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Copper (Cu) stress affects carbon and antioxidant metabolism in *Coffea arabica* seedlings

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Abstract

Although copper is a micronutrient essential for the normal development of plants, both insufficient and supra optimal doses can disrupt the functioning of metabolism and the production of biomass. To study the biochemical and physiological impacts of deficiency and excess of copper in coffee, we treated 6-month-old seedlings of *Coffea arabica* L. Catuaí cultivar to three copper treatments: control (0.03 ppm), excess (0.12 ppm) and deficiency (0 ppm) for 60 days. The changes in levels of photosynthetic pigments, biomass allocation, carbohydrate partitioning, antioxidant system and proline levels were evaluated. Under deficiency and excess of copper coffee seedlings showed lower levels of chlorophyll, reduction on dry weight of shoot, lower sugar levels and higher content of hydrogen peroxide. We also observed increased levels of proline and enzymatic activity of the antioxidant system, providing conditions for the reduction of oxidative stress triggered by nutritional imbalance. In general, the results showed that coffee plants invest in antioxidant defense system as an alternative to maintain redox balance when exposed to deficiency or excess copper. However, it is not effective to prevent an increase in lipid peroxidation. Authors may indicate an optimum range for application of copper in coffee.

Keywords: Antioxidant system, Proline, Carbohydrate.
Abbreviations: APX_Ascorbate peroxidase; CAT_Catalase; MDA_Malondialdehyde, SOD_Superoxide dismutase.

Introduction

Copper (Cu) is an essential element for the plants growth. The Cu occurrence is related to the presence of several minerals and compounds in the soil. These copper compounds are generally complexed in the form of organic compounds with low molecular weight (Marschner, 2011). Copper acts as a cofactor for numerous enzymes involved in the cellular redox state (Cohu and Pilon, 2010). In addition that Cu participate as a constituent of plastocyanin, a protein that carries electrons during photosynthesis, and superoxide dismutase (SOD) an enzyme involved in detoxification of reactive oxygen species (ROS). Copper comprises one of the superoxide dismutase isoforms, Cu/Zn SOD and S-glutathione transferase (Ravet and Pilon, 2013; Yruela, 2009). Therefore, copper is an essential micronutrient for the normal development of plants. However, infra-optimal doses of Cu cause symptoms of deficiency and dysfunction of various metabolic processes. On the other hand, supra-optimal doses are potentially toxic and may disrupt metabolic functioning and the production of plant biomass (De Kok et al., 2015).

In *Arabidopsis thaliana* copper deficiency causes symptoms of chlorosis, which is initially observed in young leaves, and may evolve necrotic lesions (Abdel-Ghany and Pilon, 2008). In *Medicago sativa*, copper deficiency causes reduced expression of genes associated with the cell wall (Printz et al., 2016). Plants grown under copper insufficiency show signs of depletion in electron transport chain of photosynthesis, leading to a reduction in non-photochemical electron extinction, mainly due to impairment of plastocyanin (Abdel-Ghany and Pilon, 2008).

On the other hand, copper excess triggers phenotypic changes, such as reduced root and shoot development, reduced cell viability at root ends and premature induction of root lignification (Lequeux et al., 2010). The excess copper can also be accumulated in the cell walls inducing the formation of tracheids, which in xylem vessels are related to the greater activity of the enzymes involved in lignin biosynthesis, called peroxidase (Bouaziziet al., 2011). In addition, both excess and deficiency of Cu^{2+} ions can induce formation of reactive oxygen species (ROS), leading to the formation of hydrogen peroxide in the cell walls, causing loosening of the cell wall (Fry et al., 2002). In addition, EROs damage the structure of the photosynthetic apparatus, causing damage to the lipid membranes, and possible extravasation of the cellular content (Yamamoto et al, 2008). High levels of copper in the cells may lead to the replacement of the Mg^{2+} in the center of the chlorophyll molecule, giving rise to the so-called cupric chlorophyll. Due to this low photosynthetic assimilation, there will be a reduction in sucrose synthesis and deprivation in the supply of soluble sugars to support the metabolism. These di-saccharides can be hydrolysed to hexoses and allocated to reserve organs for later use (Hammond and Whiter, 2008).

