

Exogenous superoxide dismutase mitigates cell damage in drought-sensitive peanuts

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ABSTRACT. The superoxide dismutase (SOD) enzyme initiates the process of neutralization of cytotoxic effects caused by reactive oxygen- species (ROS). In drought-tolerant plants, SOD is rapidly supplied due to their ability to adjust when they sense a lack of water, unlike in drought-sensitive plants. To investigate whether exogenous application of SOD could benefit drought-sensitive plants by ameliorating water stress, we tested two contrasting cultivars, BR 1 and IAC Caiapó, which are drought tolerant and sensitive cultivars, respectively. Plants of both cultivars were grown in pots in a greenhouse and submitted to nine days of water suppression starting at V1 phase, and treated with SOD at different concentrations, 11, 23 and 34 $\mu\text{g mL}^{-1}$, applied to both adaxial and abaxial surfaces of leaves. The status of water stress was estimated through verification of soil moisture in stressed treatments, in comparison with controls. Additionally, stomatal conductance was estimated to evaluate stomata closing. The plants were assessed for growth, gas exchange, and activity of antioxidative enzymes. Overall, the contribution of exogenous SOD in mitigating the effects of water stress was greater in stressed plants of drought-sensitive cultivars. Gas exchange recovery was observed in the plants even with the lowest applied SOD concentration (11 $\mu\text{g mL}^{-1}$), whereas higher exogenous SOD

supply was required to prevent oxidative damage. More in-depth studies regarding the potential benefits of this type of treatment on the phenology of runner genotypes are merited, since these cultivars are very productive, though highly sensitive to water restriction.

Key words: Antioxidative enzymes; Detoxifying enzymatic systems; Metabolism; Osmotic adjustment; Tolerant plants; Water deficit

INTRODUCTION

Water deficit is one of the main limiting factors in agricultural production and may drastically compromise crops, depending on its duration and intensity. Climate change has been occurring at a fast pace and does not allow cultures to adapt at the same rate. Genetic improvement programs have been proactive in identifying and selecting tolerant germplasm, whose genotypes are often used in hybridization works to enhance the genetic basis of commercial cultivars (Rodríguez-Uribe, 2014; Dutra et al., 2018).

Plant weakness caused by water shortage is observed phenotypically due to alterations in metabolism, affecting growth and CO₂ absorption, as consequences of changes in cellular water potential and in photosynthesis. Consequently, several oxygen-reactive species (ROS) are generated, represented by free radicals that impact cellular defense. Under normal conditions, ROS may act as beneficial molecules that balance the cellular medium. However, under cellular stress conditions, they act as markers of oxidative stress, besides compromising lipids, proteins, and nucleic acids and causing cell death (Sharma et al., 2019).

Naturally, plants have evolved defense mechanisms over time to mitigate environmental adversities so as to maintain homeostasis. Stomatal closure is one of the signs of reaction, as is cellular adjustment based on the storage of osmoregulatory substances that prevent water imbalance in the cell and water loss to the soil or atmosphere (Farooq et al., 2019). Plants react by increasing the production and storage of several osmolytes and by activating detoxifying enzymatic systems able to neutralize the cytotoxicity of ROS (Gill and Tuteja, 2010; Choudhury et al., 2017).

Antioxidative enzymes play a key role in the defense against ROS. Superoxide dismutase (SOD) is a key enzyme in such processes as it is the most effective antioxidant barrier present in the subcellular compartments (Gill and Tuteja, 2010). First reported in 1969 in bovine erythrocytes, SOD is considered a landmark in studies on the action of free radicals from oxygen metabolism, being found in most prokaryote and eukaryote organisms, as well as the production in superoxide biological systems (Del Río et al., 2018).

In cells, SODs remove O₂^{•-}, catalyzing dismutation, where one O₂^{•-} is reduced to H₂O₂ and another is oxidated to O₂. With the removal of O₂^{•-}, the risk of OH[•] formation inside the cell decreases. In tissues under environmental stress, the increase in SOD activity is directly related to tolerance in plants, which speed up the defense process against the imminent oxidative damage as a response to excess ROS generated in the cells (Dutra et al., 2018; Pereira et al., 2016). Under water stress conditions, tolerant plants speed up stomatal closure to reduce water loss (Hao et al., 2013; Dutra et al., 2018; Vison et al., 2018). Consequently, ROS production in chloroplasts increases and is fought by two types of SOD, namely, *Cu/Zn-SOD* and *Fe-SOD* (Pilon et al., 2011; Huseynova et al., 2014).

