Nitric Oxide as an Attenuator of Ecophysiological Changes in Corn (Zea mays L.) Plants Submitted to Copper Toxicity

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Received: October 11, 2020  Accepted: December 10, 2020  Published: January 6, 2021
doi:10.5296/jas.v8i4.18157  URL: https://doi.org/10.5296/jas.v8i4.18157

Abstract
The aim of this work was to evaluate the attenuating effect of the nitric oxide donor (sodium nitroprusside-NPS) on the ecophysiological responses of corn plants (Zea mays L.) submitted to copper toxicity. The corn seeds of the K9606 VIP3 variety were soaked for 48 hours in Germitest with solution containing treatment with sodium nitroprusside Na2[Fe(CN)5NO]2H2O (0, 200 and 300µM), sodium ferrocyanide Na4Fe(CN)6 (300, 100 and 0 µM) respectively and deionized water (control), sown in buckets with 15 kg of soil incubated for 50 days containing copper concentrations CuSO4.5H2O (0, 60 and 200 mg kg⁻¹). The design
consisted of randomized blocks with 12 treatments and 4 repetitions, making a total of 48 plants. In ecophysiological variables: height, leaf area and number of leaves, stem diameter increased by 32, 66% and 11.29% in the treatments with 60 mg kg\(^{-1}\) of copper and 200 mg kg\(^{-1}\) of copper, respectively. There was no effect of treatments on the chlorophyll content measured by the SPAD index and gas exchange. The chlorophyll a fluorescence variables indicate that the concentration of 200 mg kg\(^{-1}\) of copper caused damage to the structure of the PS II reaction center complexes and indicate a slightly protective effect of nitric oxide-NO present in sodium nitroprusside and cyanide present in sodium ferrocyanide, reflecting a smooth functioning of the maximum activity of photosystem II and the electron transport chain.

**Keywords:** phytotoxicity, ecophysiological variables, contamination

1. Introduction

Corn is a fundamental product for Brazilian agriculture, grown in all regions of the country, being the second crop with the highest grain production in the national territory with a large share in exports (Usda, 2019), being the most produced and consumed cereal in the country. World (Contini et al., 2019), with national average productivity of 5,719 kg ha\(^{-1}\) and 5,682 kg ha\(^{-1}\) in the off-season (Conab, 2020).

Abiotic stresses are the main environmental problems that negatively influence plant growth and development (Dresselhaus and Hückelhoven 2018). The phytotoxic effect of heavy metals can be characterized by a decrease in the total phytomass of plants, in lengths of aerial part and root and a decrease in the concentration of chlorophyll (Pinto, 2017). The levels of heavy metals in soils (Komárek et al., 2010; Ali et al., 2013; Sarwar et al., 2017).

Cu is classified as a heavy metal, however it is considered an essential element in plants (Yruela, 2009), can become toxic in high concentrations (Rodrigues et al., 2016), causing disturbances in the structure of proteins and inhibition of cell stretching (Yruela, 2013). The excess of Cu can cause damage to the photosynthetic apparatus (González-mendoza et al., 2013), and changes in photosynthesis may represent the physiological state of the plant (Kalaji et al., 2016).

Environmental stresses can interfere with the efficiency of photosynthesis and inactivate PS II-photosystem II (P680) and the electron transport chain for the production of ATP and NADPH2 (Costa et al., 2003). Fluorescence analysis has advantages for studies of electron transport during photosynthesis and it is possible to record electron transport in photosystem II (Franco, 2015).

The application of exogenous nitric oxide-NO can mitigate the decrease in photosynthetic capacity in plants, caused by a variety of abiotic stresses (Wang et al. 2014), is able to diffuse through membranes (Lamattina & García Mata, 2016), is a molecule that acts as a signaler involved in the regulation of plant growth and development, in defense against pathogens and in responses to abiotic stress (Sanz et al., 2015).
Thus, the objective of this study was to evaluate the attenuating effect of nitric oxide (NO) on biometrics, chlorophyll content, gas exchange and chlorophyll a fluorescence in K9606 VIP3 corn plants subjected to copper concentrations.

