



# Seedlings of *Garcinia brasiliensis* (Clusiaceae) subjected to root flooding: Physiological, morphoanatomical, and antioxidant responses to the stress



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## ABSTRACT

*Garcinia brasiliensis* (Mart.) is a native Amazonian tree cultivated throughout Brazil. This plant can tolerate flooding or submergence for several days, during certain periods of the year. The morphophysiological changes of *G. brasiliensis* (Mart.) seedlings were assessed that may favor their survival in flooded environments. Seedlings with six fully expanded leaves were placed in tanks so that their roots were submerged for 90 days. Antioxidant enzymatic activity and the contents of H<sub>2</sub>O<sub>2</sub>, soluble sugar, starch, and amino acid of the roots were evaluated on six harvesting occasions. At the end of the experiment, the dry mass and root morphology of the seedlings were determined. Flooding lead to a decrease in dry mass of roots and aboveground parts, as well as root length (58%), surface area (51%) and volume (43%), especially of roots with smaller diameter. The roots of the flooded seedlings presented thicker exodermis and greater xylem number, thicker phloem and fewer xylem fibers. There was a small amount of aerenchyma in the roots and hypertrophied lenticels were detected at the base of the stem. Superoxide dismutase activity was significantly higher in flooded roots at all harvesting times, and ascorbate peroxidase and catalase activities were highest during the last two harvestings. H<sub>2</sub>O<sub>2</sub> content increased after 40 and 55 days of flooding, followed by a drastic decrease. After 70 and 90 days of flooding there was an expressive increase in soluble sugars, and at 90 days, a reduction in starch content. No differences were observed in amino acid content.

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## 1. Introduction

*Garcinia brasiliensis* (Mart.), is native to the Amazon region but is cultivated throughout Brazil. A number of phytochemical studies have confirmed the medicinal potential of this plant (Martins et al., 2008; Gontijo et al., 2012).

*G. brasiliensis* is an evergreen species and can tolerate long periods of flooding or submergence (Duarte et al., 2005). Although there are a lot of researches being carried out concerning morphophysiological adaptation of plants under flooding (Armstrong et al., 1994; Jackson et al., 2009) very few work has been done with *G. brasiliensis*. Oliveira-Wittmann (2007) reported an increase in the quantity of tocopherol (vitamin E) in the latex and leaves of this tree. Parolin (2009) reported changes in the submerged leaves,

including an increase in the size of stomata and the presence of thick epidermal walls (typical of xeromorphic plants).

Flooding represents a situation of excess water in the root zone (Colmer and Voesenek, 2009). Under non-flooding conditions, the root system is in direct contact with oxygen. The decrease of oxygen in the soil due to the excess of water results in hypoxia (low oxygen concentration) or anoxia (absence of oxygen) (Bailey-Serres et al., 2012). The low level of oxygen in the rhizosphere caused by flooding is one of the major abiotic stresses that can lead to a decrease in the productivity of plants (Jackson and Colmer, 2005).

Root growth is inhibited under stress conditions caused by hypoxia (Armstrong et al., 1991). Under flooding conditions, roots can only grow near the soil surface and cannot totally explore the soil volume. Without oxygen in the roots, energy production is restricted to fermentation, which yields only two ATPs (Sairam et al., 2008). Additionally, hypoxia decreases hydraulic conductivity and affects the aquaporins, diminishing absorption of water and nutrients by roots (Dell'Amico et al., 2001; Tournaire-Roux et al., 2003; Horchani et al., 2008).

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Under hypoxia or anoxia, reactive oxygen species (ROS) are formed. They can cause damage to cell metabolism by oxidizing proteins and lipids (Moller et al., 2007). Plant tissues use both non-enzymatic and enzyme systems (Mitler, 2002; Karuppanapandian et al., 2011), to control ROS levels and protect their cells.

Plants adapted to flooding conditions, such as those found in the Amazon region, modify their morphology, anatomy, and cell metabolisms to survive these unfavorable conditions (De Simone et al., 2003; Parolin, 2009; Oliveira and Joly, 2010). The present work was designed to characterize physiological and morphoanatomical adaptations of Amazonian plants to flooding conditions and to aid in screening processes to select plants for revegetation of riparian zones of artificial reservoirs (formed during the construction and operation of hydroelectric plants). This revegetation can avoid the silting-up caused by erosion. This way, understanding the physiological characteristics of species, whose natural habitats are marked by conditions similar to those observed at the edges of hydroelectric reservoirs (with periodic inundations) will be of significant importance in protecting these sites (Silva et al., 2001). Our hypothesis was that *G. brasiliensis* seedlings survive for longer periods of flooding, this surviving occurs due to anatomical and metabolic modifications in the roots.

