas, Brazil

2 Instituto de Ciências Agrárias, Universidade Federal de Uberlândia, Monte Carmelo, Brazil

3 Setor de Olericultura e Experimentação, Universidade José do Rosário Vellano (UNIFENAS), Alfenas, Brazil

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