Abid et al. (2017) submitted 11 contrasting genotypes of beans (*Vicia faba*) to 30 days of water stress and observed increases in SOD between 99 and 289% among the tolerant genotypes and from 27 to 30% among the sensitive ones Shafiq and Ashraf (2019) found increases in SOD in corn between 31 and 37% among tolerant plants and from 4 to 21% among sensitive ones after 21 days of water suppression. The literature features several other papers in which drought tolerance can be related to increases in SOD in rice (Wang et al., 2019), soybean (Iqbal et al., 2019), and cotton (Prajapat et al., 2018), among others.

Peanut (*Arachis hypogaea*) is an oilseed plant of high dietary value that is grown in several regions of temperate and tropical climate worldwide. In semiarid environments, the occurrence of intermittent drought (also called *Indian summer* or *veranico*) during the rainy season disturbs the phenology of the crop, despite the tolerance against drought found in some cultivars (Pereira et al., 2016; Dutra et al., 2018).

Botanically, peanut is differentiated into two subspecies, namely, *fastigiata* and *hypogaea*, representing the upright and runner types, respectively. Cultivars of the *fastigiata* type have a shorter cycle and greater genetic variation for drought tolerance, whereas runners have longer cycles and are often more sensitive to water-restricted environments (Santos et al, 2012; Pereira et al, 2016; Dutra et al, 2018). Several works in the literature show the ability of peanut plants to perform osmotic adjustment to prevent oxidative damage when under drought conditions. Runner cultivars usually respond more slowly, which impacts gas exchanges and, consequently, the balance of antioxidative enzymes in the cellular defense process, especially SOD, which is responsible for initiating the detoxification steps (Pereira et al., 2016; Shinde et al., 2018; Vison et al., 2018).

Exogenous supplementation of organic compounds in plants submitted to water deficit has been reported. The results demonstrate benefits in mitigating stress in some metabolites, such as pyruvate in *Arabidopsis thaliana* (Li et al., 2014; Shen et al., 2017), proline in *Zea mays* (Ali et al., 2007) and *Triticum aestivum* (Kamran et al., 2009), salicylic acid in *Vigna unguiculata* (Dutra et al., 2017), pyruvate in *Arachis hypogaea* (Barbosa et al., 2021), among others. With enzymes of the antioxidative complex, research is still limited; however, as they act in an integrated way to prevent cell damage when the cell recognizes some environmental stress, it is possible that an external supplementation can contribute to minimize oxidative damage. As SOD initiates the cellular defense process, dismutating superoxide radicals, we hypothesize that the exogenous application can activate the action of other enzymatic cascades, to help sensitive plants to mitigate cellular damage when facing water stress.

In peanut, it is unknown whether exogenous SOD application would contribute to ameliorating the oxidative effects of water stress in sensitive plants. Thus, the present study aimed to assess the behavior of two contrasting cultivars of peanut submitted to water stress and treated with exogenous SOD at different concentrations. The plants were assessed based on descriptors of growth, gas exchange, and antioxidative enzymes.

MATERIAL AND METHODS

Conducting the experiment

The experiment was carried out in greenhouse during the dry season, in Campina Grande, PB, Brazil (07°13' S; 53°31' W). Plants of two contrasting peanut cultivars – BR 1

(subsp. *fastigiata*, upright and tolerant to drought) and IAC Caiapó (subsp. *hypogaea*, runner and sensitive to drought) – were grown in pots (750 mL) containing organic substrate (Basaplant Base Agro, Brazil) and watered daily so as to maintain the substrate moisture at the level corresponding to that of field capacity. The assays were carried out separately as a function of the difference in the cycle of the two cultivars. When the genotypes were in phase VI (Boote, 1982), corresponding to 11 days after emergence for BR 1 and 17 days for IAC Caiapó, the treatments were differentiated into five situations: Control (NS- No water stress), Water stress (WS- water suppression during nine days), and Water stress + foliar application of SOD at 11 $\mu\text{g mL}^{-1}$ (T1), 23 $\mu\text{g mL}^{-1}$ (T2) and 34 $\mu\text{g mL}^{-1}$ (T3). A completely randomized experimental design with five repetitions was employed.

