DOI: 10.1007/s11099-013-0004-7 PHOTOSYNTHETICA 51 (1): 102-108, 2013

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

F.J.R. CRUZ, G.L.S. CASTRO, D.D. SILVA JÚNIOR, R.A. FESTUCCI-BUSELLI, and H.A. PINHEIRO+

Centro de Tecnologia Agropecuária, Instituto Sócioambiental e dos Recursos Hídricos, Universidade Federal Rural da Amazônia. Tancredo Neves Av. 2501, Belém, PA 66077-530, Brazil

Abstract

The hypothesis that application of exogenous glycine betaine (GB_{EX}) 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_{EX} 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 (Ψ_{pd}) of stressed plants reached –1.28 ± 0.34 MPa. Regardless of the watering regime, significant (P<0.05) increases in foliar glycine betaine (GB_{Leaf}) concentration were observed in response to increasing GB_{EX}; however, such increases were more expressive in stressed plants. The net photosynthetic rate, stomatal conductance to water vapor, and intercellular to ambient CO_2 concentration ratio were significantly lower in water-stressed plants independently of GB_{EX} concentration sprayed on leaves. The application of 25 and 50 mM GB_{EX} 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_{EX}. Malondialdehyde concentrations did not differ between watered and stressed plants regardless of GB_{EX} concentration. In conclusion, *C. guianensis* was able to incorporate GB_{EX} through their leaves and the resulting increases in GB_{Leaf} 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^+-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).

The ability of GB in promoting a better plant

Received 30 May 2012, *accepted* 20 September 2012.
+Corresponding author; phone: + 559132105271, e-mail: hugo.pinheiro@ufra.edu.br
Abbreviations: APX – ascorbate peroxidase; CAT – catalase; C_i/C_a – intercellular to ambient CO_2 concentration ratio; DM – dry mass; GB_{EX} – exogenous glycine betaine; GB_{Leaf} – 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; Ψ_{pd} – predawn leaflet water potential.
Acknowledgements: Scholarships were granted by "Conselho Nacional de Desenvolvimento Científico e Tecnológico/CNPq" (F.J.R. Cruz, D.D. Silva Júnior, and G.L.S. Castro).

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 (01º28'03"S, 48º29'18"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 μmol(photon) m^{-2} s^{-1}, respec-

tively. The experiment consisted of a completely randomized design with six treatment combinations forming a 3×2 factorial scheme (three GB_{EX} concentrations and two watering regimes). Each experimental replicate was constituted by one plant per pot. Treatment comparisons were assessed when predawn leaf water potential (Ψ_{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 ± 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°C until analyses (Costa *et al.* 2010).

Foliar glycine betaine (GB_{Leaf}) 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_{Leaf}.

Leaf gas exchange: P_N, stomatal conductance to water vapor (g_s), and intercellular to ambient CO_2 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_2 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 µmol(photon) m^{-2} 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). 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 µmol(ascorbate) min^{-1} and 1 U of CAT is the quantity of enzyme that oxidizes 1 µmol(H_2O_2) 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) trichloracetic acid (TCA) and the slurry was centrifuged at 15,000 × *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 × *g* for 8 min at 25°C. Sample absorbance was measured using a UV-visible spectrophotometer (*Genesys^{TM} 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 mM^{-1} cm^{-1}). The results were expressed in nmol(MDA) g^{-1}(DM).

Statistical analysis: All data were tested by analysis of variance (*ANOVA*) considering 3×2 factorial scheme (three GB_{EX} 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 ± standard deviation (SD). Each experimental replicate was constituted by one plant per pot.

Results

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

Leaf gas exchange: In GB_{EX}-untreated plants, grown under full irrigation conditions, the averages of P_N, g_s, and C_i/C_a were 7.00 µmol(CO_2) m^{-2} s^{-1}, 195 mmol(H_2O) m^{-2} s^{-1} and 0.742 mol(CO_2) mol^{-1}(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 µmol(CO_2) m^{-2} s^{-1}, 20 mmol(H_2O) m^{-2} s^{-1}, and 0.395 mol(CO_2) mol^{-1}(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_{EX} effect), one could observe that GB_{EX} 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

