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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_2O_2, 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_2O_2 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.

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 morphopysiological 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 tocochromanol (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°30′ S and 40°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 °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 kg m^{-3}), simple super phosphate fertilizer (5 kg m^{-3}), ammonium sulfate (725 g m^{-3}), and dolomite limestone powder (500 g m^{-3}). Sixty days after germination (DAG) the bags containing seedlings with six totally expanded leaves were placed outdoors into brick tanks (4 m × 1 m and 0.8 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 °C and 24 °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 H_2O_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 cm × 30 cm) with a film of water of approximately 1-cm thick. The following characteristics were determined: root length (cm), root superficial area (cm^2), root volume (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 °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 (mm g^{-1}), root fineness (mm mm^{-3}), and root tissue density (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 ± 0.5 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 °C and the