

# Exogenous glycine betaine modulates ascorbate peroxidase and catalase activities and prevent lipid peroxidation in mild water-stressed *Carapa guianensis* plants

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## Abstract

The hypothesis that application of exogenous glycine betaine (GB<sub>EX</sub>) may attenuate the effects of mild water deficit in leaf gas exchange and lipid peroxidation in *Carapa guianensis* was examined. For this reason, 110-d old plants were sprayed with 0, 25, and 50 mM GB<sub>EX</sub> and then subjected to two watering regimes. In the first, irrigation was continuously performed to maintain the soil near to field capacity (watered plants). In the second, irrigation was withheld and water deficit resulted from progressive evapotranspiration (water-stressed plants). Treatment comparisons were assessed when predawn leaflet water potential ( $\Psi_{pd}$ ) of stressed plants reached  $-1.28 \pm 0.34$  MPa. Regardless of the watering regime, significant ( $P < 0.05$ ) increases in foliar glycine betaine (GB<sub>Leaf</sub>) concentration were observed in response to increasing GB<sub>EX</sub>; however, such increases were more expressive in stressed plants. The net photosynthetic rate, stomatal conductance to water vapor, and intercellular to ambient CO<sub>2</sub> concentration ratio were significantly lower in water-stressed plants independently of GB<sub>EX</sub> concentration sprayed on leaves. The application of 25 and 50 mM GB<sub>EX</sub> caused significant ( $P < 0.05$ ) increases in ascorbate peroxidase (APX) activity in stressed plants, while significant ( $P < 0.05$ ) increases in catalase activity was observed just in the stressed plants treated with 50 mM GB<sub>EX</sub>. Malondialdehyde concentrations did not differ between watered and stressed plants regardless of GB<sub>EX</sub> concentration. In conclusion, *C. guianensis* was able to incorporate GB<sub>EX</sub> through their leaves and the resulting increases in GB<sub>Leaf</sub> attenuated lipid peroxidation in stressed plants through positive modulation of APX and CAT activities.

*Additional key words:* antioxidant enzymes; drought; gas exchange; malondialdehyde; oxidative stress.

## Introduction

Glycine betaine (GB) is an amphoteric compound derived from glycine (Sakamoto and Murata 2002) found in many plant organs, mainly under abiotic stress conditions such as water deficit (Quan *et al.* 2004, Hassine *et al.* 2008, Costa *et al.* 2010, Wang *et al.* 2010), salt stress (Hassine *et al.* 2008, Hattori *et al.* 2009, Meloni and Martinez 2009) and low temperature (Allard *et al.* 1998). GB synthesis and accumulation varies between plant species and according to stress type, severity, and duration (Quan *et al.* 2004, Wang *et al.* 2010). There are some species unable to synthesize GB even under stress condition, such as tobacco (Nuccio *et al.* 1998) and few corn genotypes (Rhodes *et al.* 1989).

In higher plants, GB is preferentially synthesized from choline oxidation through coordinate activities of betaine monooxygenase and betaine aldehyde dehydrogenase, which are mainly present in chloroplasts (Chen and Murata 2011). In the first reaction, choline is oxidized to aldehyde by betaine monooxygenase and in the second reaction, the aldehyde is oxidized to GB by NAD<sup>+</sup>-dependent betaine aldehyde dehydrogenase (Takabe *et al.* 2006). There is no evidence of GB synthesis in cytosol or other organelle, but there is evidence that GB may be transported between different cell compartments (Chen and Murata 2011).

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**Abbreviations:** APX – ascorbate peroxidase; CAT – catalase;  $C_i/C_a$  – intercellular to ambient CO<sub>2</sub> concentration ratio; DM – dry mass; GB<sub>EX</sub> – exogenous glycine betaine; GB<sub>Leaf</sub> – foliar glycine betaine;  $g_s$  – stomatal conductance to water vapor; MDA – malondialdehyde;  $P_N$  – net photosynthetic rate; PAR – photosynthetically active radiation; ROS – reactive oxygen species; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase;  $\Psi_{pd}$  – predawn leaflet water potential.