To control these imbalances caused by low photosynthetic assimilation and to maintain the cellular redox state, plants have a complex antioxidant defense system. The first line of defense is performed by the enzyme superoxide dismutase

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(SOD), which dismantles the radical O_2^- a H_2O_2 and O_2. The catalase activity (CAT) converts H_2O_2 to H_2O and O_2 (Capaldi et al., 2015). Moreover, several others enzymes contribute to the control of damage caused by ROS, including those present in the ascorbate-glutathione cycle, such as ascorbate peroxidase (APX) (Jiménes et al., 1998). In studies with *Citrus sinensis* rootstocks, it was demonstrated that low and high doses of copper reflect significant increases in SOD, CAT and peroxidases activities, which evidence for increased ROS production triggered by nutritional disturbances of copper (Hipper et al., 2016). In addition to this mechanism of enzymatic defense, plants have a non-enzymatic antioxidant enzyme mechanism, involving the accumulation of organic solutes such as proline, which maintain the osmotic adjustment (Asharaf and Harris, 2004; Burritt, 2012).

Due to the global importance of coffee, studies about the physiology of this species under micronutrients deficiency and excess is lacking. In addition, there are no studies that report the adverse effects of copper deficiency or excess on the physiological and biochemical of coffee plants. The objective of this work was to understand the physiological responses of *Coffea arabica* L. seedlings, such as photosynthetic pigment levels, biomass allocation, carbohydrate partitioning, antioxidant system, proline levels and damage to cell membranes under conditions of deficiency and excess of copper.

Results

Chlorophyll and carotenoids concentration

Plants subjected to 0 and 0.12 ppm Cu had lower chlorophyll levels (Table 1) than control plants from the 30th day. In addition, plants exposed to 0 and 0.12 ppm Cu had 34 and 20 % less chlorophyll than control plants.

Exposure of plants to exclusion and 0.12 ppm Cu resulted in reduction of carotenoids in leaf (Table 1) from the second evaluative stage. At the 60th day, coffee plants exposed to 0 and 0.12 ppm Cu showed a decrease of 30 and 14% in the concentration of carotenoids.

Dry weight accumulation

The shoots dry weight (Fig. 1A) of plants subjected to 0 and 0.12 ppm Cu was reduced by 35 and 13%, 60 days after treatments. On the other hand, the dry root weight (Fig. 1B) was unaffected by different copper concentrations.

Carbohydrate concentration

Plants subjected to 0 and 0.12 ppm Cu have shown a decrease of total soluble sugar in leaf concentrations (Fig. 2A). However, at 0 ppm Cu, this reduction has occurred since the 30th day, whereas in the 0.12 ppm Cu it was observed only at 60th day. Therefore, after 60 days, plants exposed to 0 and 0.12 ppm Cu had 36 and 42% less leaf total soluble sugar than control plants. In roots of plants exposed to 0 ppm Cu, a decrease in leaf levels of total soluble sugars was observed (Fig. 2B).

The foliar starch level (Fig. 2C) in plants exposed to 0 and 0.12 ppm Cu was increased in the 30th day and decreased in the 60th day. Thus, in the last experimental time, leaf starch concentration was 27 and 32 % less than in control plants, respectively. On the other hand, starch levels in roots (Fig. 2D) of plants under 0 and 0.12 ppm Cu increased since 30th day, achieving in the 60th day increments of 96 and 73 %.

Antioxidant system

Hydrogen peroxide level was increased in leaves (Fig. 3A) and roots (Fig. 3B) of plants exposed to 0 and 0.12 ppm Cu. Thus, in the last experimental time, H_2O_2 production in leaves of plants under 0 and 0.12 ppm Cu raised 50 and 29 % and in roots in 64 and 20 %, respectively. Similar to H_2O_2 concentration, proline was increased in shoots (Fig. 3C) and roots (Fig. 3D) of plants subjected to 0 and 0.12 ppm Cu.

Increases in leaf SOD activity (Table 2) were observed in plants exposed to 0 and 0.12 ppm Cu from the 30th day. After 60 days, this increase was 19% in plants under 0 ppm Cu and 8% in those at 0.12 ppm Cu. On the other hand, this raise happened from the 30th day just in roots of plants under 0.12 ppm Cu, achieving an increase of 81 % in plants under 0 ppm Cu and 18 % in those under 0.12 ppm Cu at the 60th day.