The lyophilized SOD (Cu/Zn-SOD, Merck/Sigma Aldrich), contained 20.3 mg in 4.506 Units mg^{-1} of protein. The product was diluted in 1 mL of phosphate buffer 10 mM pH 7.4. At the end of the period of water stress, an aliquot of 100 μL of SOD at 11, 23 and 34 $\mu\text{g mL}^{-1}$ were applied on adaxial and abaxial surface of leaves of each T1, T2 and T3 treatments, using a cotton swab, for six days on the same leaflet.

The period of water stress lasted 11 days. The status of water stress was estimated through verification of soil moisture in stressed treatments, in comparison with control ones. Additionally, the stomatal conductance was estimated in order to attest the stomata closing. Then, SOD applications started on the third day of water stress outset, when the stressed plants had stomatal closure estimated at 19.05% (g_s of the stressed plants: 0.34 $\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$; g_s of the control plants: 0.42 $\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$). In that occasion, the volume of water held in the substrate corresponded to 35%, representing 65% of the deficit to reach the field capacity.

The plants were watered daily, maintaining the substrate moisture at the level corresponding to the field capacity, equivalent to 240 mL of water retention. At the end of the assay, the water content in the substrate was estimated at 86.4 and 28.8% for the control and stress treatments, respectively, based on the methodology described by Teixeira et al., (2017). Soil moisture was controlled via daily application of 20 mL of water. That value was based on the differences between the weight of the vase at field capacity and after water loss during the day. Minimum and maximum temperature and relative air humidity measured during the assay were 21.5 and 43°C, and 40 and 86%, respectively.

Growth analysis

Plant growth was measured at the end of the assay based on the total number of leaves (NL), main stem height (MSH), dry mass of canopy (DMC), and dry mass of roots (DMR). The last two were obtained after drying in a forced air oven at 65°C for 72 h.

Gas exchanges and relative water content

Gas exchanges were analyzed between 9:00 and 11:00 A.M. using an IRGA (model LCpro-SD) with no artificial carbon source and using an artificial light source of 1,200 $\mu\text{mol m}^{-2}\text{m}^{-1}$. The following traits were analyzed: Rate of liquid photosynthesis (A), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), and transpiration (E). Such

data were used to estimate the instantaneous carboxylation efficiency ($IEC=A/C_i$) and the instantaneous water-use efficiency ($IEWU=A/E$) (Dutra et al., 2018).

Relative water content (RWC) was estimated from the mass of six foliar discs collected from mature leaves located in the upper part of the plant according to the methodology by Brito et al., (2011): $RWC=[(FM-DM)/(TM-DM)] \times 100$, where FM- fresh matter (moment of collection), TM- turgid matter (discs immersed in water for 12 h), and DM- dry matter (discs dried in an oven at 60°C until constant weight).

Antioxidant enzyme

At the end of the assay, 200 mg of leaves were collected and stored at -80°C for extraction of total proteins (Pereira et al., 2012). Proteins were quantified using the method by Bradford (1976) in a spectrophotometer at 595 nm. Antioxidant activity was estimated in a spectrophotometer using superoxide dismutase (SOD) (Bulbovas et al., 2005), catalase (CAT) (Azevedo et al., 1998), and ascorbate peroxidase (APX) (Nakano and Asada, 1981) enzymes at wavelengths of 560, 240, and 290 nm.

Statistical analysis

The data were first submitted to Shapiro-Wilk normality test (Shapiro and Wilk 1965) with transformation into Log X at base 10 of those failing to meet the criteria for normalization of the distribution of deviations (Pimentel-Gomes, 2000). With the assumptions of normality met, analysis of variance using F-test ($p < 0.05$) was performed based on orthogonal contrasts of mean estimates from treatments and comparisons by Student's t -test at 5% confidence (Barbosa and Maldonado Júnior, 2015). The following contrasts were compared:

$$\hat{Y}_1 = 4 * 0NS - 0WS - T1 - T2 - T3$$

$$\hat{Y}_2 = 3 * 0WS - T1 - T2 - T3$$

$$\hat{Y}_3 = 0WS - T1$$

$$\hat{Y}_4 = 0WS - T2$$

$$\hat{Y}_5 = 0WS - T3$$

In the \hat{Y}_1 contrast, the mean of the control treatment is compared with the group of means of the stress treatments and SOD doses; for \hat{Y}_2 , the mean of the stress treatment is compared with the SOD doses; \hat{Y}_3 , \hat{Y}_4 , and \hat{Y}_5 , compare the mean of the stress treatment with the mean of the stress treatment with the plots treated with 11, 23 and 34 $\mu\text{g mL}^{-1}$, respectively. The analyses were carried out using the computational system for statistical analyses Sisvar 5.6 (Ferreira, 2019).