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response to abiotic stresses has been attributed to different mechanisms. Depending on plant species, GB may act as osmotically active compound in cytosol (Pimentel 1999, Lv *et al.* 2007, Hassini *et al.* 2008, Iqbal *et al.* 2008) and as structural stabilizer of proteins, enzymes (Sakamoto and Murata 2002), and cellular membranes (Mansour 1998). GB may also attenuate the effects of water deficit on leaf gas exchange (Ma *et al.* 2006, Yang and Lu 2006, Ma *et al.* 2007, Farooq *et al.* 2008) and oxidative stress (Raza *et al.* 2007).

*Carapa guianensis* (Aubl.) is an evergreen, woody species typically found in the Amazonian biome, whose seeds produce an oil used in the manufacturing of medicines, cosmetics, biofuels, and repellents (Mendonça and Ferraz 2007). Due to its economical relevance, this species has been considered for planting in agroforestry systems in degraded Amazonian lands. However, these areas are prone to suffer prolonged dry season (Brando *et al.* 2010). Therefore, the water deficit conditions may limit plant growth and production. Indeed, the water-stressed *C. guianensis* plants showed decreased net photosynthetic rate ( $P_N$ ) and at least in part it may be caused by slight decreases in chlorophyll *a* fluorescence parameters (Gonçalves *et al.* 2009). This indicates that decreases in  $CO_2$  fixation under stress conditions may occur in parallel to continued electron flux between photosystems. As consequence, an imbalance between photochemical and biochemical pathways of net photosynthesis may favor direct reactions between electrons coming from photosystem I with molecular oxygen (Asada 1999). The resulting reactive oxygen species (ROS) are potential oxidants and if plants are not endowed with an efficient enzymatic and nonenzymatic antioxidant system to cope with ROS, then oxidative damages to membrane lipids, DNA, proteins, and

enzymes may be expected in some extent (Mittler 2002). The occurrence of oxidative damages to membrane lipids in water-stressed *C. guianensis* plants was evident under mild drought conditions and it was adequately mitigated under more prolonged drought (Costa *et al.* 2010). These responses were respectively associated to lower and higher ascorbate peroxidase (APX) and catalase (CAT) activities (Costa *et al.* 2010).

A GB-mediated positive modulation of antioxidant enzymes activities and attenuation of oxidative damages in response to abiotic stresses have been proposed for different plant species (Lv *et al.* 2007, Raza *et al.* 2007, Farooq *et al.* 2008, Nawaz and Ashraf 2010). In *C. guianensis*, an increase in GB concentration under more prolonged water-deficit conditions was coincident to higher APX and CAT activities. This is an evidence for GB-mediated modulation of antioxidant enzymes in this species (Costa *et al.* 2010). If GB is really able to modulate the antioxidant enzymes' activities, we hypothesized that an increase in  $GB_{Leaf}$  concentration mediated by  $GB_{EX}$  application in *C. guianensis* plants under mild water-deficit conditions could result in higher activities of antioxidant enzymes, especially of APX and CAT. As consequence,  $GB_{EX}$  application could contribute to mitigation of damages to membrane lipids in *C. guianensis* plants subjected to mild water deficit. To test this hypothesis, *C. guianensis* plants previously sprayed with different  $GB_{EX}$  concentrations were subjected to full irrigation and water deficit conditions. Plant comparisons were performed aiming to examine the effects of  $GB_{EX}$  application on  $GB_{Leaf}$  concentration, leaflet gas-exchange variables, lipid peroxidation, and APX and CAT activities in *C. guianensis* plants subjected to full irrigation and water-deficit conditions.

## Materials and methods

**Plant material and experimental setup:** This research was carried out in a greenhouse placed in the campus of Federal Rural University of Amazon, Belém-PA, north Brazil (01°28'03"S, 48°29'18"W). Ninety-day-old *Carapa guianensis* (Aubl.) plants showing similar stem diameter and height and equal leaf and leaflet number were selected from seedling nursery and planted in 20 L polyethylene pots (one plant per pot) filled with sifted yellow loam latosol as substrate. The substrate pH, macro and micronutrient concentrations were adjusted as recommended for tropical trees (Brasil and Cravo 2007). The irrigation was performed daily to replace evapotranspired water and weeds were manually removed as necessary. When plants were 130-d-old, the 36 most uniform plants according to their stem height and diameter and leaf number were chosen for experimental setup. The selected plants were divided to three groups and each group was sprayed with a different  $GB_{EX}$  solution (0, 25, or 50 mM  $GB_{EX}$ ). GB concentrations