## 2. Materials and methods

### 2.1. Plant material, growth conditions, treatments and experiment design

The fruits of *G. brasiliensis* were harvested from trees growing on the Retiro Farm in the municipality of Campo Formoso, state of Bahia, Brazil ( $10^{\circ}30' S$  and  $40^{\circ}19' W$ ) and were taken to the Plant Growth and Development Laboratory of the Plant Physiology Sector of the Federal University at Lavras, Lavras, MG. The seeds were isolated from the fruits, washed with running water and subsequently germinated on moist Germitest® paper in a BOD incubator at  $30^{\circ}C$  under a 12-h photoperiod. After germinating, the seedlings were transferred to plastic bags (one plant per bag) containing soil (B horizon) and sand in the proportion of 3:1. Based on soil analysis, the following substances were incorporated into the substrate: potassium chloride ( $2.5 \text{ kg m}^{-3}$ ), simple super phosphate fertilizer ( $5 \text{ kg m}^{-3}$ ), ammonium sulfate ( $725 \text{ g m}^{-3}$ ), and dolomite limestone powder ( $500 \text{ g m}^{-3}$ ). Sixty days after germination (DAG) the bags containing seedlings with six totally expanded leaves were placed outdoors into brick tanks ( $4 \text{ m} \times 1 \text{ m}$  and  $0.8 \text{ m}$  deep) and exposed to two conditions: flooded and non-flooded (control).

In the flooded treatment, the water level in the tank was maintained 2 cm above the level of the soil in the plant bags throughout the experiment, totally covering the roots with water. Under non-flooded conditions, the plants were watered on a daily basis. The treatment and control tanks were covered with a black screen that reduced solar radiation by 70%. The average relative humidity of the air in the tanks throughout the experiment was 75% and the average maximum and minimum temperatures were  $32^{\circ}C$  and  $24^{\circ}C$ , respectively. Evaluations were initiated eight days after the imposition of the flooding regime and undertaken thereafter on the 16th, 40th, 55th, 70th, and 90th days of the experiment. We evaluated the antioxidant enzymatic activity and the content of  $\text{H}_2\text{O}_2$ , soluble sugars, starch, and amino acids. Additional parameters were analyzed at the end of the experiment (after 90 days of flooding). The experimental design was completely randomized with ten replicates per treatment.

### 2.2. Physiological responses

Total soluble sugars and amino acids were determined using the same supernatant used in the analyses of antioxidant enzymes. Total soluble sugars was determined by the colorimetric reaction with anthrone at 640 nm, using a glucose standard curve as described by Yemm and Willis (1954). The ninhydrin method was used to quantify amino acids following Yemm and Cocking (1954), using a standard glycine curve. Starch was hydrolyzed using 35% perchloric acid and quantified using Somogyi method, modified by Nelson (1944).