RESULTS AND DISCUSSION

In this study, exogenous SOD use was tested aiming to investigate its mitigating effect in peanut plants submitted to water stress. To support such hypothesis, five orthogonal contrasts were established to provide a basis for the proposed experimental design. The summary of analysis of variance based on these contrasts, based on mean estimates of treatments obtained from growth and physiological traits of tolerant and sensitive cultivars are found in [Supplementary 1](#). We found that, for all 14 traits analyzed,

the contrast $\hat{Y}1$ was the most adequate as it enabled discriminating most responses relevant to the questions of the assay, with a high percentage of significance ($p \leq 0.01$). Hence, the discussion of the results was based on this contrast.

Growth of the plants treated with exogenous SOD

In general, the two peanut cultivars were affected differently during the short water stress period. However, for the early-maturing tolerant cultivar, exogenous SOD application did not have a differential effect on the traits evaluated, although a difference among treatments was found for MSH, DMC, and DMR (Supplementary 1, Figure 1). For the sensitive cultivar, significant differences were observed for MSH and DMR (Supplementary 1). However, the benefit of SOD was seen only for MSH (Figure 2), whose mean height at the three SOD concentrations was 67% higher than in the stressed treatment, suggesting a relieving effect for the stressed plants at the beginning of growth.

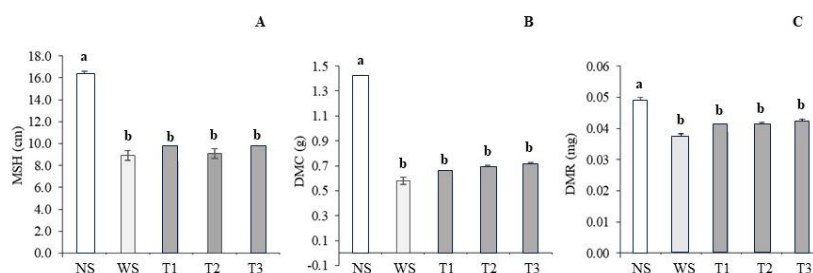


Figure 1. Vegetative growth of a drought tolerant cultivar submitted to water suppression and treated with exogenous SOD. A- MSH- Main stem height, B- DMC- Dry mass of canopy, C- DMR- Dry mass of roots. NS- No water stress, WS- water stress, T1- WS + $11 \mu\text{g mL}^{-1}$ of SOD, T2- $23 \mu\text{g mL}^{-1}$ of SOD, T3- $34 \mu\text{g mL}^{-1}$ of SOD.

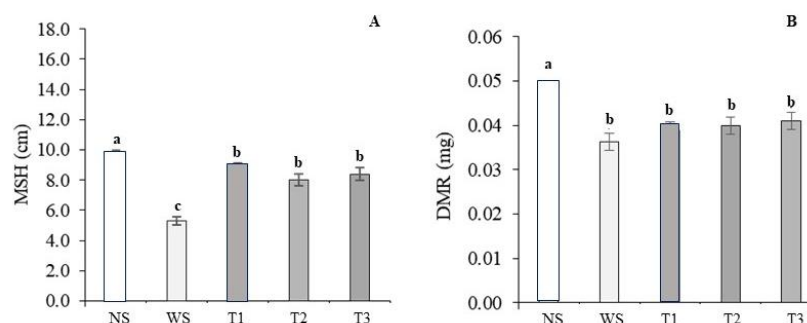


Figure 2. Vegetative growth of a drought sensitive cultivar submitted to water suppression and treated with exogenous SOD. A- MSH- Main stem height, B- DMC- Dry mass of canopy, C- DMR- Dry mass of roots. NS- No water stress, WS- water stress, T1- WS + $11 \mu\text{g mL}^{-1}$ of SOD, T2- $23 \mu\text{g mL}^{-1}$ of SOD, T3- $34 \mu\text{g mL}^{-1}$ of SOD.