were selected according to results obtained for other plant species (Raza *et al.* 2007, Farooq *et al.* 2008, Nawaz and Ashraf 2010). GB solutions were prepared in distilled water plus 0.1% (v/v) Tween 20 just before their application to plants. The 0 mM GB solution corresponded to 0.1% (v/v) Tween 20 solution. Each plant group was uniformly sprayed, between 8:00 and 9:00 h, with 100 mL of  $GB_{EX}$  using a polyethylene hand sprayer. Twenty four hours after  $GB_{EX}$  application, plants in each group were subdivided to two watering regimes. In one regime, plants were continuously irrigated as previously mentioned (full irrigation treatment; well-watered plants); and in the other, irrigation was completely withheld (water-deficit treatment, water-stressed plants) and the water deficit resulted from the progressive evapotranspiration. During the whole experimental period, the mean air temperature, relative air humidity, and photosynthetically active radiation (PAR) at midday were 34°C, 90%, and 1,044.39  $\mu\text{mol}(\text{photon})\text{ m}^{-2}\text{ s}^{-1}$ , respec-

tively. The experiment consisted of a completely randomized design with six treatment combinations forming a 3×2 factorial scheme (three GB<sub>EX</sub> concentrations and two watering regimes). Each experimental replicate was constituted by one plant per pot. Treatment comparisons were assessed when predawn leaf water potential ( $\Psi_{pd}$ ) of water-stressed plants, measured using a *Scholander*-type pressure chamber (*m670*, *Pms Instrument Co.*, Albany, USA) between 4:30 and 5:30 h, reached around  $-1.28 \pm 0.34$  MPa. Leaf gas exchange and sampling for biochemical assays were carried out between 11:00 and 13:00 h. Two mature, healthy leaflets from the third leaf from the stem apex were sampled for leaf gas-exchange measurements and fragments of leaflet tissue [*ca.* 0.3 g (fresh mass, FM)] were collected for each biochemical parameter evaluated. All samples were frozen at  $-20^{\circ}\text{C}$  until analyses (Costa *et al.* 2010).

**Foliar glycine betaine (GB<sub>Leaf</sub>)** was determined according to Grieve and Grattan (1983) exactly as modified by Costa *et al.* (2010). A GB standard curve was used to determine GB<sub>Leaf</sub>.

**Leaf gas exchange:**  $P_N$ , stomatal conductance to water vapor ( $g_s$ ), and intercellular to ambient CO<sub>2</sub> concentration ratio ( $C_i/C_a$ ) were measured using a portable open-system infrared gas analyzer (*LCpro*, *ADC Bioscientific Ltd.*, Hoddesdon, UK) under ambient CO<sub>2</sub> concentration as described in Moraes *et al.* (2011). During measurements, the mean air temperature, relative air humidity, and PAR at midday were 34°C, 90%, and 1,044.39  $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ , respectively.

**Enzyme activities:** APX (EC 1.11.1.11) and CAT (EC 1.11.1.6) extractions and activities assays were performed according to Nakano and Asada (1981) and Havir and McHale (1987) as exactly modified by Costa *et al.* (2010).

## Results

**GB<sub>Leaf</sub>.** The well-watered and water-stressed plants showed 30.6  $\mu\text{mol}(\text{GB}_{\text{Leaf}}) \text{g}^{-1}(\text{DM})$  and 37.3  $\mu\text{mol}(\text{GB}_{\text{Leaf}}) \text{g}^{-1}(\text{DM})$ , respectively, when no GB<sub>EX</sub> was added (Fig. 1). This indicated that endogenous GB synthesis was enhanced under water-deficit conditions. The GB<sub>EX</sub> application induced significant increases in GB<sub>Leaf</sub> concentrations regardless of plant water status. Therefore, the GB<sub>Leaf</sub> concentration in well-watered plants significantly ( $P<0.05$ ) increased from 32.5  $\mu\text{mol}(\text{GB}_{\text{Leaf}}) \text{g}^{-1}(\text{DM})$  in plants treated with 0 or 25 mM GB<sub>EX</sub> to 47.2  $\mu\text{mol}(\text{GB}_{\text{Leaf}}) \text{g}^{-1}(\text{DM})$  in plants treated with 50 mM GB<sub>EX</sub> (Fig. 1). In water-stressed plants, the GB<sub>Leaf</sub> concentration significantly ( $P<0.05$ ) increased from 37.3  $\mu\text{mol}(\text{GB}_{\text{Leaf}}) \text{g}^{-1}(\text{DM})$  in GB<sub>EX</sub>-untreated plants (0 mM GB<sub>EX</sub>) to 46.2  $\mu\text{mol}(\text{GB}_{\text{Leaf}}) \text{g}^{-1}(\text{DM})$  and 54.9  $\mu\text{mol}(\text{GB}_{\text{Leaf}}) \text{g}^{-1}(\text{DM})$  in plants treated with 25 and 50 mM GB<sub>EX</sub> (Fig. 1).

