Ecological Indicators 63 (2016) 154–164

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Antioxidant responses of Atlantic Forest native tree species as indicators of increasing tolerance to oxidative stress when they are exposed to air pollutants and seasonal tropical climate

Cristiane Aguiar-Silva, Solange E. Brandão, Marisa Domingos, Patricia Bulbovas*

Núcleo de Pesquisa em Ecologia, Instituto de Botânica, São Paulo, SP CEP 04045-972, Brazil

A R T I C L E   I N F O

Article history:
Received 22 April 2015
Received in revised form
23 November 2015
Accepted 24 November 2015

Keywords:
Biochemical leaf traits
Antioxidant defenses
Pigment contents
Lipid peroxidation
Tropical tree species

A B S T R A C T

Tropical forest ecosystems may be subjected to climate-origin oxidative stress as it is observed in Southeast Brazil. The region is characterized by seasonal climate with well-defined wet and dry periods. Anthropogenic air pollutants are additional oxidative stress sources in these ecosystems. However, the tolerance of Brazilian tree species to oxidative stress is still unknown. Thus, the current field study aims to comparatively establish the range of antioxidant responses of ascorbate-glutathione cycle in *Astronium graveolens*, *Croton floribundus* and *Piptadenia gonoacantha* adult trees exposed to air pollutants and seasonal tropical climate as indicators of their increasing tolerance to oxidative stress. These are the most abundant species in the semideciduous Atlantic Forest, in Southeast Brazil. Variations in biochemical leaf traits (antioxidant defenses: ascorbate peroxidase, catalase, superoxide dismutase, glutathione reductase, ascorbate and glutathione; pigments: chlorophyll a, b and carotenoid; oxidative damage indicators: hydrogen peroxide and lipid peroxidation indicator) were determined. The native Brazilian tree species revealed distinct biochemical patterns in response to environmental oxidative stress during the wet and dry seasons. Biochemical leaf traits changed mainly in response to photoxidative stress, during the wet season. This variation was stimulated by better climate conditions to photosynthesis and plant growth, such as high light energy, water availability and temperatures. Catalase seemed to be the biochemical leaf tolerance indicator in all species during the wet season. The environmental conditions during the dry season, either of natural or anthropogenic origin, were stressful to the Brazilian tree species. They induced several changes in their biochemical leaf traits. Such changes were indicated by multilinear regression analyses. Oxidative/antioxidative imbalances, such as increased lipid peroxidation and decreased glutathione as well as the chlorophyll contents, were the most appropriate oxidative stress indicators during the dry season. *C. floribundus* was the most efficient species in terms of oxidative stress tolerance and it was followed by *A. graveolens* and *P. gonoacantha*.

1. Introduction

Forest ecosystems may be subjected to climate-origin oxidative stress, such as extreme temperatures, drought and high irradiance (Tausz et al., 1998, 2007a,b; Bussotti, 2008). It is possible that the climate stress may be more intense in forest ecosystems growing under seasonal tropical climates featured by well-defined wet and dry periods. In addition, tropical forest ecosystems are also exposed to oxidative stress due to complex mixtures of anthropogenic air pollutants. Given such environmental conditions, it is possible to assume that the tolerance level to the overall oxidative stress among native plants depends on the activation of biochemical leaf traits, among other acclimation processes suggested by Bussotti (2008) for Mediterranean forests.

Abbreviations: O_3, ozone; NO_2, nitrogen dioxide; SO_2, sulfur dioxide; PM_{10}, particulate material; ROS, reactive oxygen species; AsA, reduced ascorbate; GSH, reduced glutathione; Car, carotenoid; SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; GR, glutathione reductase; H_2O_2, hydrogen peroxide; Chl-a, chlorophyll a; Chl-b, chlorophyll b; MDA, malondialdehyde; MRC, Metropolitan Region of Campinas; CA, Campinas; PA, Paulínia; CO, Cosmópolis; GSSG, oxidized glutathione; totAsA, total ascorbate; DHA, dehydroascorbate; totGSH, total glutathione; TBARS, thiobarbituric acid reactive substances; VPD, vapor pressure deficit; AsA/totAsA, ratio between reduced ascorbate and total ascorbate; GSH/totGSH, ratio between reduced glutathione and total glutathione; PCA, Principal Components Analysis; Chl-tot, total chlorophyll.