2. Material and Methods

The experiment was conducted in a greenhouse located at the Institute of Agricultural Sciences-ICA belonging to UFRA (Federal Rural University of the Amazon) - Belém, with geographical coordinates of 01° 27 '21 "S, 48° 30' 16" W and average altitude of 10 m. The chemical characteristics of the collected soil, in the 0-20 cm layer were: pH (CaCl2): 5.1, pH Buffer (SMP): 6.25, Organic Matter: 19 g / dm³; Calcium: 35 mmolc dm⁻³; Magnesium: 9 mmolc dm⁻³; Potassium: 0.8 mmolc dm⁻³; Sodium: 0.3 mmolc dm⁻³; Phosphorus: 20 mg dm⁻³; Total Organic Carbon: 11 g dm⁻³; Sulfur: 11 mg dm⁻³; Manganese: 22.5 mg dm⁻³; Iron: 20 mg dm⁻³; Copper: 0.4 mg dm⁻³; Zinc: 5.3 mg dm⁻³; Boron: 0.54 mg dm⁻³; Total Organic Carbon: 11 g dm⁻³; Silt: 120 g kg⁻¹; Total sand: 782 g kg⁻¹; Type texture: Medium. The soil was removed at UFRA / Campus Belém, sieved and placed in 15kg buckets of soil. Solutions containing copper concentrations of CuSO₄·5H₂O (0, 60 and 200 mg kg⁻¹) were placed and allowed to incubate for a period of 50 days, irrigating daily keeping at 60% field capacity.

The seeds of corn of the K9606 VIP3 variety from the company KWS SAAT SE & Co. KGaA seeds were soaked for 48 hours in Germitest paper with a solution containing the treatments with sodium nitroprusside Na₂[Fe(CN)₅NO]·2H₂O (0, 200 and 300 µM) and sodium ferrocyanide compensator Na₄Fe(CN)₆ (300, 100 and 0 µM) respectively, deionized water (control). The seeds were sown in 15 kg buckets containing soil incubated for 50 days with CuSO₄·5H₂O at concentrations 0, 60 and 200 mg kg⁻¹. The experiment lasted 93 days until the ears were removed.

2.1 Gas exchanges and Chlorophyll Fluorescence a

Measurements of gas exchange liquid photosynthetic rate (A), stomatal conductance (gs), transpiration (E) and chlorophyll a fluorescence were performed with the portable infrared gas analyzer (IRGA, LI-COR 6400-XT, Lincon, USA ) with fluorescence chamber (6400-40) with an area of 2 cm².

They were performed at 55° and 56° DAS in blocks 1 and 2 and 3 and 4, respectively. Being measured under favorable environmental conditions, between 9:00 am and 11:00 am, PARi 1000.41 µmol m² s⁻¹ One leaf per plant was inserted into the equipment chamber, always in the middle region of the leaf (6th leaf fully expanded), the same used to analyze the chlorophyll content through the SPAD index. The relationship between intracellular carbon and the environment (Ci / Ca), internal CO₂ concentration (Ci), water use efficiency (USA) and leaf temperature (Tleaf) were also quantified.
After reading gas exchanges, aluminum foil was placed on the same sheets to keep them in the dark for at least 30 minutes to carry out the chlorophyll a fluorescence reading.

2.2 Biometric Measurements

At the VT stage (weighing) (Ritchie et al., 1993), the following biometric characteristics were evaluated: height (cm) using a graduated ruler, being measured from the neck to the apex of the flag leaf; stem diameter (mm) at 8-10 centimeters from the stem of the plant with the aid of a caliper; and, for number of leaves, all those present in the plant were counted.

2.3 Chlorophyll Content

For the determination of chlorophyll content, we used the Minolta SPAD-502 chlorophyll meter. The evaluations were carried out at the 53rd DAS when more than 50% of the plants were in the VT-Pendoamento phase, where the last tassel branch is visible at the top of the plant. The style-stigmas ("hairs") of corn did not yet appear in all plants at this stage. Were made 10 readings on the 6th fully expanded sheet.

3. Results and Discussions

3.1 Gas Exchange and Chlorophyll Fluorescence at

The measurements of gas exchange and fluorescence of chlorophyll a can serve as tools to verify the integrity of the photosynthetic apparatus in the face of environmental adversities.