The protein quantification was performed according to Bradford (1976). To calculate enzyme activity, it was considered that 1 unit (U) of APX is the quantity of enzyme that oxidizes 1  $\mu\text{mol}(\text{ascorbate}) \text{min}^{-1}$  and 1 U of CAT is the quantity of enzyme that oxidizes 1  $\mu\text{mol}(\text{H}_2\text{O}_2) \text{min}^{-1}$ .

**Lipid peroxidation** was estimated as 2-thiobarbituric acid (TBA) reactive substances and expressed as equivalents of malondialdehyde (MDA) according to Cakmak and Horst (1991). Leaflet samples were ground in 3 mL of 0.1% (w/v) trichloroacetic acid (TCA) and the slurry was centrifuged at  $15,000 \times g$  for 15 min at 4°C. A 0.5 mL aliquot from the supernatant reacted with 1.5 mL of 0.5% 2-thiobarbituric acid (TBA; prepared in 20% TCA). Samples were homogenized and colorimetric reaction was performed at 90°C for 20 min. After this time, samples were immersed in an ice bath and they were centrifuged at  $13,000 \times g$  for 8 min at 25°C. Sample absorbance was measured using a UV-visible spectrophotometer (*Genesys<sup>TM</sup> 10series*, *Thermo Electron Co.*, Madison, USA) at 532 nm and corrected for nonspecific turbidity by subtracting the absorbance at 600 nm. Lipid peroxidation was estimated as the content of total TBA reactive substances and expressed as equivalents of MDA, calculated from their extinction coefficient ( $155 \text{ mM}^{-1} \text{ cm}^{-1}$ ). The results were expressed in  $\text{nmol}(\text{MDA}) \text{g}^{-1}(\text{DM})$ .

**Statistical analysis:** All data were tested by analysis of variance (*ANOVA*) considering 3×2 factorial scheme (three GB<sub>EX</sub> concentrations and two watering regimes). Comparisons between averages were performed by *Tukey's test* ( $P<0.05$ ). Data showed in the figures represents the average of six replicates  $\pm$  standard deviation (SD). Each experimental replicate was constituted by one plant per pot.

**Leaf gas exchange:** In GB<sub>EX</sub>-untreated plants, grown under full irrigation conditions, the averages of  $P_N$ ,  $g_s$ , and  $C_i/C_a$  were 7.00  $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ , 195  $\text{mmol}(\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}$  and 0.742  $\text{mol}(\text{CO}_2) \text{mol}^{-1}(\text{CO}_2)$ , respectively (Fig. 2). When these plants were subjected to mild water-deficit conditions,  $P_N$ ,  $g_s$ , and  $C_i/C_a$  averages were respectively decreased to 1.83  $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ , 20  $\text{mmol}(\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}$ , and 0.395  $\text{mol}(\text{CO}_2) \text{mol}^{-1}(\text{CO}_2)$  (Fig. 2). Therefore, the water deficit caused 74%, 90%, and 47% reductions in  $P_N$ ,  $g_s$ , and  $C_i/C_a$  (Fig. 2).