### 2.3. Morphoanatomical responses

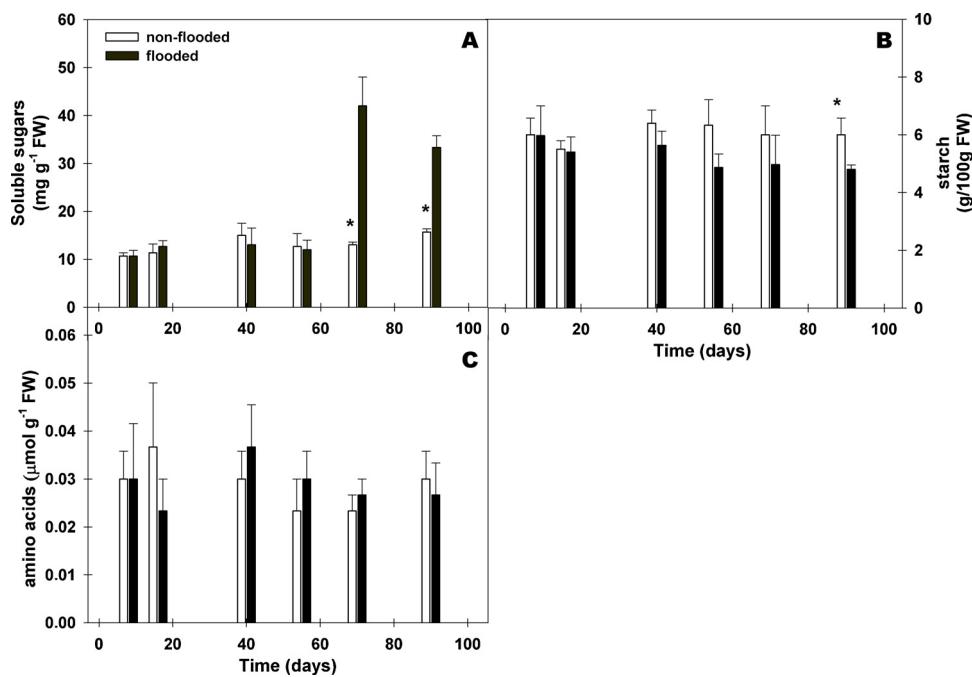
The plants in each sack were harvested, and then washed and separated into root and shoot (leaves + stems). The characteristics of roots were determined using WinRhizo Pro 2007a image analysis system (Regent Instruments, Sainte-Foy, QC, Canada) coupled to a professional scanner (Epson, Expression 10,000 XL, Epson America, Inc., USA) equipped with an additional light unit (TPU). Images of root morphology were obtained by scanning the roots at 400 dpi (Bouma et al., 2000) in an acrylic box ( $20 \text{ cm} \times 30 \text{ cm}$ ) with a film of water of approximately 1-cm thick. The following characteristics were determined: root length (cm), root superficial area ( $\text{cm}^2$ ), root volume ( $\text{cm}^3$ ), root medium diameter (mm), and the number of root tips. Root length, volume, and surface area were also classified by diameter class (0–4.5 mm) using the same software. The plant material was placed into paper bags and dried to constant weight in a forced-air circulation oven at  $72^{\circ}C$ , and the following dry weight attributes were evaluated: root dry mass, stem dry mass, leaf dry mass, shoot dry mass (leaves + stem), and root/shoot ratio (relationship between root dry mass and shoot dry mass). Other attributes of the morphological and dry mass data were calculated, including: specific root length ( $\text{mm g}^{-1}$ ), root fineness ( $\text{mm mm}^{-3}$ ), and root tissue density ( $\text{g mm}^{-3}$ ).

For root anatomy study, two complete roots per plant (including the apical, elongation, piliferous, and basal regions) were randomly harvested from each replication and washed in running water. The roots were then fixed in formaldehyde, acetic acid, and 70% ethanol solution (FAA 70) for 48 h and subsequently preserved in 70% ethanol. Transverse sections were cut  $2 \pm 0.5 \text{ cm}$  from the root apex using a table-mounted microtome. The sections were cleared with 5% sodium hypochlorite for 10 min, rehydrated for 10 min, stained with Astrablau (safranine and Astra blue, 7.5:2.5), and subsequently mounted on slides in 50% glycerin. The sections were photographed with an Olympus BX-60 light microscope coupled to a digital camera. The resulting photomicrographs were used to measure the following parameters: cortex width, exodermis width, proportion of aerenchyma in the cortex, phloem width, xylem vessel number, and xylem fibers area. The proportion of aerenchyma in the cortex was calculated by dividing total aerenchyma area by total cortex area.

These measurements were made using image analysis software (UTHSCSA ImageTool, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA), based on calibrations made with a microscope ruler photographed at the same magnification as the photomicrographs. The mean of five measurements was used for each anatomical character.

### 2.4. Antioxidant responses

Enzyme extracts were prepared by macerating 250 mg of root material in liquid nitrogen and adding 1.5 mL of an extraction buffer (100 mM potassium phosphate buffer, pH 7.0, 1 mM EDTA, 2 mM DTT, 0.8 mM PMSF, 1% PVPP, and 1 mM ascorbic acid). The extracts were centrifuged at 14,000 rpm for 30 min at  $4^{\circ}C$  and the



**Fig. 1.** Time course of total soluble sugar (A), starch (B), and amino acid (C) content in flooded and non-flooded *G. brasiliensis* seedlings. Each bar indicates the mean value  $\pm$  SE.  
\* Indicates statistically significant results at  $p \leq 0.05$ .

supernatants collected and stored at  $-80^{\circ}\text{C}$  until the time of enzyme analysis. Enzyme activity was expressed as milligrams (mg) of protein, as determined by the Bradford method (1976) using bovine serum albumin (BSA) as the standard.

Superoxide dismutase activity (SOD, EC 1.15.1.1) was evaluated by its capacity to inhibit the photoreduction of nitro blue tetrazolium (NBT), as proposed by Giannopolitis and Ries (1977). The reaction mixture was composed of 100  $\mu\text{L}$  enzyme extract and 1.9 mL reaction medium (containing 50 mM potassium phosphate buffer pH 7.8, 14 mM methionine, 0.1  $\mu\text{M}$  EDTA, 75  $\mu\text{M}$  NBT, and 2  $\mu\text{M}$  riboflavin). The mixture was illuminated with a 20-W bulb for 10 min. The same mixture was used for the control, but without the enzyme extract, and maintained in the dark. The absorbance readings were made at 560 nm, and one SOD unit was defined as the amount of enzymes capable of inhibiting NBT photoreduction by 50% under the assay conditions.