Reduction in height is one of the main indicators of the behavior of peanut plants when facing a water suppression situation, even in the early phase. Such strategy aims at minimizing energy in the photosynthetic machinery during growth, even because plant height is negatively correlated with pod production in upright plants (Luz et al., 2010;

Arruda et al., 2015). As stress advances, other growth traits are also impacted, leading to different disorders as a function of the tolerance of the genotype. The present work noticed that the height of tolerant cultivar was more impacted (43%) than in sensitive one (29%), which is justified by the upright and runner phenotypes, respectively. Runner plants often have a main stem between 8 and 12 cm, whereas upright ones range from 25 to 50 cm (Santos et al., 2012). Therefore, the differences become more noticeable in *fastigiata* plants than in *hypogaea* ones. Since water suppression occurred early in the cycle, the tolerant cultivar did not need additional substrate to alleviate the water stress as it is tolerant to drought. The opposite took place with the runner cultivar, whose management greatly depends on water (Santos et al., 2012; Duarte et al., 2013). Hence, exogenous SOD application, irrespective of concentration, relieved the growth of the plants, which recovered 84% of their height when compared with the control plants.

Gas exchanges and relative water content

Seven physiological traits were selected to evaluate stressed plants treated with exogenous SOD. Excepting *IEWU* for tolerant cultivar and *Ci* for sensitive one, both genotypes exhibited significant differences ($p \leq 0.01$) for all traits evaluated ([Supplementary 1](#)). The evaluation of gas exchange of the early-maturing tolerant cultivar showed a reduction in g_s and E by 72 and 80%, respectively, compared with the control plants (Figure 3). Such drastic behavior is very characteristic of that cultivar, which properly adjusts the regulation of guard cells to promote stomatal closure, thereby favoring water conservation (Pereira et al., 2016; Dutra et al., 2018).

SOD application had no effect on those traits in stressed plants, however, at $34 \mu\text{g mL}^{-1}$, an increase by 9% was found in photosynthesis rate compared with the average of the stressed treatment. This caused an increase in the activity of the cell's antioxidant detoxification mechanism, suggesting that the supply of exogenous SOD provided to the plants under stress contributed to maintaining the activities of the photosynthesis process, aiding in breaking down stored ROS, since SOD is present at higher concentration in ROS-producing cell organelles, particularly in chloroplasts (Farooq et al., 2019).

Naturally, SOD acts on the antioxidative metabolism by eliminating ROS during environmental stress conditions (Farooq et al., 2019). The agility of such process depends on how much SOD the cell is able to expression to maintain the defense process, which is what differentiates tolerant and sensitive plants. The response observed in the present study shows that exogenous SOD helped on re-establish cell equilibrium since the brief recovery of A at $34 \mu\text{g mL}^{-1}$ contributed to increase the CO_2 concentration within substomatal chambers by 13% with the application of just $11 \mu\text{g mL}^{-1}$ of exogenous SOD, indicating that this low concentration was sufficient for mobilize the photosynthetic machinery, even with a great reduction in g_s and E .

The lack of *IEC* response in the SOD treatments suggests the carbon available in the stomatal chambers was not fully demanded since the supply of $11 \mu\text{g mL}^{-1}$ was sufficient to recover nearly all of the C_i at 89%, based on the average of the control treatment. All this gas balance benefited the *RWC* of tolerant cultivar because, by preventing water loss by E , the plants possibly adjusted their water potential, leaving it more negative and allowing more water retention in the cell. This situation enabled full re-establishment of *RWC* with

low SOD supply, which, when absorbed by foliar cuticles, helped to eliminate ROS, thus enabling the maintenance of physiological functions.

