Chemosphere 181 (2017) 551–561

Contents lists available at ScienceDirect

Chemosphere

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

Antioxidant responses of peanut roots exposed to realistic groundwater doses of arsenate: Identification of glutathione S-transferase as a suitable biomarker for metalloid toxicity

Eliana Bianucci [a, c, *], Ana Furlan [a], María del Carmen Tordable [a], Luis E. Hernández [b], Ramón O. Carpena-Ruiz [c], Stella Castro [a]

[a] Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta 36 Km 601, 5800 Río Cuarto, Córdoba, Argentina
[b] Laboratorio de Fisiología Vegetal, Departamento de Biología, Universidad Autónoma de Madrid, 28049, Madrid, Spain
[c] Departamento de Química Agrícola Universidad Autónoma de Madrid, 28049, Madrid, Spain

HIGHLIGHTS

- This is the first report showing the effect of arsenic on peanut plant in hydroponics.
- Root histological structure modification and damage to macromolecules are induced by As.
- Glutathione S-Transferase exhibits a remarkable increase upon exposure to As.
- GST activity is proposed as a suitable biomarker of peanut arsenic contamination.

ARTICLE INFO

Article history:
Received 3 February 2017
Received in revised form
10 April 2017
Accepted 23 April 2017
Available online 24 April 2017

Handling Editor: X. Cao

Keywords:
Arsenic
Glutathione S-transferase
Oxidative stress
Peanut

ABSTRACT

Arsenic (As)-polluted groundwater constitutes a serious problem for peanut plants, as roots can accumulate the metalloid in their edible parts. Characterization of stress responses to As may help to detect potential risks and identify mechanisms of tolerance, being the induction of oxidative stress a key feature. Fifteen-day old peanut plants were treated with arsenate in order to characterize the oxidative stress indexes and antioxidant response of the legume under realistic groundwater doses of the metalloid. Superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) histochemical staining along with the activities of NADPH oxidase, superoxide dismutase (SOD), catalase (CAT) and thiol (glutathione and thioredoxins) metabolism were determined in roots. Results showed that at 20 μM $H_2AsO_4^-$, peanut growth was reduced and the root architecture was altered. $O_2^{\bullet-}$ and H_2O_2 accumulated at the root epidermis, while lipid peroxidation, NADPH oxidase, SOD, CAT and glutathione S-transferase (GST) activities augmented. These variables increased with increasing As concentration (100 μM) while glutathione reductase (GR) and glutathione peroxidase/peroxiredoxin (GPX/PRX) were significantly decreased. These findings demonstrated that the metalloid induced physiological and biochemical alterations, being the NADPH oxidase enzyme implicated in the oxidative burst. Additionally, the strong induction of GST activity, even at the lowest $H_2AsO_4^-$ doses studied, can be exploited as suitable biomarker of As toxicity in peanut plants, which may help to detect risks of As accumulation and select tolerant cultivars.

1. Introduction

Arsenic (As) is an ubiquitous metalloid present in many ecosystems. The average concentration of As in the lithosphere ranges between 20 and 30 μM, occupying the position number 52 in the list of the most abundant elements in nature (Adriano, 2001).

* Corresponding author. Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta 36 Km 601, 5800 Río Cuarto, Córdoba, Argentina.
E-mail address: ebianucci@exa.unrc.edu.ar (E. Bianucci).

http://dx.doi.org/10.1016/j.chemosphere.2017.04.104
0045-6535/

Abbreviations:

CDNB	1-chloro-2, 4-dinitrobenzene
DAB	3,3-diaminobenzidine
As	Arsenic
CAT	Catalase
GSH	Glutathione
GPX/PRX	Glutathione peroxidase/peroxiredoxin
GR	Glutathione reductase
GST	Glutathione S-transferase
H_2O_2	Hydrogen peroxide
NBT	Nitroblue tetrazolium
KI	Potassium iodide
$O_2^{\bullet-}$	Superoxide anion
SOD	Superoxide dismutase
TBARS	Thiobarbituric-reactive substances

Arsenate ($H_2AsO_4^-$, As(V)) and arsenite ($H_2AsO_3^-$, As(III)) are the prevalent inorganic chemical species, being more toxic than the organic forms (Mandal and Suzuki, 2002; Zhao et al., 2010). These contaminants are receiving much attention due to human population chronic poisoning caused by drinking water polluted in several regions of Asia and South America (Zhao et al., 2010). In addition, crop plants can accumulate large amounts of As in edible parts when irrigated with As-containing water; acting as the first stage of As distribution in the trophic chain (Smedley and Kinniburgh, 2002). In Argentina, particularly in Córdoba province, As has become a public health problem due to numerous cases of human poisoning. Francisca et al. (2006) found that 90% of this region is affected by groundwater As concentrations that exceeds the maximum allowed level of the metalloid in drinking water (0.1 μM) (FAO, 2015), reaching in some areas up to 20 μM (Cabrera et al., 2005). Peanut is a very important crop in Argentina, specifically in Córdoba, as it represents approximately 90% of the Argentinean production (Ministry of Agroindustry, Argentina, 2006). The fruit of this legume is a pod with one to five seeds that develops underground, in putative intimate contact with soil contaminants. One of the major constraints of Córdoba province, given the water scarcity in the area, is that crops require artificial irrigation by sprinkler systems using groundwater rich in As (Bustingorri and Lavado, 2013, 2014) representing a potential risk for crops. This is aggravated by the deep penetration of plant roots in soil strata, which is a typical response of plants suffering from drought stress (Sharp and LeNoble, 2002).