CAT activity (Table 2) in leaves was unaffected by 0.12 ppm Cu, while plants submitted to 0 ppm showed an increment of 30 % in CAT activity at the 60th day. Nevertheless, this increase was happened in the roots of plants exposed to 0 and 0.12 ppm Cu since 30th day.

Leaves of plants exposed to 0 and 0.12 ppm Cu showed an increase in APX activity (Table 2) at the 30th day. However, only plants under 0 ppm maintained this increment in leaves APX activity until 60 days. On the other hand, the increase in APX activity was happened in roots since 30th day only for plants exposed to 0 ppm Cu. At 60th day, roots of plants subjected to 0 and 0.12 ppm Cu have shown an increase of 150 and 122 % in APX activity.

Lipid peroxidation (Table 2) was higher in leaves and roots of plants under 0 and 0.12 ppm Cu since 30th day. At 60th day, this increase in leaves was 177 % in plants under 0 ppm Cu and 26 % in plants subjected to 0.12 ppm Cu. On the other hand, lipid peroxidation level was increased by 79% in roots of plants under 0 ppm Cu and 172 % in plants exposed to 0.12 ppm Cu.

Discussion

Due to deficiency and excess copper in coffee seedlings cv. Catuaí, a significant reduction in leaf dry weight production was observed 60 days after treatment induction (Fig. 1). Inhibition of cellular metabolic processes are observed in plants subjected to copper stress, which culminate in a reduction of biomass accumulation (Dey et al., 2014; Elleuch et al., 2013; Jouili and Ferjani, 2003). In addition, growth reduction due to the inadequate supply of copper results from several physiological changes such as mitotic disturbance (Bouazizi et al., 2010), decrease in auxin synthesis (Yuan et al., 2013), reduced photosynthetic activity (Droppa et al., 1987; Henriques, 1989) and lower carbohydrate synthesis (Yruela, 2009). At the end of the experiment, decrease in leaf biomass was also accompanied by a significant reduction of soluble sugars (Fig. 2a) and starch (Fig. 2c), which may have compromised the carbon allocation to shoot from roots when compared to coffee seedlings under normal conditions. At the end of the experiment, it was observed that copper stress did not interfere in dry weight production of roots, compared to control, indicating that plant developed efficient mechanisms to support these copper concentrations.

Despite the presence of stress, the dry weight of roots was constant compared to leaves under conditions of deficiency and excess. As a result of these findings, we can associate biomass preservation in roots to the progressive accumulation of starch from 30 days after induction of treatments (Fig. 2d).

Table 1. Effect of different copper concentrations in leaf and roots copper levels, chlorophyll and carotenoids concentration in *Coffea arabica* L. plants, at 0, 30 and 60 days after treatments induction. Capital letters compare treatments (0.03, 0 and 0.12 ppm of copper) at each sampling time; lowercase effect between times (0, 30 and 60 days) within each treatment. Different letters indicate significant differences with 0.05 probability.

	Cu ppm			Chlorophyll µg g^{-1} FW	Carotenoids µg g^{-1} FW
Days	Cu ppm	Leaf	Root		
0	0.03	18 Aa	19 Aa	2991 Ac	564 Ac
	0.00	18 Aa	19 Aa	2991 Aa	564 Aa
	0.12	18 Ac	19 Ac	2991 Aa	564 Ab
30	0.03	20 Ba	28 Ba	4166 Aa	847 Aa
	0.00	11 Cb	6 Cb	2951 Ba	551 Ba
	0.12	28 Ab	62 Ab	2926 Ba	459 Cc
60	0.03	22 Ba	28 Ba	3636 Ab	748 Ab
	0.00	12 Cb	6 Cb	2402 Cb	526 Ca
	0.12	42 Aa	107 Aa	2915 Ba	643 Ba