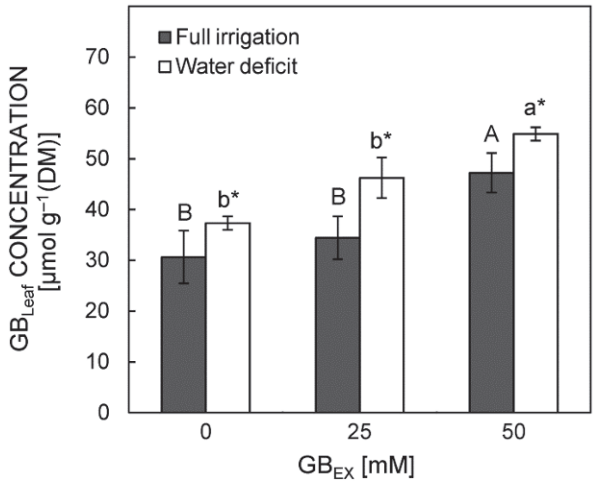

Fig. 1. Foliar glycinebetaine (GB_{Leaf}) concentration in *C. guianensis* plants, sprayed with different exogenous glycine betaine (GB_{EX}) concentration (0, 25, and 50 mM) and subjected to two watering regimes (full irrigation and water deficit). Different *capital letters* denote significant differences 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_{EX} 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 ± SD ($n = 6$).

plants treated with 0, 25, or 50 mM GB_{EX} (Fig. 2). Similar responses to GB_{EX} application were also observed in water-stressed plants (Fig. 2).

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

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

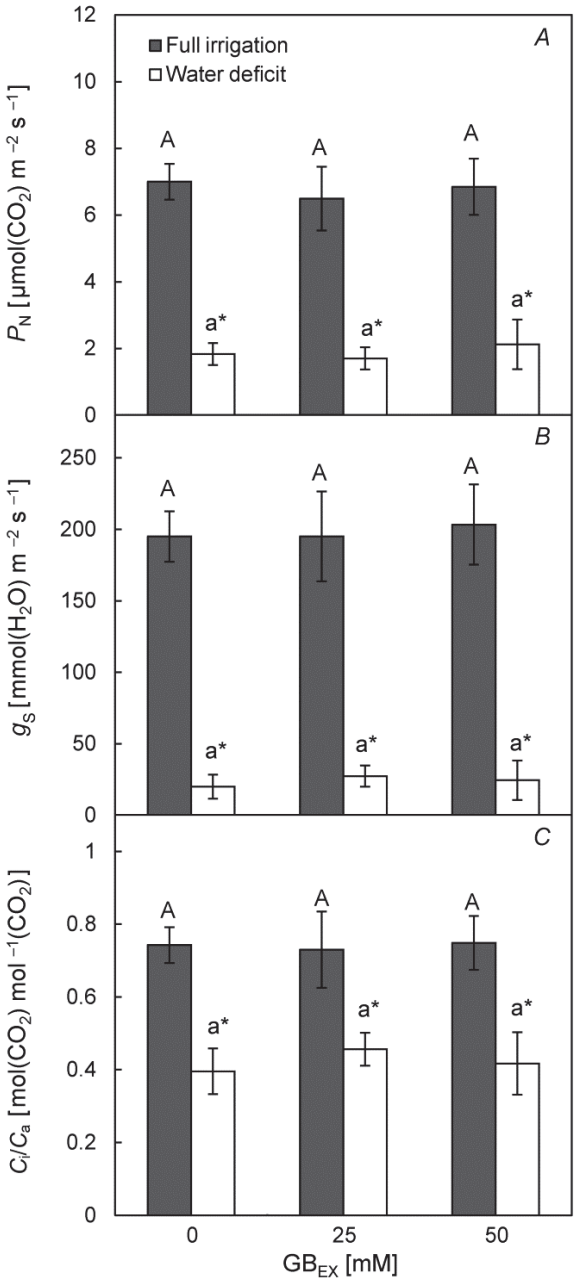

Fig. 2. Net photosynthetic rate (P_N), stomatal conductance to water vapor (g_s), and intercellular to ambient CO_2 concentration ratio (C_i/C_a) in *C. guianensis* leaves sprayed with different exogenous glycine betaine (GB_{EX}) concentration (0, 25, and 50 mM) and subjected to two watering regimes (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_{EX} concentration applied on leaves (Fig. 4).