When plants were compared within the same water regime (GB<sub>EX</sub> effect), one could observe that GB<sub>EX</sub> application (0, 25, or 50 mM) did not cause any effect (increases or decreases) in leaflet gas-exchange variables (Fig. 2). Therefore, nonsignificant ( $P>0.05$ ) differences in  $P_N$ ,  $g_s$ , and  $C_i/C_a$  were observed between well-watered

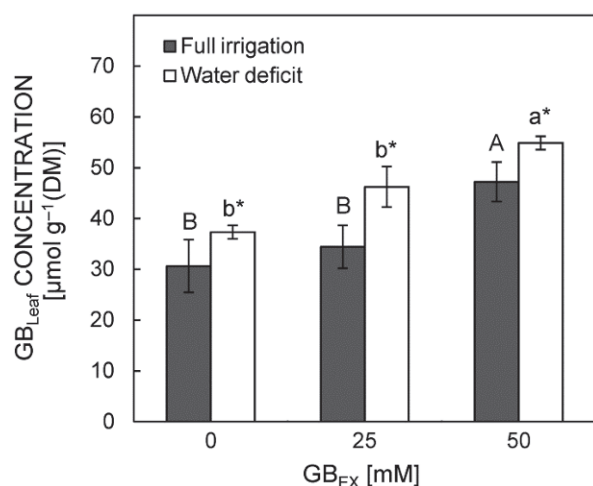


Fig. 1. Foliar glycinebetaine (GB<sub>Leaf</sub>) concentration in *C. guianensis* plants, sprayed with different exogenous glycine betaine (GB<sub>EX</sub>) concentration (0, 25, and 50 mM) and subjected to two watering regimes (full irrigation and water deficit). Different capital letters denote significant differences among means for full-irrigated plants, and different small letters represent significant differences among means for water-stressed plants by the Tukey's test at  $P < 0.05$  (GB<sub>EX</sub> effect). Means for water-stressed plants marked with an asterisk differ from those for full-irrigated plants by the  $F$ -test at  $P < 0.05$  (watering-regime effect). Values are means  $\pm$  SD ( $n = 6$ ).

plants treated with 0, 25, or 50 mM GB<sub>EX</sub> (Fig. 2). Similar responses to GB<sub>EX</sub> application were also observed in water-stressed plants (Fig. 2).

**Enzyme activities:** In plants sprayed with 0 mM GB<sub>EX</sub>, the mild water-deficit significantly ( $P < 0.05$ ) increased APX activity (Fig. 3A) with no effect (increase or decrease) in CAT activity (Fig. 3B). Both APX and CAT activities in well-watered plants were not influenced by GB<sub>EX</sub> application (Fig. 3). In these plants, the average of APX activity was  $0.42 \text{ U mg}^{-1}(\text{protein})$  (Fig. 3A) and the average of CAT activity was  $0.85 \text{ U mg}^{-1}(\text{protein})$  (Fig. 3B). In water-stressed plants, the APX activity significantly ( $P < 0.05$ ) increased from  $0.65 \text{ U mg}^{-1}(\text{protein})$  in plants treated with 0 or 25 mM GB<sub>EX</sub> to  $1.26 \text{ U mg}^{-1}(\text{protein})$  in plants treated with 50 mM GB<sub>EX</sub> (Fig. 3A). For these plants, significant ( $P < 0.05$ ) changes in CAT activity were just observed after spraying plants with 50 mM GB<sub>EX</sub> (Fig. 3B).

**Lipid peroxidation:** The MDA concentration was unchanged by GB<sub>EX</sub> application, independently of plant water status (Fig. 4). Thus, the average of MDA concentration was  $249 \text{ nmol(MDA) g}^{-1}(\text{DM})$  in well-watered plants and  $264 \text{ nmol(MDA) g}^{-1}(\text{DM})$  in stressed plants