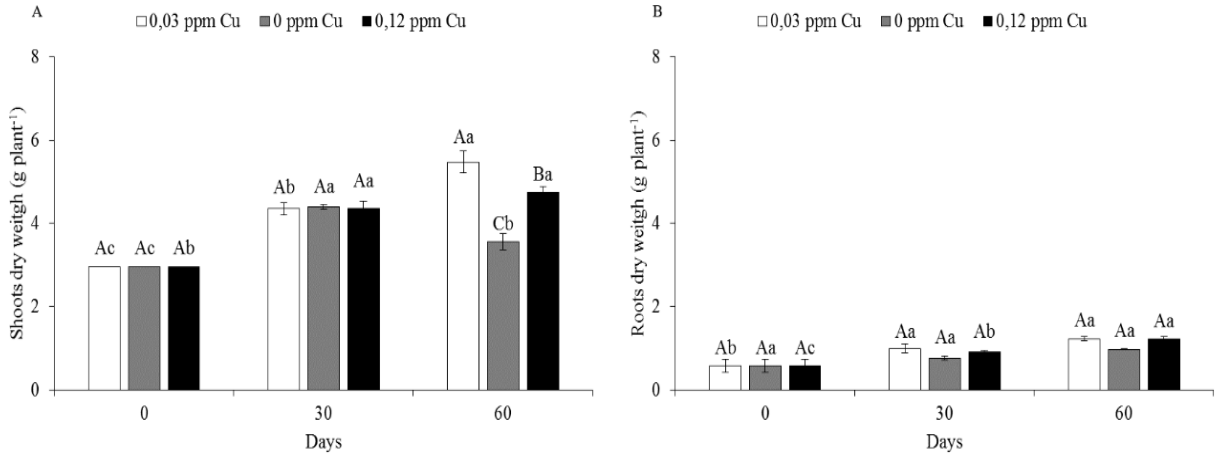

Fig 1. Effect of different copper concentrations on dry weight of shoots (A) and roots (B) of *Coffea arabica* L. plants. Capital letters compare treatments (0.03, 0 and 0.12 ppm of copper) at each sampling time; lowercase effect between times (0, 30 and 60 days) within each treatment. Different letters indicate significant differences with 0.05 probability. Bars correspond to the mean standard error.

Table 2. Effect of different copper concentrations on superoxide dismutase, catalase and ascorbato peroxidase activity and in lipid peroxidation in leaf and root of *Coffea arabica* L. plants. Capital letters compare treatments (0.03, 0 and 0.12 ppm of copper) at each sampling time; lowercase effect between times (0, 30 and 60 days) within each treatment. Different letters indicate significant differences with 0.05 probability.

		SOD U mg^{-1} protein		CAT µmol H_2O_2 min^{-1} mg^{-1} protein		APX µmolAsA min^{-1} mg^{-1} protein		Lipid peroxidation ηmol MDA g^{-1} FW	
Days	Cu ppm	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
0	0.03	1257 Ab	491 Ab	4 Ac	3 Aa	21 Aa	17 Aa	500 Aa	72 Aa
	0.00	1257 Ac	491 Ab	4 Ab	3 Ab	21 Ab	17 Ab	500 Ac	72 Ac
	0.12	1257 Ab	491 Ac	4 Ac	3 Aa	21 Aa	17 Ab	500 Ab	72 Ac
30	0.03	1594 Ba	471 Bb	7 Ba	2 Ba	18 Ba	13 Bb	540 Ba	66 Ba
	0.00	1714 Ab	508 Bb	7 Aa	4 Aa	23 Ab	27 Aa	767 Ab	95 Ab
	0.12	1674 Aa	568 Ab	7 Ba	3 Aa	23 Aa	15 Bb	699 Aa	110 Ab
60	0.03	1574 Ca	547 Ca	6 Bb	3 Ba	21 Ba	10 Cc	524 Ba	80 Ca
	0.00	1875 Aa	995 Aa	7 Aa	4 Aa	43 Aa	25 Aa	1454 Aa	144 Ba
	0.12	1703 Ba	645 Ba	5 Bb	4 Aa	21 Ba	22 Ba	662 Ba	218 Aa

