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Original Articles

Sapindus saponaria bioindicator potential concerning potassium fluoride exposure by simulated rainfall: Anatomical and physiological traits

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ABSTRACT

Fluoride is one of the main phytotoxic pollutants released into the atmosphere. It can be released in the form of potassium fluoride by brick, ceramic, phosphate mineral and aluminum industries, causing damage to different plant species. *Sapindus saponaria* L. (Sapindaceae) is widely distributed throughout Brazil, and has been used both in the recovery of degraded areas and in urban afforestation. In this context, the aim of the present study was to determine *S. saponaria* bioindicator potential through morpho-anatomical and physiological responses after simulated rainfall with potassium fluoride. Young *S. saponaria* individuals, approximately 50 days old, were treated daily with KF through simulated rain at 0, 10, 20, 30 and 40 mg L^{-1}, for 19 consecutive days. Necrosis and chlorosis in *S. saponaria* were evidenced at the 4th day of KF application, for all treatments, followed by alterations of parenchyma tissues, cell collapse, accumulation of phenolic compounds and damage to the photochemical and biochemical stages of photosynthesis with increasing KF doses. Visible symptoms associated with non-invasive variables, such as F_v/F_m, $\Delta F/F_m'$, ETR, *A* and *A*/*Ci*, were shown to be important biomarkers of fluoride action. In addition, the evaluated characteristics indicate that *S. saponaria* is highly sensitive to KF, with high bioindicator potential regarding this pollutant.

1. Introduction

As industrialization has progressed, the incidence of air pollutant contamination in humans and the environment has increased worldwide (Mondal, 2017, Yepu et al., 2017). Among other important pollutants, fluoride (F) is considered one of the most phytotoxic for the environment (Saini et al., 2012; Panda, 2015) and is mainly released by aluminum smelters, phosphate fertilizers and brick industries (Cape et al., 2003). Even with government regulations and the use of filters, the emission of these compounds into the atmosphere continues to be an environmental problem, leading to toxic effects in plants even at low concentrations (Walna et al., 2013). This is due to the high reactivity, biodegradability and gradual accumulation in plants exposed to the pollutant F, causing severe damage to the environment (Fornasiero, 2003).

Fluoride can be absorbed into plants from contaminated air through leaves (Divan Junior et al., 2008) as well as from contaminated soil and water through roots (Zouari et al., 2014). However, the main F absorption route in plants is through leaves, through the stomata when in its gaseous state (Sant'Anna-Santos et al., 2014) and, throughout the leaf surface when exposed as an aqueous solution, such as rainfall (Miller, 1993; Chaves et al., 2002). Upon penetrating the leaf, this compound moves through apoplastic pathways, reaching the margins and apex of the leaves, and can also accumulate in the mesophyll, causing lesions such as the collapse of parenchyma cells, leading to chlorosis and necrosis (Pita-Barbosa et al., 2009; Rodrigues et al., 2017). F effects also include alterations in primary plant metabolism, such as photosynthetic (Kamaluddin and Zwiazek, 2003) and respiration (Miller and Miller, 1974) processes, and triggering oxidative stress by the production of reactive oxygen species (ROS) (Zouari et al., 2017).

In order to control the emission of pollutant gases, such as F, it is

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important to install air pollution monitoring techniques in industrialized areas, such as active or passive air samplers (Jochner et al., 2015). However, these techniques are costly, and no equipment can estimate the toxicity potential of pollutants to the environment (Kovalchuk and Kovalchuk, 2008). Thus, the use of air pollution bioindicators, such as plants already growing in the vicinity of the industries, or even planted for this purpose, becomes an effective and low-cost means of monitoring and mitigating contamination by air pollutants (Sant'Anna-Santos et al., 2014; Louback et al., 2016). In order to carry out environmental monitoring, it is necessary to recognize the effects of the pollutant on the species surrounding the potentially polluting areas and to estimate the bioindicator potential of these species.

Sapindus saponaria L, belonging to the Sapindaceae family, is a tree native to the Americas (Lorenzi, 2000; ITIS, 2018; USDA, 2018). It has been used for both urban landscaping and recovery models of degraded areas (Román-Dañobeytia et al., 2011, Ferreira and Santos, 2012, Izquierdo et al., 2015, Horstman et al., 2018). Among the species belonging to this family, *Allophylus edulis* has been described as a bioremediator for contaminated soils (Nogueira et al., 2011). However, to date, no studies on the effect of fluoride on *S. saponaria* morphology, anatomy and physiology are available. In this context, the aim of the present study was to evaluate the effects of potassium fluoride (KF), via simulated rainfall, on *S. saponaria* morphological and physiological leaf responses.

2. Material and methods

2.1. Plant material, growing conditions and treatments

Sapindus saponaria L. seeds were obtained from 10 adult plants in full production, located in the municipality of Rio Verde, Goiás, Brazil (latitude 17° 46′ 30″ S – longitude 50° 57′ 59″W, and altitude of 784 m). A specimen was deposited at the Goiano Federal Institute Herbarium (IFGoiano, Rio Verde Campus) under number No. 1008/2018. The seeds were initially immersed in concentrated sulfuric acid for 90 min to break dormancy, treated with Vitavax®-Thiram fungicide (30%) and subsequently seeded in beds containing washed sand as substrate. The experiments were conducted under controlled conditions in a greenhouse located at the IFGoiano (latitude 17° 48′16″S, longitude 50° 54′ 19″ W and altitude of 753 m).