Discussion

The GB-constitutive synthesis in plants sprayed with no GB_{EX} 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_{Leaf} concentrations in GB-sprayed plants indicated that *C. guianensis* was able to uptake GB_{EX} through its leaflet surface, regardless of plant water status. Particularly for water-stressed plants assessed under Ψ_{pd} of –1.28 MPa, the increases in GB_{Leaf} concentration in response to foliar application of 25 or 50 mM GB_{EX} were comparable with GB_{Leaf} concentration in *C. guianensis* plants assessed under Ψ_{pd} of –3.2 MPa (Costa *et al.* 2010). Therefore, the GB_{Leaf} concentrations in plants sprayed with 25 or 50 mM GB_{EX} 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_{EX} was really able to attenuate the effects of mild water-deficit in leaf gas exchange and lipid peroxidation in this species.

The GB_{EX} 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_{EX} 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

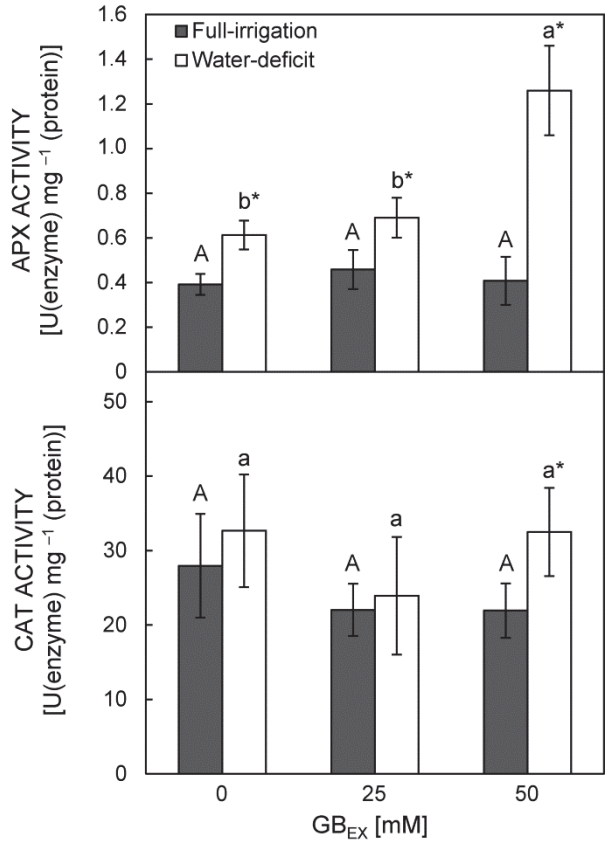

Fig. 3. Ascorbate peroxidase (APX) and catalase (CAT) activities in *C. guianensis* leaves sprayed with different exogenous glycine betaine (GB_{EX}) concentration (0, 25, and 50 mM) and subjected to two watering regimes (full irrigation and water deficit). Statistics as in Fig. 1.

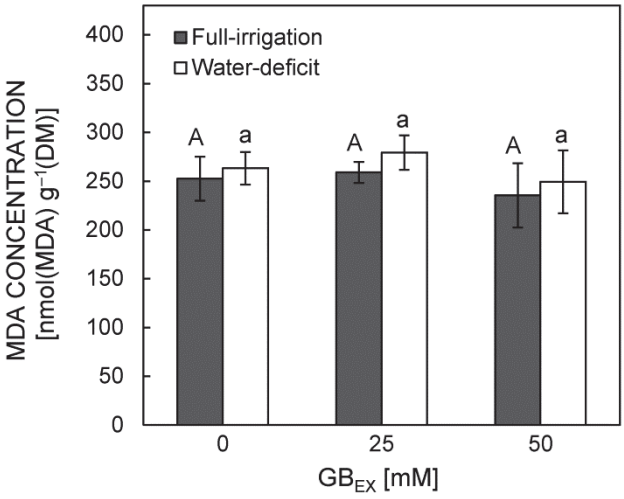

Fig. 4. Malondialdehyde (MDA) concentration in *C. guianensis* leaves sprayed with different exogenous glycine betaine (GB_{EX}) concentration (0, 25, and 50 mM) and subjected to two watering regimes (full irrigation and water deficit). Statistics as in Fig. 1.