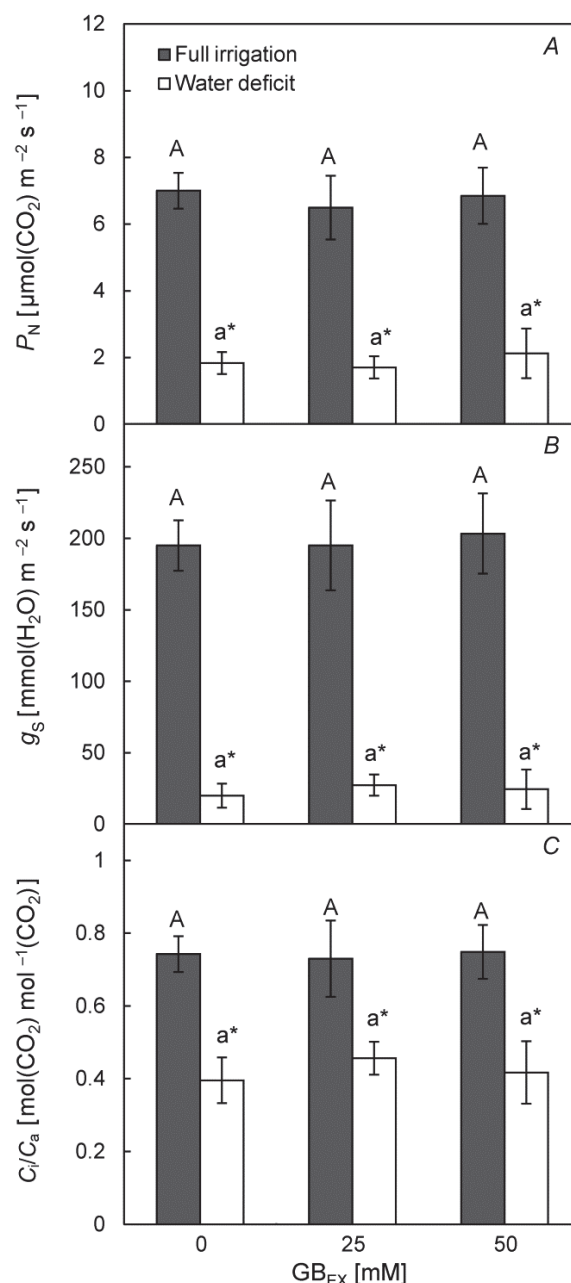


Fig. 2. Net photosynthetic rate ( $P_N$ ), stomatal conductance to water vapor ( $g_s$ ), and intercellular to ambient CO<sub>2</sub> concentration ratio ( $C_i/C_a$ ) in *C. guianensis* leaves sprayed with different exogenous glycine betaine (GB<sub>EX</sub>) concentration (0, 25, and 50 mM) and subjected to two watering regimes (full irrigation and water deficit). Statistics as in Fig. 1.

(Fig. 4). Furthermore, nonsignificant differences in MDA concentration between well-watered and water-stressed plants were observed, regardless of GB<sub>EX</sub> concentration applied on leaves (Fig. 4).

## Discussion

The GB-constitutive synthesis in plants sprayed with no GB<sub>EX</sub> was evident under full irrigation or under water-deficit conditions. The higher GB concentration in water-stressed plants confirmed our previous study (Costa *et al.* 2010) and indicated that Carapa plants induced GB synthesis and its foliar accumulation as a strategy to tolerate water-deficit conditions. Similar results were observed previously for different plant species (Ma *et al.* 2006, Lv *et al.* 2007, Ma *et al.* 2007, Farooq *et al.* 2008, Iqbal *et al.* 2008), and they were at least in part related to GB-mediated decreases in foliar osmotic potential, causing an osmotic adjustment that stimulate root water uptake from dried soils and its transport to aboveground tissues. This osmotic adjustment contributes to an attenuation of the water-deficit effects on plant turgor as observed in *Phaseolus vulgaris* (Weibing and Rajashekar 1999).

The increased GB<sub>Leaf</sub> concentrations in GB-sprayed plants indicated that *C. guianensis* was able to uptake GB<sub>EX</sub> through its leaflet surface, regardless of plant water status. Particularly for water-stressed plants assessed under  $\Psi_{pd}$  of  $-1.28$  MPa, the increases in GB<sub>Leaf</sub> concen

tration in response to foliar application of 25 or 50 mM GB<sub>EX</sub> were comparable with GB<sub>Leaf</sub> concentration in *C. guianensis* plants assessed under  $\Psi_{pd}$  of  $-3.2$  MPa (Costa *et al.* 2010). Therefore, the GB<sub>Leaf</sub> concentrations in plants sprayed with 25 or 50 mM GB<sub>EX</sub> and subjected to mild water-deficit conditions were similar to those naturally found in *C. guianensis* plants subjected to prolonged water-deficit conditions (Costa *et al.* 2010). These results allowed examine if GB<sub>EX</sub> was really able to attenuate the effects of mild water-deficit in leaf gas exchange and lipid peroxidation in this species.