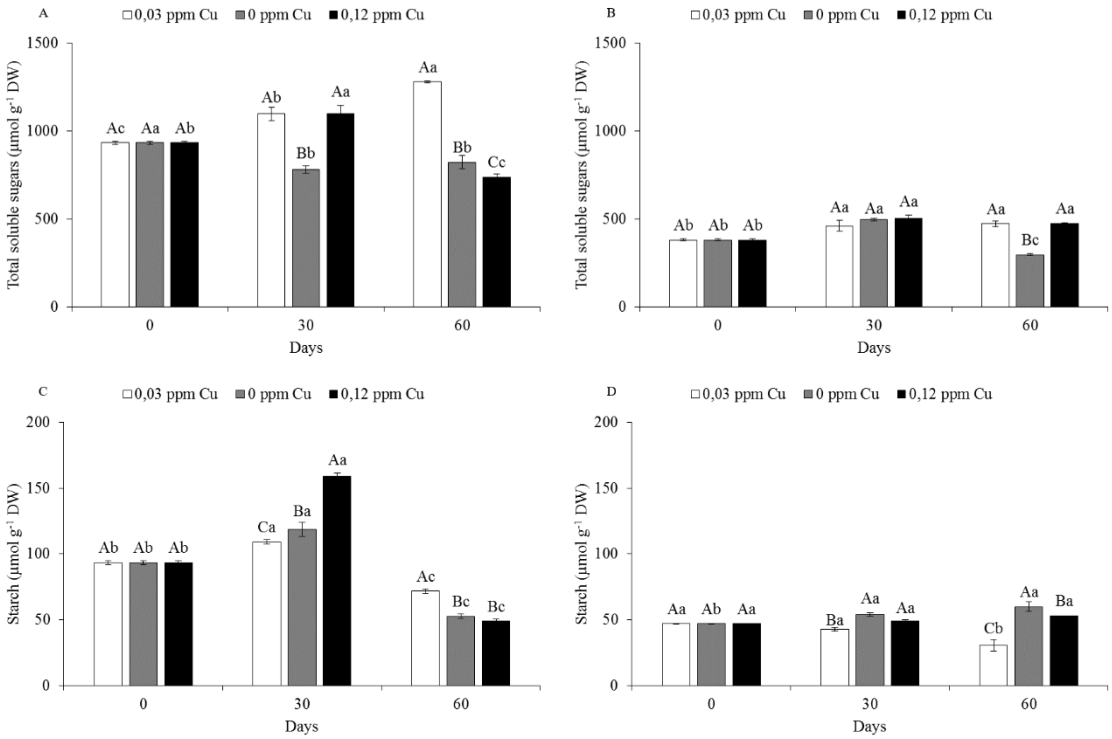

Fig 2. Effect of different copper concentrations on total soluble sugars and starch concentrations in shoots (A and C) and roots (B and D) of *Coffea arabica* L. plants. Capital letters compare treatments (0.03, 0 and 0.12 ppm of copper) at each sampling time; lowercase effect between times (0, 30 and 60 days) within each treatment. Different letters indicate significant differences with 0.05 probability. Bars correspond to the mean standard error.