Regarding gas exchange, there was no interaction between the dosages of sodium nitroprusside (SPL) and sodium ferrocyanide (FCS) with copper concentrations or the isolated effect of treatments, as can be seen in Table 1. The excess of copper can cause abiotic stress causing damage to photosystems, resulting in the decline of photosynthesis (Küpper & Andresen, 2016), which may impact the chlorophyll concentration (Cambrollé et al., 2015), decrease in Rubisco's carboxylative activity (Siedlecka & Krupa, 2004), competition with other metal ions such as Fe, Ni and Zn and increased lipid peroxidation (Küpper & Andresen, 2016).

Different results were verified in this work, in which the dosages of 60 and 200 mg kg-1 were not enough to alter the gas exchange variables in corn plants, possibly Cu acted as a micronutrient within the favorable limits (Souza et al., 2014), with direct participation in electron transport (Dalcorso et al., 2014), between cytochrome b6f and photosystem I (PSI) in the photochemical phase (Yruela, 2013).

Mateos-Naranjo et al. (2008) researching the effect of copper on the growth and photosynthesis of Spartina densiflora Brongn. describe that the quantum efficiency of FSII, the rate of liquid photosynthesis, stomatal conductance and pigment concentration decreased with the increase in Cu concentration.
Table 1. Summary of photosynthesis analysis of variance (A), stomatal conductance (gs), internal CO2 concentration (Ci), transpiration (E), water use efficiency (USA), leaf temperature (Tleaf), internal CO2 concentration ratio / external CO2 concentration (Ci / Ca) of corn plants treated with sodium nitroprusside and sodium ferrocyanide submitted to copper toxicity

| Causes of variation | THE (µmol m-2 s-1) | gs (µmol m-2 s-1) | Ci (mol m-2 s-1) | AND | USA (µmol mmol-1) | Tleaf (° C) | Ci / Ca (mol mol-1) |
|---------------------|-------------------|-----------------|-----------------|-----|-----------------|------------|-----------------|
| Nitroprusside       | 4.58ns            | 0.56ns          | 6095.00ns       | 1.81ns | 0.69ns | 0.10ns | 0.20ns          |
| Copper              | 5.58ns            | 0.14ns          | 4141.10ns       | 0.04ns | 0.18ns | 0.00ns | 0.13ns          |
| Nitroprusside x Copper | 7.86ns        | 0.22ns          | 1608.60ns       | 0.52ns | 0.34ns | 0.10ns | 0.05ns          |
| Blocks              | 41.67 *           | 4.02 *          | 15054.00 *      | 21.56 * | 10.4 * | 1.30 * | 0.51 *          |
| Residue             | 10.05             | 0.44            | 4070.70         | 0.76   | 0.99ns | 0.20  | 0.13            |
| Average             | 12.80             | 0.14            | 208.15          | 4.00   | 3.53   | 36.10  | 0.53            |
| CV (%)              | 25.6              | 6.2             | 23.8            | 17.2   | 35.8   | 0.4   | 25.0            |

CV: Coefficient of variation;
* Significant at the 5% probability level by the Tukey test
ns not significant at the 5% probability level by the Tukey test
Source: The author (2020)

Regarding chlorophyll fluorescence a it was found that there was no significant effect on the parameters Fs (steady state fluorescence), Fm ' (maximum fluorescence adapted to the light), ΦPSII (effective quantum efficiency), NPQ (non-photochemical dissipation), Fo' (minimum fluorescence of the sheet adapted to light), Fv' / Fm' (Genty parameter), ETR (electron transport rate), as shown in Tables 2 and 3.

Table 2. Summary of analysis of variance of F0 (initial fluorescence), Fm (maximum fluorescence), Fv (variable fluorescence), Fv / Fm (PSII potential quantum efficiency), Fs (steady state fluorescence), Fm ' (maximum fluorescence adapted to the of course), ΦPSII (effective quantum efficiency), NPQ (non-photochemical dissipation), Fo / Fm ' (basal quantum efficiency of PS II) of corn plants (Zea mays L.) treated with sodium nitroprusside and sodium ferrocyanide submitted to copper toxicity