The GB<sub>EX</sub> application in *Oryza sativa* (Farooq *et al.* 2008) and *Nicotiana tabacum* (Ma *et al.* 2007) attenuated the negative effects of water deficit in  $P_N$ . Similar effect was observed in stressed *Gossypium hirsutum* plants expressing the *beta* gene, responsible for greater endogenous GB synthesis (Lv *et al.* 2007). This response was in part associated with the protective role of GB as structural stabilizer agent of cellular membranes, including the thylakoid membranes (Robinson and Jones 1986, Genard *et al.* 1991). Thus, the exogenous GB application may prevent expressive reductions in  $P_N$  and for this reason its usage has been proposed as strategy to maintenance or improvement of plant growth and yield under stress conditions (Agboma *et al.* 1997). In this experiment, however, the magnitudes of decreases in  $P_N$  were not attenuated by GB<sub>EX</sub> application in water-stressed plants. Nevertheless, the protective role of this compound on photochemical apparatus could not be excluded in this species because (1) nonsignificant changes in chlorophyll *a* and *b* and total carotenoids concentrations were observed in stressed *C. guianensis* plants subjected to leaf water potential around  $-3.2$  MPa (Costa *et al.* 2010), and

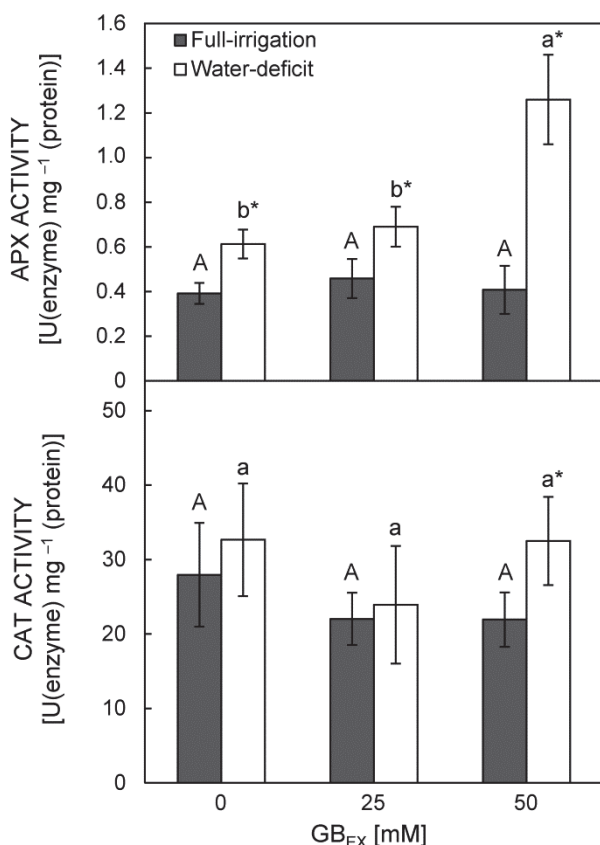


Fig. 3. Ascorbate peroxidase (APX) and catalase (CAT) activities in *C. guianensis* leaves sprayed with different exogenous glycine betaine (GB<sub>EX</sub>) concentration (0, 25, and 50 mM) and subjected to two watering regimes (full irrigation and water deficit). Statistics as in Fig. 1.

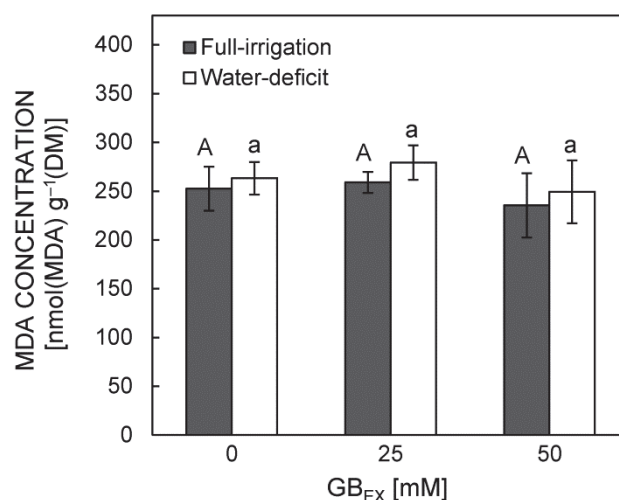


Fig. 4. Malondialdehyde (MDA) concentration in *C. guianensis* leaves sprayed with different exogenous glycine betaine (GB<sub>EX</sub>) concentration (0, 25, and 50 mM) and subjected to two watering regimes (full irrigation and water deficit). Statistics as in Fig. 1.