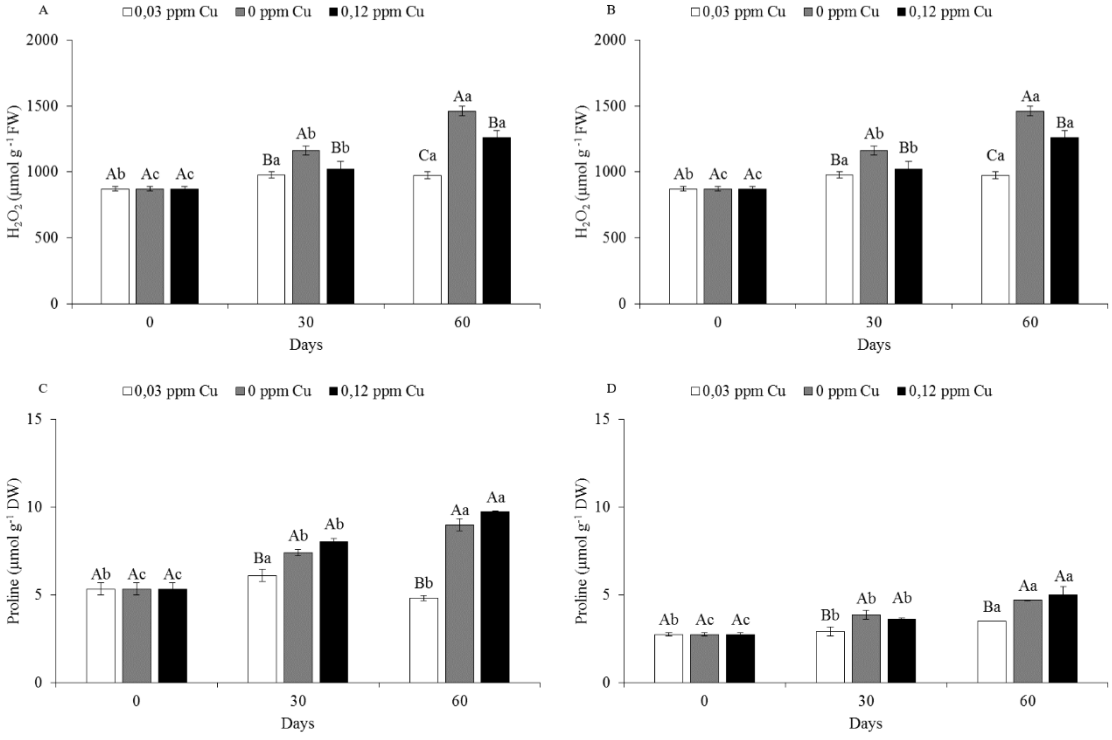

Fig 3. Effect of different copper concentrations on hydrogen peroxide and proline concentrations in shoots (A and C) and roots (B and D) of *Coffea arabica* L. plants. Capital letters compare treatments (0.03, 0 and 0.12 ppm of copper) at each sampling time; lowercase effect between times (0, 30 and 60 days) within each treatment. Different letters indicate significant differences with 0.05 probability. Bars correspond to the mean standard error.

and total soluble sugars (Fig. 2b) in plants subjected to supra-optimal doses of copper.

In Poaceae, an increase in soluble sugars content under copper stress was observed (Chai et al., 2011, 2014), suggesting the role of these molecules in cell protection. In addition, these carbohydrates are considered important organic metabolites that improve tolerance to osmotic stress and protect against photo-damage (Rodrigues-Calcerrada et al., 2011).

In leaves, chlorophyll and carotenoids levels were reduced due to deficiency and excess copper, compared to control. The results are in agreement with those observed in studies with *Spartina alterniflora* and *Astragalusneo-mobayenii*, in which a decrease in the photosynthetic pigments content induced by the increase of copper concentration (Chai et al., 2014; Karimi et al., 2012). In *Hordeum vulgare* L. we demonstrated that the excess of Cu^{2+} inhibits the chlorophyll and carotenoids biosynthesis and impairs the incorporation of these pigments into photosystems (Caspi et al., 1999). In addition to this effect, copper competes with iron uptake because it possesses a similar assimilation pathway, as it is observed a reduction in chlorophyll content due to iron deficiency induced by excess copper (Tanyolaç et al., 2007). Copper can also replace the Mg ion at the central position in chlorophyll molecule, leading to inhibition of chlorophyll synthesis (Yruela, 2009; Boojar; Goodarzi, 2007). Peng et al. (2013) also observed a reduction in photosynthetic pigments content under copper deficiency. Also, Droppa et al. (1987), concluded that these conditions cause a change in thylakoid membrane and modify the environment on the acceptor side of photosystems II. In addition, the reduction in pigment content is accompanied by reduction of plastoquinone synthesis.

Exposure of coffee seedlings to different copper concentrations altered cellular homeostasis, leading to higher generation of reactive oxygen species (ROS). In this sense, although higher H_2O_2 production occurred in plants with deficiency and excess copper than in control plants, H_2O_2 levels were higher in deficient plants at the end of the experiment (Fig. 2A and 2B). Similar results were observed in *Phaseolus vulgaris* (Bouazizi et al., 2010), *Morusalba* (Tewari et al., 2006), *Beta vulgaris* (Henriques, 1989) and *Nicotiana tabacum* (Raeymaeker et al., 2003). H_2O_2 acts as a signal in response to plant stress and in supra-optimal concentrations becomes toxic to membranes, affecting its permeability, which can lead to cell death (Soares et al., 2016; Capaldi et al., 2015).