* Corresponding author.
E-mail address: pbulbovas@hotmail.com (P. Bulbovas).

http://dx.doi.org/10.1016/j.ecolind.2015.11.060
1470-160X/

Air pollution has increased in developing countries located in the tropical zone due to city growth, industrial activities and agriculture (Deniz and Duzenli, 2007; Singh et al., 2014). The Brazilian Southeast region is the most developed and richest in the country. Many high biodiversity Atlantic forest relicts are still found in this part of Brazil. Indeed, since the colonization period this tropical biome is at great extinction risk due to different land uses that led to forest fragmentation and environmental pollution (Ribeiro et al., 2009; Forzza et al., 2012; Lira et al., 2012).

After uptake by plants, air pollutants such as ozone (O_3), nitrogen dioxide (NO_2), sulfur dioxide (SO_2) and particulate material (PM_{10}) react with water or other cell constituents to form reactive oxygen species (ROS). These species initiate multiple oxidation events in the cell and trigger many destructive changes, such as protein, pigment, lipids, and nucleic acid damages (Cheng et al., 2007; Feng and Kobayashi, 2009; Singh et al., 2010; Wujeska et al., 2013). Consequently, plant growth changes, tree death (in extreme cases) and changes in the competition of species, in population and community structures and in successional processes may be observed (Karnosky et al., 2003; Shriner and Karnosky, 2003).

However, the intensity of injury level in the plants depends on how efficiently they mobilize antioxidant defenses, which scavenge the ROS and minimize the mentioned oxidative effects on plants (Foyer and Shigeoka, 2011; Gill et al., 2013; Iriti and Faoro, 2008). ROS detoxification in plants may occur by antioxidant activation in the ascorbate-glutathione cycle and the activation of other associated compounds such as hydrophilic (e.g., ascorbate/AsA, glutathione/GSH) and lipophilic substances (e.g., the carotenoid pigment/Car) as well as of other detoxifying enzymes (e.g., superoxide dismutase/SOD, catalase/CAT and ascorbate peroxidase/APX). ROS detoxification is also supported by enzymatic repair (e.g., glutathione reductase/GR) and regeneration processes (Tausz et al., 2007a,b; Gill and Tuteja, 2010; Foyer and Shigeoka, 2011). Thus, measuring the range of antioxidant responses in plants growing in their natural environment is an efficient indication of their redox potential and induced-stress resistance against multiple oxidative stresses. These measurements reveal their ability to perpetuate in disturbed ecosystems (Tausz et al., 2001, 2003; Bussotti, 2008; Wujeska et al., 2013). Analyzing the indicators of plant tolerance to oxidative stress at cell level, such as hydrogen peroxide (H_2O_2), other pigments (e.g., chlorophyll a and b, chl-a and chl-b), and lipid peroxidation derivatives (e.g., malondialdehyde, MDA) may add important information on the susceptibility of plants to natural and anthropogenic oxidative stress (Pignata et al., 2002; Gratão et al., 2012).

Several studies have been dedicated to evaluate both the antioxidant responses and the oxidative damage indicators of plant species growing in forest ecosystems subjected to air pollution and climate oscillations. Among these studies, it is worth highlighting those carried out in the Canary Islands (Tausz et al., 1998, 2005), San Bernardino Mountains, California (Tausz et al., 2001), India (Agrawal and Singh, 2000) and Germany (Tausz et al., 2007b). However, such approach has never been used in Brazil.