| Causes of variation | Fo (µmol e- m-2 s-1) | Fm (µmol e- m-2 s-1) | Fv 0.08ns | Fv / Fm (µmol e- m-2 s-1) | Fs (µmol e- m-2 s-1) | Fm' (µmol e- m-2 s-1) | ΦPSII | NO WHY | Fo / Fm' |
|---------------------|---------------------|---------------------|----------|-------------------------|---------------------|---------------------|-------|--------|----------|
| Nitroprusside       | 1.36ns              | 3.96 *              | 0.08ns   | 0.30ns                  | 0.12ns              | 0.29ns              | 8.68ns | 0.50ns | 0.69ns   |
| Copper              | 3.42 *              | 2.13ns              | 0.17 *   | 0.38 *                  | 0.14ns              | 0.04ns              | 4.91ns | 0.27ns | 1.66 *   |
| Nitroprusside x Copper | 0.56ns          | 0.84ns              | 0.08ns   | 0.06ns                  | 0.14ns              | 0.27ns              | 9.00ns | 1.28ns | 0.56ns   |
| Blocks              | 1.49ns              | 0.70ns              | 0.03ns   | 0.14ns                  | 0.05ns              | 0.26ns              | 3.08ns | 0.57ns | 0.59ns   |
| Residue             | 6.23                | 1.00                | 0.04     | 0.10                    | 0.20                | 0.25               | 5.12   | 0.79   | 0.24     |
| Average             | 187.48              | 1154.15             | 976.11   | 0.84                    | 371.02              | 523.55             | 0.30   | 1.18   | 0.35     |
| CV (%)              | 5.8                 | 0.7                 | 0.6      | 24.1                    | 2.4                 | 2.5                 | 20.7   | 6.19   | 17.7     |

CV: Coefficient of variation;
Table 3. Summary of analysis of variance of \( F_0 \) (minimum leaf fluorescence adapted to light), \( qP \) (photochemical dissipation), \( Fv' / Fm' \) (Genty parameter), ETR (electron transport rate), \( qL \) (photoinhibition coefficient) of corn plants (Zea mays L.) treated with sodium nitroprusside and sodium ferrocyanide submitted to copper toxicity

| Causes of variation   | \( F_0 \) | \( qP \) | \( Fv' / Fm' \) | ETR (\( \mu \text{mol e} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)) | \( qL \) |
|----------------------|---------|---------|----------------|---------------------------------|---------|
| Nitroprusside        | 1.07ns  | 1.06 *  | 0.76ns         | 1.25ns                          | 0.49 *  |
| Copper               | 3.43ns  | 0.68 *  | 0.47ns         | 0.91ns                          | 1.01 *  |
| Nitroprusside \& Copper | 0.95ns  | 0.22ns  | 0.09ns         | 1.30ns                          | 0.28ns  |
| Blocks               | 1.86ns  | 0.31ns  | 0.15ns         | 0.30ns                          | 0.32ns  |
| Residue              | 1.20    | 0.18    | 0.28           | 1.00                            | 0.13    |
| Average              | 155.39  | 0.44    | 0.67           | 60.37                           | 1.11    |
| CV (%)               | 8.9     | 23.7    | 28.1           | 1.9                             | 18.9    |

CV: Coefficient of variation;

* Significant at 5% probability by F

ns not significant at 5% probability by F

Source: The author (2020)

F0 (initial fluorescence) is the minimum constant fluorescence in the dark when all reaction centers are open is one of the parameters of chlorophyll fluorescence used to assess plant stress to abiotic factors, for example, toxic metals (Yadav et al., 2018). The increase in F0 (initial fluorescence) indicates damage in the reactions of PS II - photosystem II (Paunov et al., 2018) or decrease in the capacity of transferring the excitation energy from the antenna to the reaction center (Baker & Rosenqvist, 2004), which was not observed in the present study, verifying that the treatment with 60 mg kg\(^{-1}\) of copper provided an average of 163.42, reducing F0 by 16.53% (initial fluorescence) when compared to the treatment with 0 mg kg\(^{-1}\) copper that obtained an average of 195.78, as verified in Table 4.