Catalase activity (CAT, EC 1.11.1.6) was determined by the consumption of  $\text{H}_2\text{O}_2$  at 240 nm for 3 min. The reaction mixture was incubated at  $28^{\circ}\text{C}$  and was constituted of 25  $\mu\text{L}$  enzyme extract and 0.975 mL reaction medium containing 50 mM potassium phosphate buffer (pH 7.0) and 12.5 mM  $\text{H}_2\text{O}_2$ . The molar extinction coefficient used was  $36 \text{ mM}^{-1} \text{ cm}^{-1}$  (Havir and Mchale, 1987).

Ascorbate peroxidase activity (APX, EC 1.11.1.11) was determined by measuring the oxidation of ascorbate at 290 nm for 3 min. The reaction mixture was incubated at  $28^{\circ}\text{C}$  and was composed of 60  $\mu\text{L}$  enzyme extract and 0.94 mL reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 1 mM  $\text{H}_2\text{O}_2$ , 0.8 mM L-ascorbic acid, and distilled water. The molar extinction coefficient used was  $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$  (Nakano and Asada, 1981).

Samples containing 250 mg of root tissue were macerated in liquid nitrogen with 20% PVPP, homogenized in 5 mL 0.1% trichloroacetic acid (TCA), and subsequently centrifuged at  $10,000 \times g$  for 10 min at  $4^{\circ}\text{C}$ . The supernatant was used to determine the content of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and MDA.

$\text{H}_2\text{O}_2$  content was measured using a spectrophotometer, according to Alexieva et al. (2001). The reaction mixture consisted of 0.5 mL supernatant extract and 0.5 mL 100 mM potassium phosphate buffer (pH 7.0) with 2 mL 1 M potassium iodine (KI). The

reaction was developed for 1 h in the dark and absorbance was measured at 390 nm. Concentration of  $\text{H}_2\text{O}_2$  was calculated using a standard curve.

## 2.5. Data analysis

Data were tested for normality by Shapiro-Wilk and for homoscedasticity by the Levine test, and were found to meet the criteria. To compare the characteristics between flooded and non-flooded plants we used the F Test at a 0.05 significance level ( $P \leq 0.05$ ), using Sisvar software, version 4.3 (Federal University of Lavras, Lavras, Brazil). Means were calculated for all the parameters  $\pm$  standard errors (SE).