(2) only slight changes in chlorophyll *a* fluorescence parameters were registered for *C. guianensis* plants subjected to leaf water potential around  $-3.4$  MPa (Gonçalves *et al.* 2009). On the other hand, our results showed that water deficit caused significant decreases in  $g_s$  and  $C_i/C_a$ . Thus, the  $P_N$  decreases in stressed plants might be at least in part explained by lower intercellular  $CO_2$  availability under stress conditions, which would decrease the Rubisco carboxylase activity and would stimulate photorespiration.

Under water-deficit conditions, the continuous electron transport through photosystems associated with a lower biochemical  $CO_2$  fixation may result in an increased ROS production inside chloroplasts (Asada 1999). Moreover, if  $CO_2$  availability inside chloroplasts is diminished in response to limited  $g_s$ , a higher Rubisco oxygenase activity is stimulated. As consequence, an overproduction of  $H_2O_2$  inside peroxisomes is expected (Mittler 2002). Considering the previous studies about *C. guianensis* responses to water deficit (Gonçalves *et al.* 2009, Costa *et al.* 2010), one can infer this species is prone to suffer oxidative damages under mild water deficit. In fact, when plants of different water regimes in this experiment were sprayed with 0 mM GB, the highest activity of APX in stressed plants was a clear evidence of the occurrence of oxidative stress mediated by mild water deficit in this species. The higher activity of APX explained the absence of significant differences in MDA concentration between well watered and stressed plants treated with 0 mM GB. Therefore, the possible oxidative damages due to water deficit were adequately mitigated by the constitutive APX activity. This result contrasts with that previously observed by Costa *et al.* (2010), who observed no significant increases in activity of APX under moderate water deficit. It is noteworthy, however, that the climatic conditions in the two experiments were quite different, especially the photosynthetically active radiation, which was higher during our experiment. Furthermore, considering that no changes in CAT activity

were observed for plants sprayed with 0 mM GB, one can also infer that oxidative stress was more properly related to the metabolic changes in net photosynthesis than photorespiration. This result is in line to previous data reported by Costa *et al.* (2010).

Both APX and CAT activities were modulated by GB<sub>EX</sub> application. However, these effects were only observed in water-stressed plants, possibly because an overproduction of ROS was just observed under stressful conditions. By comparison, the exogenous GB application improved *Oryza sativa* tolerance to salt stress (Demiral and Türkan 2004) and water stress (Farooq *et al.* 2008) by promoting increase in APX activity. On the other hand, divergent data concerning the exogenous GB effects in CAT activity have been reported. Therefore, significant decreases (Demiral and Türkan 2004, Ma *et al.* 2006, Raza *et al.* 2007) and increases (Ma *et al.* 2006, 2007, Raza *et al.* 2007, Farooq *et al.* 2008) in CAT activity may be observed according to the species, genotype, stress type, and intensity. Our results also showed that APX activity seemed to be better modulated by GB<sub>EX</sub> application than CAT activity. This differential response might be explained, because GB is mainly synthesized in chloroplasts (Chen and Murata 2011) and almost all GB<sub>EX</sub> absorbed by leaves is translocated to cytosol and chloroplasts (Park *et al.* 2006). On the other hand, it remains unknown if GB could be translocated to peroxisomes. From the foregoing, both the cellular localization of APX and CAT and the differential ability of GB translocation inside cells might have influenced the observed response pattern. Anyway, the greater APX and CAT activities in stressed plants sprayed with 25 or 50 mM GB<sub>EX</sub> might have contributed to keep MDA concentration similar to control plants. Taken together, the presented data supported that GB<sub>EX</sub> attenuated the effects of mild water deficit on lipid peroxidation by causing a positive modulation in both APX and CAT activities.

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