Table 4. Effect of copper concentrations on stem diameter and chlorophyll a fluorescence parameters of corn plants treated with sodium nitroprusside and sodium ferrocyanide subjected to copper toxicity

| Copper (mg kg\(^{-1}\)) | Diameter (mm) | Fo | Fv | \( Fv' / Fm' \) | Fo / Fm | Fo / Fm' | \( qP \) | \( qL \) |
|-------------------------|---------------|----|----|----------------|---------|---------|---------|---------|
| 0                       | 10.27 ± 1.14  | 195.78 ± 11.24 | 972.87 ± 36.95 | ab | 0.84 ± 0.01 ab | 0.33 ± 0.03 b | 0.44 ± 0.02 ab | 1.14 ± 0.08 a |
| 60                      | 13.62 ± 1.25  | 163.42 ± 13.09 | 1047.68 ± 44.63 | a  | 0.86 ± 0.01 a  | 0.31 ± 0.03 b | 0.41 ± 0.01 b  | 1.20 ± 0.08 a  |
| 200                     | 11.43 ± 1.48  | 204.96 ± 13.21 | 907.56 ± 30.45 | b  | 0.81 ± 0.01 b  | 0.41 ± 0.03 a  | 0.46 ± 0.02 a  | 1.00 ± 0.04 b  |

Means followed by the same letter in the column do not differ by Tukey's test at the 5% probability level. Values described correspond to the means of four repetitions and SD.
Chlorophyll a fluorescence is a tool used to detect damage to the photosynthetic apparatus caused by abiotic stresses (Stirbet et al., 2018).

In the present work it was found that there was no effect of the interaction between the dosages of sodium nitroprusside (SPL) and sodium ferrocyanide (FCS) with the copper concentrations in the parameter Fv (variable fluorescence), verifying the effect of the treatment with 200 mg kg\(^{-1}\) copper which had an average of 907.56 with a reduction of 6.71% compared to treatment with 0 mg kg\(^{-1}\) of copper which had an average of 972.87. This reduction may be due to the replacement of Mg by Cu in the chlorophyll molecule, causing structural changes in the photosynthetic pigments of the PSII (Zvezdanovic et al., 2007).

The higher the Fv (variable fluorescence), the greater the plant's capacity to transfer the energy of the electrons ejected from the pigment molecules to produce ATP, NADPH and reduced ferrodoxin (Fdr) (Baker, 2008).

There was no effect of the interaction between the dosages of sodium nitroprusside (SPL) and sodium ferrocyanide (FCS) with the copper concentrations in the parameter F0 / Fm (basal quantum production of the non-photochemical process in PSII) verifying the effect of the treatment of 200 mg kg\(^{-1}\) of copper, which presented an average of 0.41 providing an increase of 24.54% when compared to the treatment with 0 mg kg\(^{-1}\) of copper which presented an average of 0.33, as shown in Table 4, with the ratio between F0 (minimum or initial fluorescence of chlorophyll a in the dark-adapted state) and Fm (maximum leaf fluorescence adapted to light). Several authors cite the increase in this relationship as indicative of stress, suggesting normal values, that is, values recommended as standard, between 0.14 and 0.20 (Roháček, 2002).

Cambrollé et al. (2015), observed a reduction in the maximum quantum yield of PSII (Fv / Fm) and in the effective quantum efficiency of PSII (Y (II)) due to the phytointoxication caused by Cu, which causes the degradation of the internal content of the chloroplast and the replacement of Mg by Cu in chlorophyll.

There was no effect of the interaction between the dosages of sodium nitroprusside (SPL) and sodium ferrocyanide (FCS) with the copper concentrations in the parameter qP (photochemical quenching) verifying the effect of the treatment of 60 mg kg\(^{-1}\) of copper,
which presented an average of 0.41 providing a reduction of 8.24% in relation to the treatment with 0 mg kg\(^{-1}\) of copper, which presented an average of 0.44 as verified in Table 4.

The qP parameter (photochemical quenching) indicates the proportion of reaction centers open in the PSII (Minagawa, 2008), it is the dissipation caused by the photochemical process, that is, it is caused by the use of energy to reduce NADP, being directly related to the redox state of plastoquinone and decreases in proportion to the closing of the reaction centers (Sousa, 2012).

Cu acts as cofactors in several enzymes such as cytochrome c oxidase, Cu / Zn superoxide dismutase and plastocyanine (Nazir et al., 2019; Zhang & Li, 2019), that transports electrons in primary photosynthetic reactions (Dalcorso et al., 2014; Yruela, 2013).