Increase of ROS concentration leads to lipid peroxidation, causing membrane damage, extravasation of electrolytes, loss of protein function and ion transport channels. Malondialdehyde (MDA), a lipid peroxidation product, is considered an indicator of cellular damage under stress conditions. In spite of the increase in ROS levels, the level of MDA was increased substantially at the end of the experiment (Table 2), indicating severe damage to membranes in coffee plants exposed to Cu stress. Our observation is in agreement with previous reports (Thounaojam et al., 2013, Mostofa et al., 2014) suggesting that the production of ROS induced by Cu is the main cause of lipid peroxidation.

Generally, stress conditions due to lack or excess of nutrients in plants are related to the increase in antioxidant system activity (Li et al., 2013; Michael; Krishnaswamy, 2011; Jain et al., 2010). The increase in the detoxifying activity of enzymes of antioxidant system can be attributed, in large part, to the role of these compounds in ROS neutralization, contributing to cellular homeostatic metabolism (höller et al., 2014; Tripathi; Gaur, 2004).

In coffee seedlings, the variation of copper concentrations significantly altered SOD, CAT and APX activity in roots (Table 2). In leaves, changes in SOD levels were observed in both deficiency and excess treatments, whereas CAT and APX enzymes only changed in seedlings submitted to copper deficiency (Table 2).

Copper deficiency in coffee seedlings showed an increase in enzymatic activity of the superoxide dismutase, when compared to plants under normal copper supply. This result can be justified by the compensation of activity of other isorforms. This fact was observed in works carried on *Citrus sinensis*, when supplemented with low copper concentration, a lower Cu/Zn-SOD, but showed an increase in Mn-SOD activity, a mitochondrial SOD, which compensated for the reduction of Cu/Zn-SOD activity (Hipper et al., 2016).

Arabidopsis thaliana showed an increase in Fe-SOD isoform activity under the same condition. In chloroplasts, a compensation mechanism of superoxide radical elimination was observed, due to the reduction of Cu/Zn-SOD activity (Yamasaki et al., 2008).

In coffee seedlings exposed to excess copper, high SOD activity (Table 2) may be related to Cu/Zn-SOD isoform, since the cofactor for its action was in greater availability. Plants of *Citrus sinensis* submitted to high cupper levels showed a greater Cu/Zn-SOD and Mn-SOD activity, which was responsible by superoxide radical removal (Hippler et al., 2016).

The increase in CAT and APX activity in leaves and roots of Cu-deficient seedlings is related to the need to neutralize the high H_2O_2 content of (Fig. 2A and 2B), avoiding possible cellular damages. Similar results were also observed in *Oriza sativa* L. (Dionisio-sese and Tobita 1998) and in *Zea mays* L. (Tanyolac et al., 2006).

However, it was observed that CAT and APX activity was maintained in coffee leaves under copper excess, and an increase in CAT and APX activity in roots under the same conditions. In studies with *Ipomoea batatas* L. plants, it was verified that roots was the main organ that mostly affected by the increase in copper concentration, due to a higher activity of antioxidant enzymes accompanied by the increase of H_2O_2 production (Cuchiara et al., 2015).

Another mechanism used by plant cells under stress conditions is the synthesis of low molecular weight compounds that, besides acting on osmoregulation, may present antioxidant action (Verlues; Sharma, 2010). Under these conditions, the enzymatic systems (superoxide dismutase, catalase and peroxidases) and non-enzymatic (proline, etc.) are activated. In supra-optimal copper concentration, coffee seedlings showed a significant increase in proline content (Fig 2C and 2D), which is in agreement with *Silene vulgaris* (Schat et al., 1997), *Oriza sativa* (Chen et al., 2001) and *Zea mays* (Wen et al., 2013). However, an interesting fact in the present study was the increase of proline levels in seedlings under copper deficiency in both leaf and root (Fig. 2C and 2D). Similar results were found in *Cucumis sativus* (Fariduddin et al., 2013), where proline levels were substantially increased as a result of copper deficiency. The decrease in proline levels may be due to degradation reactions of this amino acid, which are catalyzed by mitochondrial enzymes such as proline dehydrogenase in various stress conditions (Pavlikova et al., 2008; Kavi-Kishor et al., 1995).