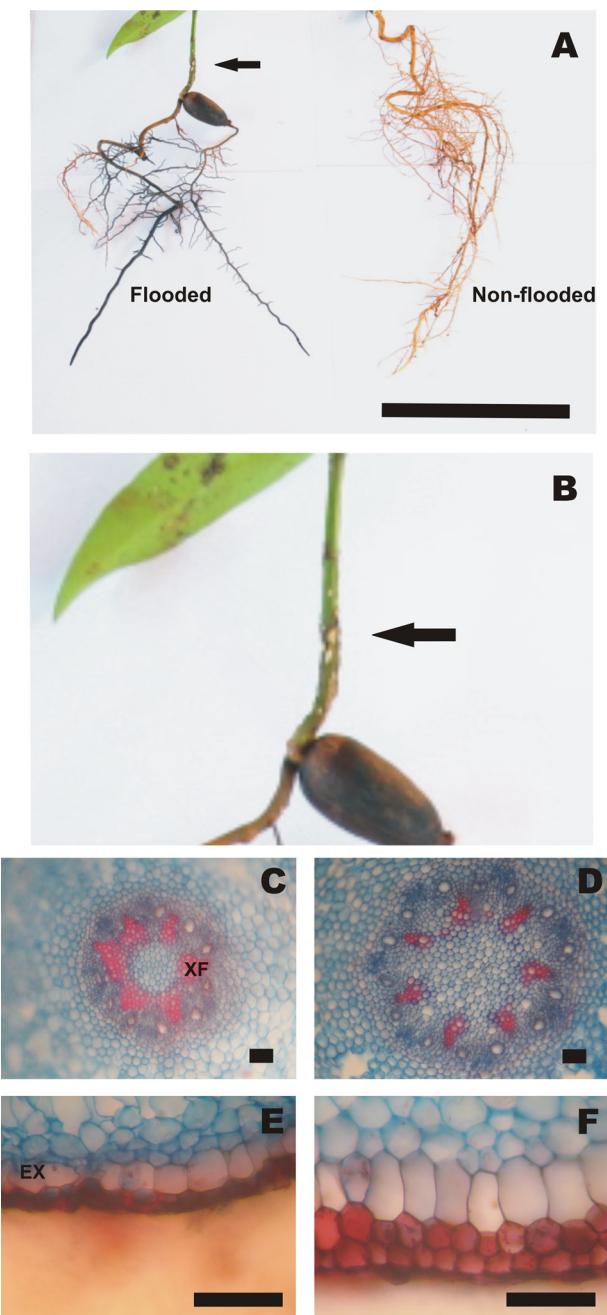
## 3. Results

### 3.1. Physiological responses

There was no difference between the two treatments in terms of total soluble sugars for the seedlings harvested at 8, 16, 40 and 55 days. However, at 70 and 90 days, the flooded seedlings showed a significant increase in total soluble sugars (Fig. 1A). Starch content was statistically significant only at 90 days, with consumption being noted (decrease) in the flooded seedlings (Fig. 1B). There was no difference between the two treatments at any of the harvesting times in terms of total amino acids present in the roots (Fig. 1C).

### 3.2. Morphoanatomical responses

All the plants were still alive after 90 days under flooded conditions. Hypertrophied lenticels were observed on the basal region of the stem (Fig. 2A and B) and the roots were characteristically dark from flooding stress (Fig. 2A). No adventitious roots were observed. Flooded plants, when compared to non-flooded plants, presented a drastic decrease in their root length (57.6%), root superficial area (51%), root volume (42.85% decrease), and numbers of root tips (61.85% decrease) (Table 1 and Fig. 2A). Plants that



**Fig. 2.** Morphology and anatomy of *G. brasiliensis* seedlings. (A) Roots of flooded and non-flooded seedlings, arrow indicates the location of hypertrophied lenticels; (B) amplification of the stem base, arrow indicates the location of the hypertrophied lenticels; (C) photomicrograph of the vascular cylinder of non-flooded roots; (D) photomicrograph of the vascular cylinder of flooded roots; (E) photomicrographs of the exodermis of non-flooded roots and (F) photomicrograph of the exodermis of flooded roots. XF = xylem fibers; EX = exodermis. Bar in (A)=5 cm; bar in (C), (D), (E) and (F)=100 µm.

experienced flooding significantly increased their root medium diameter (**Table 1**).

As far as morphological and dry mass data is concerned, we observed thinnest roots for *G. brasiliensis* under flooding conditions (**Table 1**). There was no difference between the treatments in terms of root tissue density and specific root length (**Table 1**).

Flooding decreased seedling dry mass production. This decrease was statistically significant for root dry mass (36.3% decrease), dry leaf mass (44.44% decrease), and shoot dry mass (35.7% decrease)

**Table 1**

Root morphology, dry weight attributes, and root anatomy of flooded and non-flooded *G. brasiliensis* seedlings over time (90 days).

Characteristics/treatments	Non-flooded	Flooded
Root length (mm)	6943 ± 540*	2942 ± 210
Root surface area (mm <sup>2</sup> )	15,580 ± 1100*	7630 ± 600
Root volume (mm <sup>3</sup> )	2800 ± 200*	1600 ± 130
Average root diameter (mm)	0.72 ± 0.02	0.83 ± 0.03
Numbers of root tips	1434 ± 166*	547 ± 78
Specific root length (mm g <sup>-1</sup> )	6280 ± 550	4533 ± 650
Root thinness (mm mm <sup>-3</sup> )	2.49 ± 0.18*	1.93 ± 0.19
Root tissue density (g mm <sup>-3</sup> )	410 ± 20	450 ± 30
Root dry mass (g)	1.13 ± 0.07*	0.72 ± 0.07
Stem dry mass (g)	0.42 ± 0.03	0.40 ± 0.04
Leaf dry mass (g)	0.9 ± 0.05*	0.5 ± 0.04
Shoot dry mass (g)	1.4 ± 0.07*	0.9 ± 0.08
Root/shoot ratio	0.865 ± 0.06	0.861 ± 0.11
Cortex width (µm)	147.6 ± 7.2	160.9 ± 10.6
Exodermis width (µm)	85.6 ± 3.8*	112.7 ± 4.7
PA (%)	0.06 ± 0.02	1.5 ± 0.6
Phloem width (µm)	153.0 ± 17.8*	189.7 ± 24.9
Xylem number (µm)	5.4 ± 0.3*	7.1 ± 0.7
Xylem fibers area (mm <sup>2</sup> )	362.6 ± 61.8*	165.5 ± 36.5

Means ± SE, n = 10.