There was no effect of the interaction between the dosages of sodium nitroprusside (SPL) and sodium ferrocyanide (FCS) with the copper concentrations in the parameter qL (quenching related to photosynthesis photoinhibition), verifying the treatment effect of 200 mg kg\(^{-1}\) of copper that showed an average of 1.00 providing a reduction of 12.43% when compared to the treatment with 0 mg kg\(^{-1}\) of copper that showed an average of 1.14, as verified in Table 4. This parameter is a protection mechanism that allows dissipate excess thermal energy. This dissipation of energy can prevent the formation of reactive oxygen species (ROSs), which can irreversibly damage proteins, lipids and pigments in photosynthetic membranes (Horton & Ruban, 2004).

Regarding the chlorophyll fluorescence parameters that were significant in relation to the dosages of sodium nitroprusside (SPN) and sodium ferrocyanide (FCS) it was found that the treatment with (300 \(\mu\)M) sodium nitroprusside + (0 \(\mu\)M) sodium ferrocyanide showed an average of 1122.76 providing a reduction of 2.81% in parameter Fm (maximum fluorescence) when compared to the control treatment (deionized water), which had an average of 1155.26, as shown in Table 4.

The parameter Fm (maximum fluorescence) indicates the maximum fluorescence intensity that occurs when practically all quinone A (QA) is reduced and the reaction centers reach their maximum capacity (Baker & Rosenqvist, 2004). This decrease may be associated with the inactivation of PSII in the membranes of the thylakoid directly affecting the flow of electrons between the photosystems (Strasser et al., 2004).

NO can function as a positive and negative regulator of responses to stress depending on its local concentration (Mur et al. 2012), its effects depend on chemical changes in proteins, such as S-nitrosylation (Malik et al., 2011; Fungillo et al., 2014).

Misra et al. (2014) suggested that chloroplast proteins like the proteins of the reaction center of PSII D1 and D2, are targets of NO, and may prevent electron transfer and consequent inactivation of the PSII reaction center (Yamamoto et al., 2008).
Table 5. Effect of sodium nitroprusside (NPS) and sodium ferrocyanide (FCS) on the chlorophyll a fluorescence parameters of corn plants treated with sodium nitroprusside and sodium ferrocyanide subjected to copper toxicity

| Treatment                          | $F_m$       | $q_P$      | $q_L$      |
|-----------------------------------|-------------|------------|------------|
| Water                             | 1155.26 ± 19.22 a | 0.40 ± 0.02 b | 1.12 ± 0.05 a |
| SPL (0 µM) + FCS (300 µM)         | 1157.97 ± 20.53 a | 0.47 ± 0.02 a | 1.21 ± 0.06 a |
| SPL (200 µM) + FCS (100 µM)       | 1177.84 ± 15.55 a | 0.47 ± 0.02 a | 0.96 ± 0.03 b   |
| SPL (300 µM) + FCS (0 µM)         | 1122.76 ± 9.97 b  | 0.43 ± 0.02 ab  | 1.15 ± 0.07 a   |

Means followed by the same letter in the column do not differ by Tukey's test at the 5% probability level. Values described correspond to the means of four repetitions and SD.

Treatments with (300 µM) Sodium nitroprusside+ (0 µM) sodium ferrocyanide, (200 µM) Sodium nitroprusside+ (100 µM) sodium ferrocyanide and (0 µM) Sodium nitroprusside+ (300 µM) sodium ferrocyanide showed averages of 0.43, 0.47 and 0.47 respectively, in the $q_P$ parameter (photochemical quenching), providing an increase of 7.55%, 20% and 17.70% when compared to the control treatment (deionized water), which presented an average of 0.40, as verified in Table 5.

The slightly protective effect of NO in relation to the maximum activity of photosystem II, can reflect on a good functioning of the electron transport chain, generating ATP and NADPH (Silveira et al., 2016).

The release of cyanide can reduce the photochemical activity of photosystem II-PS II, affecting plant productivity. Sodium nitroprusside (SNP) and sodium ferrocyanide (FSC) release cyanide during their photolysis, reducing the photochemical activity of PSII. (Wodala et al., 2010).