Arsenate form ($H_2AsO_4^-$ As(V)) is an analog of phosphate ($H_2PO_4^-$) and it can be easily absorbed by plant by $H_2PO_4^-$ transporters (Tripathi et al., 2007; Verbruggen et al., 2009). Once inside the cell, most of $H_2AsO_4^-$ is reduced to $H_2AsO_3^-$, by different $H_2AsO_4^-$reductases (Dhankher et al., 2006; Duan et al., 2007; Sánchez-Bermejo et al., 2014). Therefore, As is present predominantly in roots and shoots as $H_2AsO_3^-$, which binds to sulfhydryl groups of thiol-rich peptides such as glutathione (GSH) and phytochelatins (PCs) (Pickering et al., 2000). Although the precise mechanisms of toxicity are not completely understood, plants exposed to both As species showed substantial redox homeostasis imbalance, with accumulation of reactive oxygen species (ROS) (Requejo and Tena, 2005; Talukdar, 2013), and changes in antioxidant responses (Mylona et al., 1998). It is feasible that the As high affinity for sulfhydryl groups causes GSH depletion and changes in enzymatic activity that leads to oxidative stress (Hernández et al., 2015). Biomolecules such as lipids, proteins and nucleic acids are principal targets of ROS, which cause the characteristic oxidative damage symptoms (Verbruggen et al., 2009). Another source of ROS is constituted by the plasma membrane-associated NADPH oxidase family that has a low redox potential able to reduce O_2 to superoxide anion ($O_2^{\bullet-}$) (Mittler et al., 2011). Moreover, under metal(-loid)s stress these enzymes are activated increasing ROS accumulation (Hernández et al., 2015).

Plants possess an effective antioxidant defense system that scavenges ROS accumulated by aerobic metabolism, and is comprised of antioxidant enzymes and metabolites (Noctor et al., 2012). Superoxide dismutase (SOD) constitutes the first line of defense dismutating $O_2^{\bullet-}$ into hydrogen peroxide (H_2O_2), which is then reduced to water and oxygen by ascorbate peroxidase (APX) and catalase (CAT) (Mishra et al., 2006). These enzymes constitutes the major ROS scavenging mechanisms of plants (Mittler, 2002) and their induction under different metal(loid) treatments have been demonstrated in several species (Sharma and Dietz, 2009). One of the most studied antioxidant metabolite essential to cope with the oxidative stress induced by toxic elements such as Cd, Hg and As is the tripeptide GSH (Li et al., 2006; Bianucci et al., 2012; Sobrino-Plata et al., 2013). GSH transits from its reduced form (thiol) to its oxidized form (GSSG, disulfide), keeping a strong reducing environment in the cell which plays an important role in preventing excessive oxidation of cellular biomolecules under different stressing conditions (Jozefczak et al., 2012). Besides, a series of antioxidant enzymes related to GSH metabolism are involved in the detoxification of free radicals (Foyer and Noctor, 2011). Glutathione reductase (GR) regenerates GSH from GSSG at the expense of NADPH to maintain a high GSH/GSSG ratio (Gill et al., 2013). Glutathione-S-transferases (GSTs) are a large and diverse group of enzymes involved in the detoxification of dangerous xenobiotics catalyzing their conjugation with GSH. Moreover, GSTs can bind GSH with organic peroxides and radicals generated by ROS attack, which can amplify in a chain reaction the oxidative damage caused by toxic elements, preventing their reaction with many important biomolecules (Gill and Tuteja, 2010). The enzyme named misleadingly glutathione peroxidase (GPX), a peroxidase able to use GSH and thioredoxin (TRX), constitutes a special case (Herbette et al., 2002). Particularity, TRX is known to be a more efficient reducing substrate than GSH in certain plant species (Iqbal et al., 2006). Thus, these enzymes should be considered as peroxiredoxins (PRX) rather than GPX (Navrot et al., 2006) and are named accordingly in our work as GPX/PRX.

Arsenate and arsenite are highly soluble and stable in water under a wide range of pH and redox potential conditions. However, under oxidizing environments as occur in many areas of Córdoba province, the prevalent arsenic form is arsenate (Blarasin et al., 2014). Thus, our experiments were conducted to characterize oxidative stress indexes and the antioxidant response of peanut (*Arachis hypogaea* L.) plants exposed to environmentally realistic As(V) levels, considering the economic relevance of this legume crop in Córdoba province. This study will provide information about the mechanisms of toxicity evoked by As(V), which can be used as bioindexes of putative risks to consumers. Most sensitive stress indexes could be used to select tolerant cultivars with lower toxic symptoms and able to exclude As from edible parts of the plants.

2. Material and methods

2.1. Plant material and experimental design

Peanut seeds, kindly supplied by "El Carmen S.A." Córdoba, Argentina, were surface sterilized following the method previously described by Vincent (1970). Then, they were germinated at 28 °C