\* Indicates statistically significant results at p ≤ 0.05. PA = proportion occupied by the aerenchyma in the cortex.

(**Table 1**). No significant difference was observed between the treatments in terms of stem dry mass and root/shoot ratio.

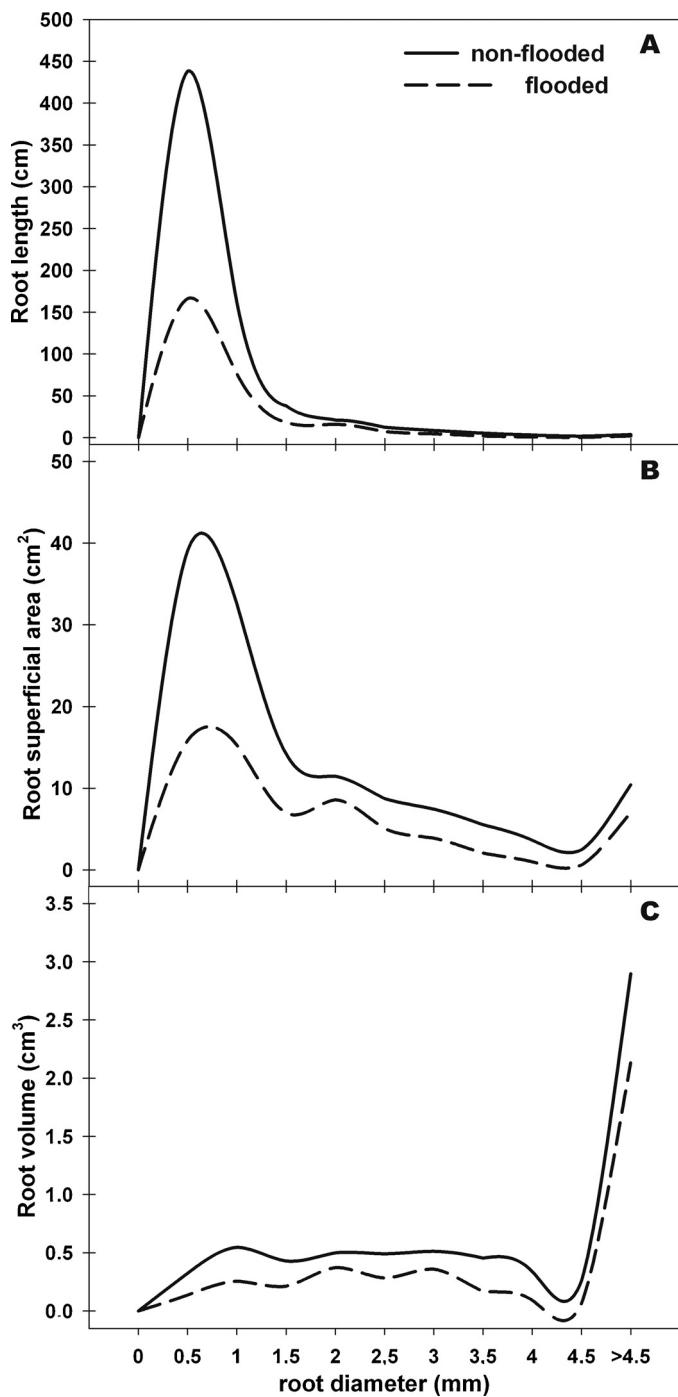
There was no difference between flooded and non-flooded roots regarding length, surface area, and volume distributed through different root diameter classes (**Fig. 3**). In general, the root system of non-flooded seedlings of *G. brasiliensis* was dominated by thin-diameter roots with longer length, larger surface area and higher volume of thinner roots. Roots of flooded seedlings, on the other hand, presented lower values for these parameters for both thin and large diameter roots (**Fig. 3**).

No difference was observed for cortex width between flooded and non-flooded seedlings (**Table 1**). However, significant difference was observed for exodermis width between the two types of treatments. With flooding, the exodermis increased in width by 23.70% in relation to non-flooded seedlings (**Table 1**; **Fig. 3E** and F). No statistical differences were detected for aerenchyma proportion in root cortex for flooded and no flooded treatments (**Table 1**). Regarding phloem width, flooded treatment lead to a significant increase in its width (**Table 1**). Similarly, an increase was observed for xylem vessel number in the roots of flooded seedlings (**Table 1**). In terms of area of xylem fibers, non-flooded seedlings showed significantly higher means (**Table 1**; **Fig. 3C** and D).