Different results were verified in this work, in which the dosages of sodium nitroprusside and sodium ferrocyanide did not have a negative effect on the chlorophyll a fluorescence parameters.

The energy in NADH is dissipated in the form of heat. The oxidation of cyanide-resistant NADH is a characteristic feature of this electron transport pathway in plants (Lenhinger, 2006).

According to Gerivani et al. (2016) due to cyanide being less toxic to plants due to the functioning of the alternative oxidase and its ability to metabolize it.

NO through S-nitrosylation triggers changes in enzyme dynamics (Farnese et al., 2016), being an important process in the response of plants to abiotic stresses, regulating a wide range of cellular functions and signaling events (Sevilla et al., 2015), being able to control protein activity (Yu et al., 2014).

Treatment with (200 µM) Sodium nitroprusside+ (100 µM) sodium ferrocyanide showed an average of 0.96 providing a 14.14% reduction in the $q_L$ parameter (quenching related to photosynthesis photoinhibition) when compared to the control treatment (deionized water), which obtained an average of 1.12. As shown in Table 5.
According to Silva (2015), NO can react quickly with O2- producing peroxynitrite (ONOO-) (Popova & Tuan, 2010), being able to act in increasing the synthesis and / or activity of antioxidant enzymes, such as ascorbate peroxidases (DEL RIO, 2015), resulting in the activation of defense mechanisms (Saxena & Shekhawa, 2013).

According to Cardoso 2013, cyanide has the capacity to generate an electrical potential by inhibiting electrogenic ionic transport, increasing the pH of the external environment, making the cytosol acid, showing the active transport of H+ out of the cell.

3.2 Biometric Measurements

There was no interaction between the dosages of sodium nitroprusside (SPL) and sodium ferrocyanide (FCS) with the copper concentrations for the biometric variables: height and number of leaves and these were not affected by the isolated effect of the treatments (Table 6).

Table 6. Summary of analysis of variance of the SPAD index, height, leaf area, number of leaves, stem diameter of corn plants treated with sodium nitroprusside and sodium ferrocyanide subjected to copper toxicity

| Causes of variation | SPAD | Height (m) | Leaf area (cm²) | Number of sheets | Diameter (mm) |
|---------------------|------|------------|----------------|-----------------|---------------|
| Sodium nitroprusside| 624.92ns | 70.99ns | 131.48ns | 0.41ns | 36.69ns |
| Copper              | 76.76ns | 8.94ns   | 109.96ns | 0.15ns | 366.46 * |
| Nitroprusside x Copper| 409.21ns | 28.03ns | 88.01ns | 0.80ns | 99.74ns |
| Blocks              | 789.58ns | 536.84 * | 77.11ns | 1.56ns | 39.88ns |
| Residue             | 816.92  | 114.17    | 95.81      | 0.69            | 62.97         |
| Average             | 24.44   | 118.70    | 1856.44    | 14.52           | 116.89        |
| CV (%)              | 15.8    | 24.8      | 10.1       | 2.8             | 17.9          |

CV: Coefficient of variation;

* Significant at the 5% probability level by the Tukey test

ns not significant at the 5% probability level by the Tukey test

Source: The author (2020)

In the present study, no visible symptoms of Cu toxicity were observed, such as chlorosis caused by damage to thylakoid membranes (Adrees et al. 2015). According to Yuan et al. (2013) observed that in higher concentrations of Cu there was inhibition of cell division and elongation in Arabidopsis thaliana, reduced growth due to biochemical and metabolic changes (Wang et al., 2017) as an increase in the concentration of ROSs (Ravet & Pilon, 2013).

Zhu et al. (2016) observed that treatment with NO is able to stimulate the cell division process, acting with auxin, mediating the transition from phase G1 to S.

The effect of copper measurements on the stem diameter variable was observed, with averages of 13.62 cm and 11.43 cm, providing an increase of 32.66% and 11.29% in
treatments with 60 mg kg⁻¹ and 200 mg kg⁻¹ copper, respectively, compared to treatment with 0 mg kg⁻¹ copper, which averaged 10.27 cm (Table 4).