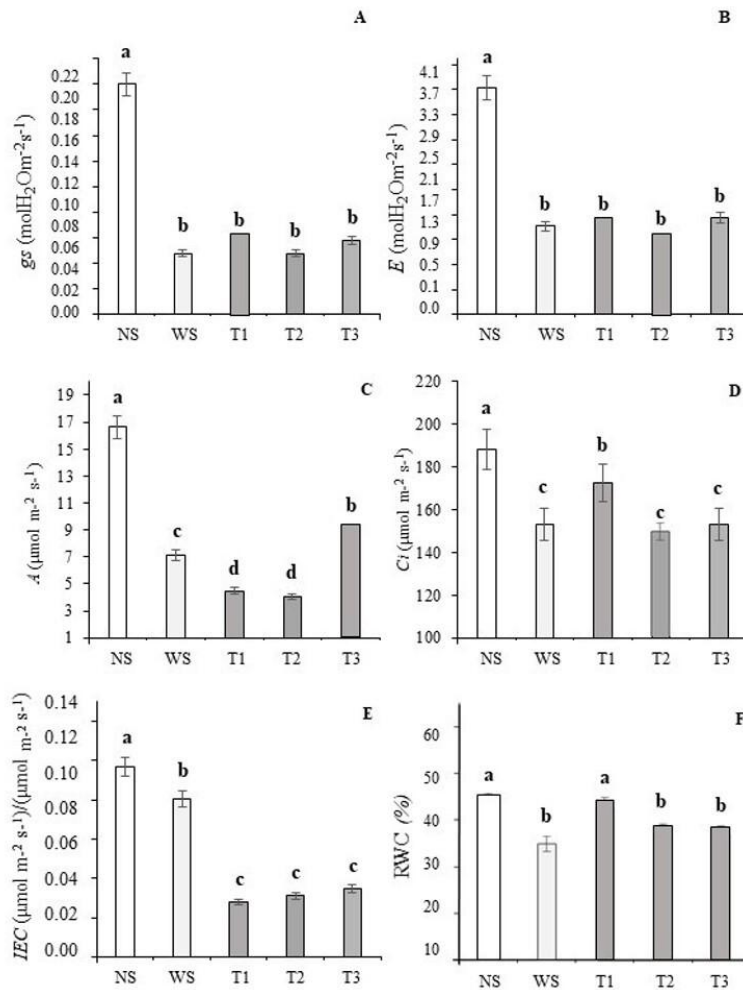


Figure 3. Gas exchange of a drought tolerant cultivar submitted to water suppression and treated with exogenous SOD. A- MSH- Main stem height, B- DMC- Dry mass of canopy, C- DMR- Dry mass of roots. NS- No water stress, WS- water stress, T1- WS + $11 \mu\text{g mL}^{-1}$ of SOD, T2- $23 \mu\text{g mL}^{-1}$ of SOD, T3- $34 \mu\text{g mL}^{-1}$ of SOD.

As for the gas exchanges of the sensitive cultivar, we was found that, just like the early-maturing tolerant cultivar, plants also were benefited from additional SOD supply. The difference is that the late-maturing cultivar benefited from SOD at the early signs of stress and at low concentration ($11 \mu\text{g mL}^{-1}$), which alleviated the g_s , T , and A of plants by 20%, 31%, and 33%, respectively, compared with the WS treatment (Figure 4). Although it seems like modest gains, such recovery was sufficient to ensure adequate carbon flow in the cells, based on the expressive increase by 160% in carboxylation efficiency, represented by the IEC . Despite the lack of statistically significant difference in the C_i among the

treatments ([Supplementary 1](#)), CO₂ stores reduced further diffusive limitations, maintaining water availability in the cells and minimizing oxidative damage.

As seen in Figure 4, the *IEWU*, which correspond to the water status of cells during stress, showed an increase of 19%, compared with the average of the treatments with exogenous SOD. This contributes to the recovery of *RWC* by around 28%, based on the averages of the T1 and T2 treatments, compared to the stressed plants (WS).