### 3.3. Antioxidant responses

Superoxide dismutase (SOD) activity was significantly higher in roots of flooded seedlings throughout the experiment, with a peak of activity at 90 days (**Fig. 4A**). On the eighth day of flooding, ascorbate peroxidase (APX) activity was equal in both flooded and non-flooded treatments. Significant increase in activity was observed after 70 and 90 days of flooding (**Fig. 4B**). Catalase activity (CAT) was significant only after 70 and 90 days of flooding (**Fig. 4C**).

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content varied throughout the experiment, with a significant increase after 40 and 55 days of flooding, followed by a drastic decrease in the mean values after that, with the two treatments having similar values at the end of the experimental period (70 and 90 days) (**Fig. 4D**).



**Fig. 3.** Means of ten replicates of (A) root length, (B) root superficial area, (C) root volume distributed by diameter classes of roots of flooded and non-flooded *G. brasiliensis* seedlings (after 90 days of flooding).

#### 4. Discussion

No differences were observed between the two treatments in terms of total sugars or starch content until the 55th day of flooding. This low variation in sugar reserve accumulated in roots demonstrates the capacity of *G. brasiliensis* seedlings to tolerate stress through diminished sugar consumption, as the lack of oxygen induces a shift from aerobic respiration to fermentation (Sairam et al., 2008). Continuous supply of fermentable sugars to roots is considered to be critical for long-term survival of plants under flooding (Jackson and Drew, 1984). Flooding did not cause drastic

protein degradation, as no change was observed in amino acid content. An increase in amino acid content can result from protein degradation due to oxygen deficiency. It is well known that proteins can undergo hydrolysis, freeing amino acids that, in turn, could be interconverted through the action of transaminase enzymes induced by anoxia, giving rise to new types of amino acids (Sousa and Sodek, 2002).

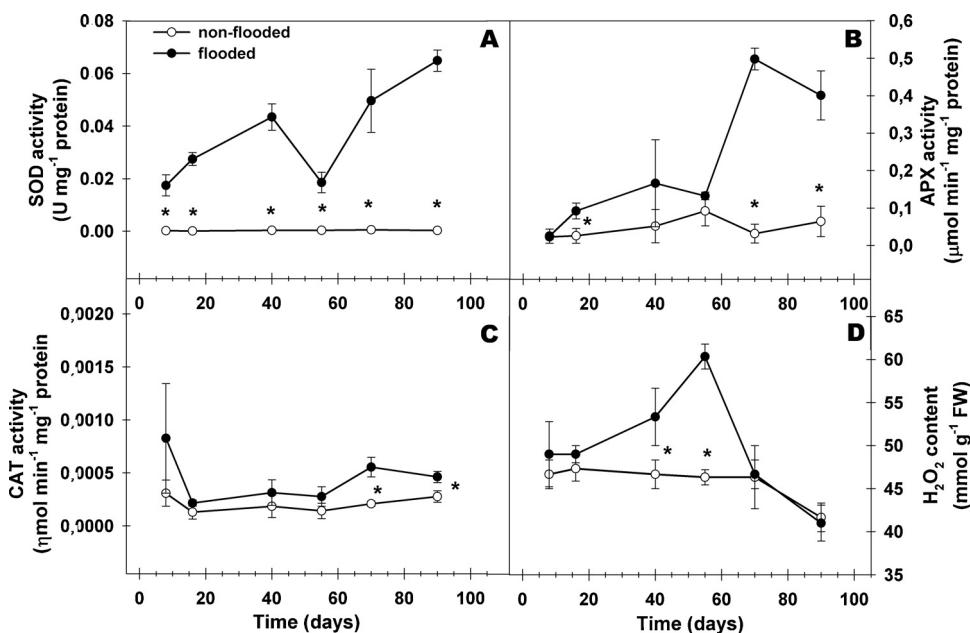
*G. brasiliensis* seedlings did not present adventitious roots. However, still regarding root aeration, hypertrophied lenticels were observed at the bases of the stems of flooded *G. brasiliensis* seedlings. They can favor ventilation in these roots, since they are under O<sub>2</sub> deficit (Shimamura et al., 2010). The flooded seedlings probably showed low development of thin roots due to their sensitivity to stress conditions, resulting in the inhibition of their development and even death, as reported in other studies (Imada et al., 2008). Regarding root medium diameter, Ryser (2006) noted that larger root diameter could lead to a smaller specific length. Root length of *G. brasiliensis* were slightly smaller on the average but there were no statistically significant alterations in the specific length. According to Eissenstat (1992), longer specific length and longer length of fine roots would be disadvantageous under flooding conditions as they would increase the root surface area, which, according to Sorrel (1994), would lead to a greater loss of oxygen from the roots to the soil. High root tissue density (root with large amount of dead tissue) is one of the characteristics of species occupying high stress environments that lead to a decrease of root specific length (Wahl et al., 2000). Although seedlings grown under flooding conditions had lesser quantities of xylem fibers (dead tissue) no significant alterations of their average root medium diameter were noted.