In view of the herein presented considerations, our hypothesis is that the native tree species from the remaining Brazilian Atlantic forest surrounded by diversified land uses have been affected by oxidative stress caused by both climate and environmental pollution. However, the redox potential and consequent induced-stress resistance against multiple oxidative stresses may vary among species due to their natural distinct biochemical leaf traits, validating the search for specific biochemical indicators of oxidative stress in those species. The current study aimed to establish comparatively the range of antioxidant responses associated to the ascorbate-glutathione cycle of the most abundant tree species from the semideciduous Atlantic Forest, in Southeast Brazil. The indicators were found by (1) knowing the profile of the antioxidant responses (APX, CAT, SOD, GR, AsA, GSH and Car) during wet and dry seasons; (2) assessing whether these defense responses are able to prevent or decrease cell damage by evaluating the redox state of AsA and GSH as well as the contents of pigments (chl-a and chl-b), H_2O_2 and lipid peroxidation.

2. Materials and methods

2.1. Study area and plant sampling

The field study was conducted in forest remnants located in the Metropolitan Region of Campinas (MRC). The MRC is located in the central-eastern region of São Paulo State, Brazil (Fig. 1). It is the second most important economical center of São Paulo State, and it is characterized by different land uses such as urban settlements, highways, industries and extensive agriculture of sugarcane, orange and ornamental plants (CETESB, 2013). MRC is also affected by sizable emissions from the Metropolitan Region of São Paulo because of the predominantly South and Southwest winds in the region (Boian and Andrade, 2012).

According to Thornthwaite's typology, the prevalent climate in the MRC is marked by a hot and rainy season, between October and March, and by a dry season, between April and September (Rolim et al., 2007). The mean temperature ranges from 23.2 °C to 24.9 °C, and the total rainfall reaches 1100 mm during the rainy seasons. Mean temperatures between 18.5 °C and 23.0 °C and a mean total rainfall of 280 mm are recorded during the dry seasons (Applied Meteorological and Climate Research for Agriculture/CEPAGRI, http://www.cpa.unicamp.br/). Wind circulation during a single day (SSE and SE in early morning and evening, SSE, SE and NE in the morning and NNE, SSE, N, SSW and S in the afternoon) as well as the flat topography favor the mixture of pollutants in the entire MRC (Boian and Andrade, 2012; Bulbovas et al., 2015; Tresmondi and Tomaz, 2004).

The natural vegetation in the MRC is classified as semideciduous forest. It is subtype of the Atlantic forest domain (Oliveira-Filho and Fontes, 2000). This type of vegetation is characterized by a number of species that in response to water shortage partially lose their leaves during the dry season. The semideciduous Atlantic forest is currently fragmented into small residual areas. These fragments are mostly isolated from each other due to the regional urban growth and development because it occurred without any environmental conservation criteria (Ribeiro et al., 2009). Although some remaining native plants are still found in the conservation unities and protected by the Brazilian environmental laws, most of them are surrounded by agricultural lands.

The present study was developed in the three largest forest remnants in the MRC. These remnants are located in the following cities: Campinas, the largest urban-industrial center at MRC (233.5 ha; 680 m altitude); Paulínia, which holds an important industrial center, composed of chemical, agrochemical and petrochemical industries (188.9 ha; 620 m altitude); and Cosmópolis (199.9 ha; 585 m altitude), which is a typical rural town. They are referred as CA, PA, and CO, respectively, in Fig. 1.

The study was conducted with three of the most abundant native tree species, *Astronium graveolens* Jacq. (Anacardiaceae), *Croton floribundus* Spreng. (Euphorbiaceae) and *Piptadenia gonoacantha* (Mart.) Macbr (Fabaceae), as it was previously indicated by a floristic survey (Domingos et al., 2015). As they belong to distinct taxonomic and successional groups, their inherent leaf traits appeared to be useful for biomonitoring the risks associated with specific pollutant categories, even when they are under the complex mixture of air contamination and seasonal climate observed in the studied region (see details in Domingos et al., 2015).