(*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_{EX} 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_{EX} application than CAT activity. This differential response might be explained, because GB is manly synthesized in chloroplasts (Chen and Murata 2011) and almost all GB_{EX} 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_{EX} might have contributed to keep MDA concentration similar to control plants. Taken together, the presented data supported that GB_{EX} attenuated the effects of mild water deficit on lipid peroxidation by causing a positive modulation in both APX and CAT activities.

References

Agboma, M., Jones, M.G.K., Peltonen-Sainio, P. *et al.*: Exogenous glycine betaine enhances grain yield of maize, sorghum and wheat grown under two supplementary watering regimes. – J. Agron. Crop Sci. **178**: 29-37, 1997.

Allard, F., Houde, M., Krol, M. *et al.*: Betaine improves freezing tolerance in wheat. – Plant Cell Physiol. **39**: 1194-1202, 1998.

Asada, K.: The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. – Annu. Rev. Plant Physiol. Plant Mol. Biol. **50**: 601-639, 1999.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. – Anal. Biochem. **72**: 248-254, 1976.

Brando, P.M., Goetz, S.J., Baccini, A. *et al.*: Seasonal and interannual variability of climate and vegetation indices across the Amazon. – Proc. Nat. Acad. Sci. USA **107**: 14685-14690, 2010.

Brasil, E.C., Cravo, M.S.: [Interpretation of soil analysis results] – In: Cravo, M.S., Viégas, I.J.M., Brasil, E.C. (ed.): [Fertilizing and Liming Recommendations for the State of Pará, Brazil] Pp. 43-47. Embrapa Amazônia Oriental, Belém 2007. [In Port.]

Cakmak, I., Horst, J.: Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). – Physiol. Plant. **83**: 463-468, 1991.

Chen, T.H.H., Murata, N.: Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. – Plant Cell Environ. **34**: 1-20, 2011.

Costa, M.A., Pinheiro, H.A., Shimizu, E.S.C. *et al.*: Lipid peroxidation, chloroplastic pigments and antioxidant

strategies in *Carapa guianensis* (Aubl.) subjected to water-deficit and short-term rewetting. – Trees **24**: 275-283, 2010.

Demiral, T., Türkan, I.: Does exogenous glycinebetaine affect antioxidative system of rice seedlings under NaCl treatment ? – J. Plant Physiol. **161**: 1089-1100, 2004.

Farooq, M., Basra, S.M.A., Wahid, A. *et al*.: Physiological role of exogenously applied glycinebetaine to improve drought tolerance in fine grain aromatic rice (*Oryza sativa* L.). – J. Agron. Crop Sci. **194**: 325-333, 2008.

Genard, H., Le Saos, J., Hillard, J. *et al*.: Effect of salinity on lipid composition, glycine betaine content and photosynthetic activity in chloroplasts of *Suaeda maritime*. – Plant Physiol. Biochem. **29**: 421-427, 1991.

Gonçalves, J.F.C., Silva, C.E.M., Guimarães, D.G.: [Photosynthesis and water potential of andiroba seedlings submitted to water stress and rewetting.] – Pesq. Agropec. Bras. **44**: 8-14, 2009. [In Port.]

Grieve, C.M., Grattan, S.R.: Rapid assay for determination of water soluble quaternary ammonium compounds. – Plant Soil **70**: 303-307, 1983.

Hassine, A.B., Ghanem, M.E., Bouzid, S., Lutts, S.: An inland and a coastal population of the Mediterranean xerohalophyte species *Atriplex halimus* L. differ in their ability to accumulate proline and glycinebetaine in response to salinity and water stress. – J. Exp. Bot. **59**: 1315-1326, 2008.

Hattori, T., Mitsuya, S., Fujiwara, T. *et al*.: Tissue specificity of glycinebetaine synthesis in barley. – Plant Sci. **176**: 112-118, 2009.

Havir, E.A., McHale, N.A.: Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. – Plant Physiol. **84**: 450-455, 1987.

Iqbal, N., Ashraf, M., Ashraf, M.Y.: Glycinebetaine, an osmolyte of interest to improve water stress tolerance in sunflower (*Helianthus annuus* L.): water relations and yield. – S. Afr. J. Bot. **74**: 274-281, 2008.

Lv, S., Yang, A., Zhang, K. *et al*.: Increase of glycinebetaine synthesis improves drought tolerance in cotton. – Mol. Breeding **20**: 233-248, 2007.