Approximately 50 days after emergence, the seedlings were selected by height standardization (~20 cm) and individually transplanted into 5 L pots containing a substrate composed of vermiculite, washed sand and Bioplant® (1:1:1). After 10 days of acclimation, the plants were exposed to the application of a liquid potassium fluoride solution (KF, pH 6.0) at 0, 10, 20, 30 and 40 mg L^{-1}, as adapted from Silva et al. (2000) and Rodrigues et al. (2017). The pH values of each solution as adjusted with HCl (2.0 mol L^{-1}) and NaOH (2.0 mol L^{-1}). The fluoride application occurred by means of simulated rainfall with manual sprays, 60 mL day^{-1} per plant, based on average plant evapotranspiration volume. After 19 days of KF exposure, visual and physiological evaluations were performed, and plant material was sampled for morpho-anatomical analyses.

A completely randomized design was applied, with 5 treatments (KF concentrations) and 4 replicates, each replicate composed of one pot containing 1 plant (4 plants per treatment).

2.2. Visible leaf symptoms

Visible symptoms were identified through photographs of the entire surface of completely expanded *Sapindus saponaria* leaves, taken with a digital camera (Cyber-Shot HX100V, SONY, Japan), monitored throughout the experimental period. The figures were prepared with the leaves that best represented the KF treatment effects.

2.3. Morphoanatomical leaf characterization

For the morphoanatomic analyses, 3 cm^2 samples from the middle region of the last fully expanded *Sapindus saponaria* leaf from all repetitions (n = 4) from each treatment (n = 4) were collected. The samples were first fixed in Karnovsky solution (1965) for 24 h. The plant material was then prewashed in a phosphate buffer (0.1 M, pH 7.2) and dehydrated in an increasing ethylic series (30–100%) and pre-infiltrated and infiltrated in historesine (Leica, Germany), as recommended by the manufacturer. Subsequently, the samples were cross-sectioned at 5 µm thickness in a rotary microtome (Model 1508R, Logen Scientific, China) and the sections stained with toluidine blue - polychromatic coloration (0.05% 0.1 M phosphate buffer, pH 6,8) (O'Brien et al., 1964). The obtained images were photographed under an Olympus microscope (BX61, Tokyo, Japan), coupled to a DP-72 camera using the clear field option. Morphoanatomical observations of the adaxial and abaxial epidermis, palisade and spongy parenchyma and mesophyll were then performed.

2.4. Histolocation of phenolic compounds

The histochemical detection of phenolic compounds was performed in the median region of the last fully expanded *S. saponaria* leaf, fixed in a ferrous sulphate solution in formalin for 48 h (Johansen, 1940). The samples were then prewashed in a phosphate buffer (0.1 M, pH 7.2) and dehydrated in an increasing ethylic series (30%–100%) and pre-infiltrated and infiltrated in historesine (Leica, Germany). Subsequently, the samples were cross-sectioned at 5 µm thickness in a rotary microtome (Model 1508R, Logen Scientific, China) and analysed under an Olympus microscope (BX61, Tokyo, Japan).

2.5. Evaluation of chlorophyll a fluorescence

Chlorophyll *a* fluorescence variables were measured with a leaf chamber fluorometer (6400-40, Li-cor, Nebraska, EUA) coupled to an IRGA (IRGA, LI-6400xt, Li-cor, Nebraska, EUA) in the last totally expanded leaf of each plant. Initially, minimum (F_0) and maximum (F_m) fluorescence were recorded in the dark-adapted state (when all PSII centers are open), after the measuring light (~0.03 µmol m^{-2} s^{-1}) and a 8 s saturation pulse (> 3.000 µmol m^{-2} s^{-1}) were switched on, respectively. The maximum quantum efficiency of PSII photochemistry was determined as $F_v/F_m = (F_m - F_0)/F_m$ (Kitajima and Butler, 1975). After sample illumination with a continuous actinic light (~1000 µmol m^{-2} s^{-1}) for 40 s, a saturation pulse was applied in order to determine the maximal (F_m') and steady-state (Fs) fluorescence in light-adapted leaves. The obtained data allowed for the calculation of the quantum efficiency of the PSII ($\Delta F/F_m' = [F_m' - F_s]/F_m'$) and the apparent electron transport rate (ETR = $\Delta F/F_m' \times PAR \times 0.5 \times 0.84$, where PAR is the photon flux (µmol m^{-2} s^{-1}) in the leaves, 0.5 is the excitation energy fraction directed to the PSII (Laisk and Loreto, 1996) and 0.84 is the amount corresponding to the fraction of incident light that is absorbed by the leaves. The non-regulated non-photochemical quenching ($qN = [F_m - F_m']/[F_m - F_o]$ (Lichtenthaler et al., 2005), and the regulated non-photochemical dissipation ($NPQ = [F_m - F_m']/F_m'$), which estimates the rate constant for heat loss from the PSII (Maxwell and Johnson, 2000; Baker, 2008), were also calculated.

2.6. Gas exchanges

Gas exchanges were measured in the same leaf as the chlorophyll a fluorescence measurements, to record photosynthetic rates (*A*, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (*gs*, mol H_2O m^{-2} s^{-1}), transpiration (*E*, mmol H_2O m^{-2} s^{-1}) and ratio between the internal and external CO_2 concentrations (*Ci/Ca*). From these values, the instantaneous efficiency of the carboxylation (*A/Ci*) and the relation between the rate of electron transport and CO_2 assimilation (ETR/*A*;