In spite of the fact that flooded plants showed significantly lower leaf dry mass and shoot dry mass, no difference in the relationship between root dry mass and shoot dry mass was observed. Plants submitted to root flooding could send signals from roots to shoot to induce stomatal closure (Else et al., 2009). When stomata close, gas exchange is interrupted as well as photosynthesis, and biomass production tends to diminish. Ferreira et al. (2009) observed a strong decrease in biomass accumulation in *Himatanthus sucuuba* (Apocynaceae) when exposed to long flooding periods.

There was no difference in cortex width between the two treatments in *G. brasiliensis*. However, a decrease in cortex width was reported in *Cecropia pachystachya* (Cecropiaceae) and *Caesalpinia peltophoroides* (Fabaceae) by Batista et al. (2008) and Henrique et al. (2010), respectively, under flooding conditions.

One of the anatomical characteristics of *G. brasiliensis* that appears to favor flooding tolerance is the presence of a thick layer of hypodermal cells (exodermis). This cell layer prevents radial loss of oxygen to the rhizosphere while increasing longitudinal oxygen diffusion in the root (Colmer, 2003). Additionally, this suberized layer protects against phytotoxin absorption (Soukup et al., 2002) and pathogen exposure (De Simone et al., 2003).

No aerenchyma presence was observed in flooded roots. Santiago and Paoli (2007), studying the effects of flooding on the tree species *Guibourtia hymenifolia* (Fabaceae), also did not observe the presence of any significant amount of root aerenchyma. Nevertheless, this tissue has frequently been observed in other native Amazon plants (Ferreira et al., 2009; Parolin, 2009). This tissue, which is essentially composed of air chambers, facilitates gas transfer between aerial organs and roots (Bailey-Serres and Voesenek, 2008). Plants growing under flood conditions experience inhibition of photosynthate transport to the roots (Chen et al., 2005). Thicker phloem tissue, as seen in roots of *G. brasiliensis*, may favor flooding tolerance as this tissue would contribute to an increased photosynthate transport from the aerial organs of these plants to their roots (Souza et al., 2009), which experience energy deficit due to their shift to anaerobic metabolism.



**Fig. 4.** Time course of antioxidant enzyme activity and hydrogen peroxide ( $H_2O_2$ ) content in flooded and non-flooded *G. brasiliensis* seedlings. Each point indicates the mean value  $\pm$  SE. \* Indicates statistically significant results at  $p \leq 0.05$ . (A) Superoxide dismutase activity (SOD); (B) ascorbate peroxidase activity (APX); (C) catalase activity (CAT); (D)  $H_2O_2$  content.

A large xylem vessel number found in the roots of flooded seedlings in the present experiment may be related to a guarantee in the maintenance of water flow, as the ability of roots to absorb water is inhibited by lack of oxygen (Dell'Amico et al., 2001; Tournaire-Roux et al., 2003). Reduction in sclerenchyma tissue (xylem fibers) was observed in flooded roots, which may be relevant as an energy-saving strategy that favors the production of more important tissues (such as phloem, which facilitates seedling survival in flooded environments) (Souza et al., 2010).

In terms of antioxidant enzyme systems, we observed significantly high superoxide dismutase (SOD) activity throughout the experiment, as well as high ascorbate peroxidase (APX) and catalase (CAT) activities in the final assays (after 70 and 90 days) in flooded roots. One form of defense against low oxygen conditions is the expression of enzymes in charge of removing detrimental reactive oxygen species (ROS), (Moller et al., 2007; Karuppanapandian et al., 2011). These results are good indications that, peroxidases of the ascorbate cycle, catalases, and superoxide dismutases are important to preserve the physiological integrity of *G. brasiliensis* seedlings. Regarding hydrogen peroxide ( $H_2O_2$ ), an increase was observed in its concentration until the 55th day of root flooding. This increase may have been due to a high SOD activity, which is responsible for transforming singlet oxygen (ROS) into  $H_2O_2$ . On the other hand, it is possible that the observed increase in APX and CAT activities after 55 days of flooding reduced  $H_2O_2$  content.

It can, therefore, be concluded that flooding stress led to a decrease in root length, root superficial area, root volume as well as in biomass attributes of *G. brasiliensis* seedlings. There were significant biochemical and anatomical changes in seedlings of *G. brasiliensis* related to flooding tolerance which favor their survival. Thus these plants can be used for ciliary reforestation of artificial reservoir formed by the construction of dams.

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