Ma, Q.-Q., Wang, W., Li, Y.-H. *et al*.: Alleviation of photoinhibition in drought-stressed wheat (*Triticum aestivum*) by foliar-applied glycinebetaine. – J. Plant Physiol. **163**: 165-175, 2006.

Ma, X.L., Wang, Y.J., Xie, S.L. *et al*.: Glycinebetaine application ameliorates negative effects of drought stress in tobacco. – Russ. J. Plant Physiol. **54**: 472-479, 2007.

Mansour, M.M.F.: Protection of plasma membrane of onion epidermal cells by glycine betaine and proline against NaCl stress. – Plant Physiol. Biochem. **36**: 767–772, 1998.

Meloni, D.A., Martínez, C.A.: Glycinebetaine improves salt tolerance in vinal (*Prosopis ruscifolia* Griesbach) seedlings. – Braz. J. Plant Physiol. **21**: 233-241, 2009.

Mendonça, A.P., Ferraz, I.D.K.: [Crapwood oil: traditional extraction, use and social aspects in the state of Amazonas, Brasil.] – Acta Amaz. **37**: 353 – 364, 2007. [In Port.]

Mittler, R.: Oxidative stress, antioxidants and stress tolerance. – Trends Plant Sci. **9**: 405-410, 2002.

Moraes F.K.C., Castro, G.L.S., Silva Júnior, D.D. *et al.*: Chloroplastidic pigments, gas exchange, and carbohydrates changes during *Carapa guianensis* leaflet expansion. – Photosynthetica **49**: 619-626, 2011.

Nakano, Y., Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach choloroplasts. – Plant Cell Physiol. **22**: 867-880, 1981.

Nawaz, K., Ashraf, M.: Exogenous application of glycinebetaine modulates activities of antioxidants in maize plants subject to salt stress. – J. Agron. Crop Sci. **196**: 28-37, 2010.

Nuccio, M.L., Russell, B.L., Nolte, K.D. *et al*.: The endogenous choline supply limits glycine betaine synthesis in transgenic tobacco expressing choline monooxygenase. – Plant J. **16**: 487-496, 1998.

Park, E.-J., Jeknić, Z., Chen, T.H.H.: Exogenous application of glycinebetaine increases chilling tolerance in tomato plants. – Plant Cell Physiol. **47**: 706-714, 2006.

Pimentel, C.: [Water relations in two hybrids of corn under two cycles of water stress.] – Pesq. Agropec. Bras. **34**: 2021-2027, 1999. [In Port.]

Quan, R., Shang, M., Zhang, H. *et al*.: Engineering of enhanced glycinebetaine synthesis improves drought tolerance in maize. – Plant Biotechnol. J. **2**: 477-486, 2004.

Raza, S.H., Athar, H.R., Ashraf, M., Hameed, A.: Glycinebetaine-induced modulation of antioxidant enzymes activities and ion accumulation in two wheat cultivars differing in salt tolerance. – Environ. Exp. Bot. **60**: 368-376, 2007.

Rhodes, D., Rich, P.J., Brunk, D.G. *et al*.: Development of two isogenic sweet corn hybrids differing for glycinebetaine content. – Plant Physiol. **91**: 1112-1121, 1989.

Robinson, S.P., Jones, G.P.: Accumulation of glycinebetaine in chloroplasts provides osmotic adjustment during salt stress. – Aust. J. Plant Physiol. **13**: 659-668, 1986.

Sakamoto, A., Murata, N.: The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. – Plant Cell Environ. **25**:163-171, 2002.

Takabe, T., Rai, V., Hibino, T.: Metabolic engineering of glycinebetaine. – In: Rai, A., Takabe, T. (ed.): Abiotic Stress Tolerance in Plants: Towards the Improvements of Global Environmental and Food. Pp. 137-151. Springer, Dordrecht 2006.

Wang, G.P., Li, F., Zhang, J. *et al*.: Overaccumulation of glycine betaine enhances tolerance of the photosynthetic apparatus to drought and heat stress in wheat. – Photosynthetica **48**: 30-41, 2010.

Weibing, X., Rajashekar, C.B.: Alleviation of water stress in beans by exogenous glycine betaine. – Plant Sci. **148**: 185-192, 1999.

Yang, X., Lu, C.: Effects of exogenous glycinebetaine on growth, CO_2 assimilation and photosystem II photochemistry of maize plants. – Physiol. Plant. **127**: 593-602, 2006.