Most of the inorganic Cu in the soil occurs as oxides and sulfides that are insoluble with low phyto availability (Mihaljević et al., 2019). According to Kumpiene et al. (2008), organic matter competes for metals with the oxide exchange sites, presenting a mitigating effect of copper toxicity.

Berton et al. (1997) found that corn plants with 335 mg kg⁻¹ of Cu in the leaves did not show symptoms of toxicity. Second, Benimeli et al. (2009), corn plants can tolerate and accumulate high concentrations of Cu without showing visible morphological changes, as verified in the present work. The accumulation of copper in the root system may represent a strategy of tolerance of the plant to the excess of heavy metals in the soil (Cambrollé et al., 2013).

According to Silva (2019) working with sorghum, he found that for the variables stem diameter, plant height and number of tillers, doses above 160 (mg of copper kg⁻¹ of soil) begin to affect negatively.

3.3 Chlorophyll Content

There was no interaction between the measurements of sodium nitroprusside (SPL) and sodium ferrocyanide (FCS) with copper concentrations nor the isolated effect of treatments on the determination of chlorophyll content measured by the SPAD index, as seen in Table 6. The Minolta SPAD-502 chlorophyll meter has been investigated as an instrument for rapid diagnosis of the nutritional status of different cultures in relation to the N content, adding advantages such as simplicity of use, in addition to enable a non-destructive assessment of leaf tissue (Argenta et al., 2001).

According to Tiecher et al. (2017) a reduction in the content of chlorophyll a, chlorophyll b and carotenoids is generally observed in plants grown in soils with high levels of Cu, this may be due to the fact that excess Cu²⁺ can replace the Mg²⁺ of the chlorophyll structure, in addition to antagonism between the elements Fe and Cu, causing a reduction in chlorophyll, PSII becomes more susceptible to photoinhibition (Yruela, 2009). Different results were found in this study in which no significant effects of the applied doses were observed in relation to the chlorophyll content measured by the SPAD index.

4. Conclusion

There was no effect of treatments on the gas exchange variables and in relation to chlorophyll fluorescence there was no significant effect on the parameters F₀, Fm', ΦPSII, Fo', Fv / Fm', ETR, NPQ. The treatment with 60 mg kg⁻¹ of copper provided a reduction of 16.53% in F₀. Treatment with 200 mg kg⁻¹ of proportional copper reduction of 6.71% in Fv, reduction of 2.92% in Fv / Fm, increase of 24.54% in F₀ / Fm', reduction of 12.43% in qL. The treatment with 60 mg kg⁻¹ of copper provided a reduction of 8.24% in qP. Treatment with (300 µM) sodium nitroprusside + (0 µM) sodium ferrocyanide provided a reduction of 2.81% in Fm. Treatments with (300 µM) Sodium nitroprusside+ (0 µM) sodium ferrocyanide, (200 µM) Sodium nitroprusside+ (100 µM) sodium ferrocyanide and (0 µM) Sodium nitroprusside+
(300 µM) sodium ferrocyanide providing an increase of 7.55%, 20% and 17.70% in qP, treatment with (200 µM) Sodium nitroprusside+ (100 µM) sodium ferrocyanide providing a 14.14% reduction in qL.

There was no effect of treatments on the chlorophyll content measured by the SPAD index, on the physiological variables: height, leaf area and number of leaves, observing the effect of copper concentrations on stem diameter, showing an increase of 32.66% and 11.29% respectively, concentrations of 60 mg kg⁻¹ of copper and 200 mg kg⁻¹ of copper.

The chlorophyll a fluorescence variables indicate that the concentration of 200 mg kg⁻¹ of copper caused damage to the structure of the PS II reaction center complexes causing the degradation of the internal content of the chloroplast, thus reducing the transfer of energy from the antennas to the reaction centers of the PSII.

The fluorescence variables of chlorophyll a indicate a slightly protective effect of nitric oxide-NO present in sodium nitroprusside and cyanide present in sodium ferrocyanide, reflecting a good functioning of the maximum activity of photosystem II and the electron transport chain generating ATP and NADPH for the photochemical step of photosynthesis.

Acknowledgement

We are grateful to Capes - Coordination for the Improvement of Higher Education Personnel, for the granting of the scholarship, UFRA - Federal Rural University of the Amazon and to the Biodiversity Studies in Higher Plants - EBPS group.

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