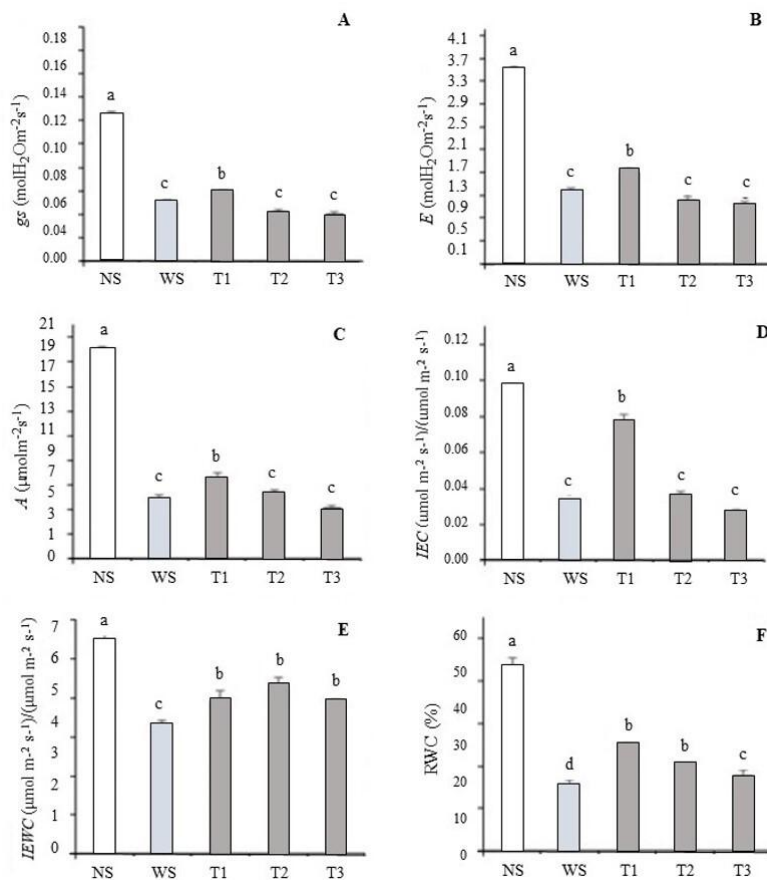


Figure 4. Gas exchange of a drought sensitive cultivar submitted to water suppression and treated with exogenous SOD. A- MSH- Main stem height, B- DMC- Dry mass of canopy, C- DMR- Dry mass of roots. NS- No water stress, WS- water stress, T1- WS + 11 μg mL⁻¹ of SOD, T2- 23 μg mL⁻¹ of SOD, T3- 34 μg mL⁻¹ of SOD.

The role of SOD in the cell medium when faced with environmental stress shows the importance of the enzyme in setting off the antioxidative metabolism. By minimizing excess ROS promoted by cell stress, SOD sets off the dismutation steps to eliminate reactive species. In tolerant species, the amount of SOD acting on the biochemical metabolism must be reasonably produced to protect the cells from oxidative damage. To the extent of our knowledge, the literature has no report on the response to the application of exogenous SOD in plants under water stress. The results of this research, based on data on

gas exchange, provide an exciting overview of the benefit of the enzyme in mitigating water stress, at least in C3 plants. Given the averages of the control treatment of the two cultivars studied, it can be seen that the results become more significant when the *RWC* of drought sensitive variety is shown to reduce by 54% in the stressed treatment (WS). However, with SOD supply between 11 and 23 $\mu\text{g mL}^{-1}$ of SOD, such loss decreased to 40% (Figure 4). In drought-tolerant cultivar, *RWC* reduction was lower at 24% compared to the stressed treatment, however, exogenous SOD application in those plants completely recovered the water condition in plant cells. We cannot state whether such response is predictable in tolerant specimens; further studies with other tolerant genotypes are needed to make such claim. However, it is well known that the lowered requirement of exogenous SOD by tolerant cultivar is precisely due to its broad ability of adjustment when facing water restriction situations, accumulating large amounts of organic solutes, especially proline, a potent osmoregulatory of plants under stress (Nogueira and Santos, 2000; Alves et al., 2016; Pereira et al., 2016; Dutra et al., 2018).

The results found here open the perspective of use of exogenous SOD to alleviate the deleterious effects of water stress, especially taking in account the results found to *RWC*, which demonstrates the percentage of water present in the tissues under stress. In the work by Bhatnagar-Mathur et al., (2009), who investigated the influence of antioxidant enzymes in different types of wild and transgenic peanut under water deficit, the authors reported that the enzymes did not influence on transpiration efficiency. Unlike this conclusion, we found different responses in gas exchange and *RWC* here. Thus, we understand that exogenous SOD supplementation may constitute an opportunity to mitigate water stress, especially in drought-sensitive germplasm.

Antioxidant enzyme

The activity of antioxidative enzymes in the treatments of this study showed a statistically significant difference between cultivars drought tolerant variety and drought sensitive variety, indicating the application of exogenous SOD contributed to the reduction in cell damage in stressed plants ([Supplementary 1](#)).

Figure 5A shows the profile of the enzyme activities in the different treatments applied to drought tolerant variety. It can be seen that SOD absorption at 34 $\mu\text{g mL}^{-1}$ of SOD in stressed plants resulted in a 50% increase in cell SOD availability when compared with the WS treatment. With the amount of SOD available in the cell, the processes of dismutation and consequent elimination of ROS were optimized with support of the activities of CAT and APX. The activity of CAT showed that, at 11 $\mu\text{g mL}^{-1}$ of SOD, the process of elimination of H_2O_2 , generated from the dismutation of the superoxide radical, decreased by 36% when compared with the mean of WS. At higher concentrations (23 and 34 $\mu\text{g mL}^{-1}$ of SOD), the reduction was by 55%, leading to greater availability of O_2 and H_2O for the cells, with lower cellular wear, considering the trend of proximity to the plants of the control treatment.