Materials and methods

Plant culture and Cu treatments

Coffea arabica L. seedlings from cultivar Catuaí 144 were cultivated for six months in 500 mL polypropylene bags filled with subsoil and cattle manure in 2:1 proportion, plus potassium chloride and superphosphate in the proportion 1:10 (Guimarães et al. 2002). After selection for uniformity in size and vigor, seedlings were transferred to 10 L plastic containers (33x31x38 - WxHxD) containing a nutrient solution (Hoagland and Arnon 1950). Plants were acclimated for 21 days when solutions with increasing concentrations were used: ¼ strength for 7 days, ½ strength for 7 days, and full strength for 7 days. After acclimatization, seedlings were subjected to three treatments: a control, Cu-deficient and excess of copper. The original concentration of the nutrient solution was used for the control and the same solution was used for the deficient treatment, but with the exclusion of copper. For copper excess treatment, the control solution was added with the four times of recommended dose of copper nutrient. Pre-tests with different concentrations of copper were made to determine values of excess copper that presented physiological changes. The volume of the nutrient solution was replenished with deionized water on a daily basis. The pH of the solution was also adjusted daily to 5.5±0.5 with NaOH and HCL solution (1 M) and solutions were completely replaced weekly. All seedlings were maintained under constant aeration throughout the experimental period.

Evaluations were performed on leaves and roots at the beginning of the experiment and after 30 and 60 days. The experimental design was completely randomized using a 3x3 factorial scheme: three treatments control (0.03 ppm), Cu-deficient (0 ppm) and Cu-excess (0,12 ppm) and three times of evaluation (0, 30, and 60 days), totaling 9 treatments with three replications. The collection times were determined from visual analysis of the plant and biochemical tests. Each experimental plot consisted of five seedlings.

Data were subjected to analysis of variance using the statistical program SISVAR 4.3 (System Analysis of Variance for Balanced Data) (Ferreira, 2011). Means between treatments were compared by the Scott and Knott (1974) test at 0.05 probability

Evaluation of Cu, chlorophyll and carotenoids

Leaf and roots copper content was determined according Malavolta et al. (1989): 500 mg of leaf dry weight were ground and placed in digestion tubes, to which 6 mL of a mix of HNO_3 and HClO4 2:1 (v/v) were added. The digestion tubes were then placed in a digestion block and temperature was increased gradually until 160 °C and kept until volume of the solution was reduced to half. Temperature was then increased to 210 °C and kept until white fumes of $HClO_4$ were obtained and the extract became colorless. After cooling, the final volume was made up to 50 mL through the addition of deionized water. Copper content was determined through atomic absorption spectrometry.

Leaf chlorophyll and carotenoids contents were determined as described by Lichtenthaler and Buschmann (2001): 0.1 g of fresh weight of leaves from each treatment were macerated in 80 % acetone. The final volume was made up to 10 mL, and spectrophotometric readings were taken at 445, 645 and 663 nm.

Shoot and root dry weight

The seedlings were divided into roots and shoots. The plant material was dried at 70 °C to constant weight and the dry weight measured.

Carbohydrates

The extraction of carbohydrates was performed according to Zanandrea et al. (2010). Starch and total soluble sugars were quantified as described by Dische (1962).

Antioxidant metabolism

H_2O_2 was determined according Velikova et al. (2000). The extract for the determination of activity of SOD, CAT and APX was obtained according to Biemelt et al. (1998). SOD activity was measured according to Giannopolitis and Ries, (1977), CAT activity was evaluated according Havir and McHale (1987) and APX activity was determined by monitoring of the rate of oxidation of ascorbate according Nakano and Asada (1981). Lipid peroxidation was determined by quantification of thiobarbituric acid reactive species, as described by Buege and Aust (1978).

Proline content determination

Proline analysis was performed according to Torello and Rice (1986) with some modifications.

Conclusion

Photosynthetic pigments shoot dry weight, total soluble sugars and starch concentration of coffee seedlings were affected by both deficiency and excess of copper at the end of experimental period. Copper stress caused an increase in hydrogen peroxide levels and consequently in lipid peroxidation, both in leaves and in roots from 30th day. Therefore, there was an activation of antioxidant metabolism as well as an increase in proline levels in both treatments of copper excess and deficiency.

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