Such condition was also observed in the activity of APX, which used a lower amount of substrate to reduce the H_2O_2 remaining from the process of neutralization into H_2O . As seen in Figure 5, the application of 11 $\mu\text{g mL}^{-1}$ of SOD contributed to reducing the effort of APX by 50% and, at higher concentrations, by more significant 67%. These results mean that an extra supply of 34 $\mu\text{g mL}^{-1}$ of SOD for stressed plants was quite adequate to

help the cascade of events of these enzymes, minimizing the wear of the cellular machinery often caused by oxidative stress.

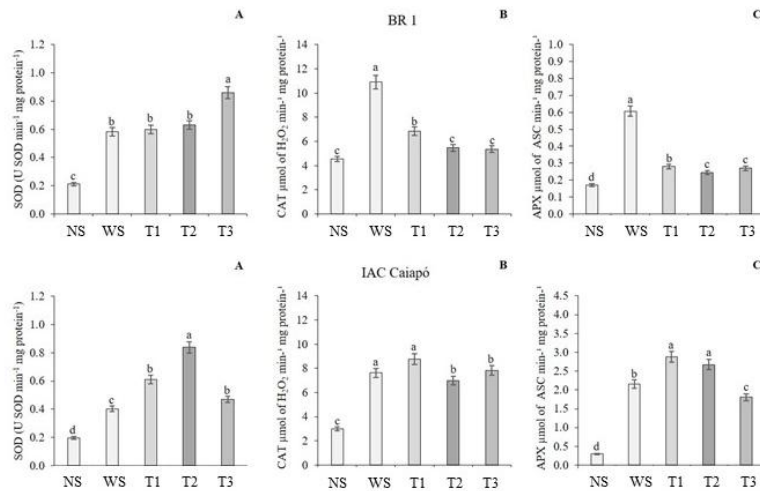


Figure 5. Antioxidative activity of drought tolerant (BR 1) and sensitive (IAC Caiapó) cultivars submitted to water suppression and treated with exogenous SOD. A- MSH- Main stem height, B- DMC- Dry mass of canopy, C- DMR- Dry mass of roots. NS- No water stress, WS- water stress, T1- WS + 11 µg mL⁻¹ of SOD, T2- 23 µg mL⁻¹ of SOD, T3- 34 µg mL⁻¹ of SOD.

In sensitive cultivar, the triggering of the enzymes followed the same pace as in tolerant plants in terms of use of exogenous SOD to minimize cell damage in plants under stress. The difference between them was based on the percentage of mitigation, which was slower in the runner cultivar precisely as it is more sensitive to drought. In addition, the sensitive variety required a higher supply of SOD to respond to the cellular defense process. Thus, the supply of 23 µg mL⁻¹ of SOD enabled a significant increase by 200% of SOD available in the cells based on the average of the plants in the stressed treatment (Figure 5). That is a very encouraging result since, as it initiates the antioxidative process, the increase in SOD allowed the steps of dismutation and neutralization of reactive species to be carried out unimpeded, without exhausting the cellular machinery. Despite this supply, the agility in H₂O₂ reprogram metabolism by CAT and APX in stressed plants was modest when considering the reduction in activity of those enzymes in stressed plants. CAT (23 and 34 µg mL⁻¹ of SOD) and APX (34 µg mL⁻¹ of SOD) reduced activity by only 19% and 22%, when compared with WS.

These results show that, in both cultivars, exogenous SOD contributed to minimizing oxidative damage in plants water stress. The rate of response of drought tolerant cultivar, however, was more agile since it managed to scavenge the effects of ROS, reaching closer to the average of the control plants, as seen in APX, which scavenging system process.

In the chain of events of antioxidative enzymes, SOD, CAT, and APX act in a coordinated manner to eliminate ROS and prevent cell damage (Sharma et al., 2019). In resistant plants, an supply is often seen in SOD of stressed plants to favor the steps of H₂O₂ neutralization and O₂ and H₂O release to the cells by CAT and APX (Pereira et al., 2012; Farooq et al., 2019). We did not find in literature reports involving application of exogenous

SOD under stress, however, as it is involved in the cellular defense processes, it is expected that, an increase in enzyme activity, benefitting plants tend to draw closer to the normality condition. This study verified such ability in the drought-resistant cultivar. However, in sensitive cultivar, despite the slower response, the mitigating effect of exogenous SOD was more beneficial if the reductions in activity exhibited by the stressed plants when compared with the control treatment are taken into account. This is a prospective study, and it provides a basis for other genotypes to be studied, including exploring the possible practical benefits of this enzyme in peanut crop management.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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