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DDTs-induced antioxidant responses in plants and their influence on phytoremediation process

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ABSTRACT

Phytoremediation is a low cost technology based on the use of plants to remove a wide range of pollutants from the environment, including the insecticide DDT. However, some pollutants are known to enhance generation of reactive oxygen species (ROS), which can generate toxic effects on plants affecting the phytoremediation efficiency. This study aims to analyze the potential use of antioxidant responses as a measure of tolerance to select plants for phytoremediation purposes. Tomato and zucchini plants were grown for 15 days in soils contaminated with DDTs (DDT + DDE + DDD). Protein content, glutathione-S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx) and catalase (CAT) activities were measured in plant tissues. Exposure to DDTs did not affect protein content or CAT activity in any of the species. GST, GR and GPx activity showed different responses in exposed and control tomato plants. After DDTs exposure, tomato showed increased GR and GPX activity in stems and leaves, respectively, and a decrease in the GST activity in roots. As no effects were observed in zucchini, results suggest different susceptibility and/or defense mechanisms involved after pesticide exposure. Finally, both species differed also in terms of DDTs uptake and translocation. The knowledge about antioxidant responses induced by pesticides exposure could be helpful for planning phytoremediation strategies and for the selection of tolerant species according to particular scenarios.

1. Introduction

The insecticide DDT (1,1,1-trichloro-2,2-bis-(4′-chlorophenyl) ethane) has been widely used for pest control because its low cost, broad spectrum activity and high residual biological activity (Turusov et al., 2002). Although, it's use has been prohibited in most countries because of the negative impact on wildlife and human health in addition to biomagnification process throughout food web, DDT is still being used in some developing countries for essential public health purposes (Foght et al., 2001; Bouwman et al., 2015).

Phytoremediation is defined as the use of green plants to remove pollutants from the environment or to render them harmless (Salt et al., 1998; Campos et al., 2008). Depending on the nature of the contaminant, plant species and soil characteristics, phytoremediation may be achieved in different ways. Phytostimulation or rhizodegradation, occurs when the organic contaminants are degraded in the root zone (rhizosphere), either by exuded plant enzymes or by the associated microbial community (Pilon-Smits, 2009). Pollutants can also be extracted and accumulated into plant tissues, followed by harvesting of the plant material, which is called phytoextraction. Finally, phytodegradation refers to the ability of plants to degrade organic pollutants directly via their own enzymatic activities. After uptake in plant tissue, certain pollutants can leave the plant in volatile form, known as phytovolatilization. These various phytoremediation routes are not mutually exclusive and can occur simultaneously.

The potential of *Cucurbita* species to accumulate significant amounts of Persistent Organic Pollutants (POPs), including dioxins, chlordane, DDT, DDE, DDD, and PCBs has been reported (Hülster et al., 1994; White et al., 2003; White, 2009). Particularly, stems and roots of *Cucurbita pepo* ssp *pepo* accumulate POP concentrations that are 5–30 times greater than present in the soil, often extracting 1–5% of the contamination in a single growing season. On the other hand, other crop species such as tomato plants have a high capacity to increase DDE bioavailability and metabolism in the rhizosphere, as well as to accumulate DDE and other organic persistent contaminants especially in roots (Gonzalez et al., 2003; Mitton et al., 2014, 2016; Mattina et al.,

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2003). In this sense, a previous study demonstrated that roots of 15 days tomato plants showed higher DDTs concentration than sunflower, soybean and alfalfa reaching levels of 2075 ng g^{-1}. Particularly, tomato plants presented the highest bioconcentration factor for DDE (2.23) (Mitton et al., 2014).

Insecticide-induced oxidative stress was shown to modify the cellular redox balance by altering antioxidant levels or the activity of the cellular defense systems (Bashir et al., 2007). Mishra et al. (2008) reported that the insecticide dimethoate triggered oxidative stress by producing reactive oxygen species (ROS). However, plants have multiple strategies to cope with the insecticide-induced toxicity. Among them, prevention of oxidative damage to cells has been suggested as one of the mechanisms of stress tolerance (Saraf and Sood, 2002; Prasad et al., 2005). Enzymes of the antioxidant system include ROS scavengers like glutathione peroxidase (GPx) and catalase (CAT) (Khan and Kour, 2007). Reduced glutathione (GSH) represents a non-enzymatic defense that protects cells from oxidative stress by scavenging ROS or by reducing oxidized components such as proteins (Pinto et al., 2003; Jan et al., 2012). Besides its antioxidant function, the occurrence and activity of detoxification enzymes is crucial for biotransformation and, eventually, degradation of the contaminants (Schröder, 2006; Schröder and Collins, 2002). In this sense, the conjugation of xenobiotics with GSH, mediated by glutathione-S-transferases (GSTs) is a xenobiotic biotransformation mechanism described in animals and plants (Marrs, 1996; Schröder and Collins, 2002; Kurasvili et al., 2016). This reaction results in both an increase of toxicant solubility that facilitates its excretion and decreases its toxicity (Brentner et al., 2008). In this context, the aim this work was to study the potential use of antioxidant responses as tolerance criteria for selecting plants with phytoremediation capabilities. This work includes the study of two species (tomato and zucchini) with different behavior towards the uptake and translocation of DDT.

2. Material and methods

2.1. Plant growth

Rectangular pots of 6000 cm^3 were filled with 1000 g of dry DDTs polluted soil obtained from an apple and peach production site located in Villa Regina, Rio Negro, Argentina (S 39°04.9′14″, W 67°02.9′59″). DDTs levels ranged between 63.5–101.3 ng g^{-1} dry weight of DDT and 381.4–455.3 ng g^{-1} dry weight of DDE (Gonzalez et al., 2010; Mitton et al., 2012, 2014). Soils are classified as Aridisols order according to Spil Survey Staff, (1999) and had 2.7% of organic carbon, 14.1% of sand, 62.9% of silt and 23% of clay (Gonzalez et al., 2010). Five seeds of *Solanum lycopersicum* "tomato" (cultivar *Platense*) and *Cucurbita pepo* "zucchini" (cultivar *Grey*) were placed in each pot separately. The plants were grown in greenhouse at temperature of 10–26 °C under natural sunlight (light:dark cycle 14:10 h) and five pots were established for each species. Planted control pots were established with non polluted soil (1.9% organic carbon, 60.7% sand, 31.8% silt and 7.3% clay, total organochlorine pesticide levels, including DDTs, lower than 2×10^{-6} mg g^{-1}, Gonzalez et al., 2010). The pots were watered on demand with tap water and weeded.

2.2. Plant sampling

Destructive harvest was done 15 days after germination, obtaining stems, leaves and roots. Attached soil particles were removed from roots by washing with distilled water. Each pot was individually analyzed and the samples were pooled. All samples were kept in ultrafreezer (− 80 °C) until analysis.

2.3. Pesticides analysis

DDTs levels in zucchini tissues were analyzed according to Metcalfe and Metcalfe (1997), as modified by Miglioranza et al. (2003). Briefly, subsamples of wet tissue were homogenized with sodium sulfate and extracted with a mixture of hexane-dichloromethane in a Soxhlet equipment. Lipids were removed by gel permeation chromatography in Bio Beads S-X3 (200–400 mesh size, Bio Rads Laboratory, Hercules, CA, USA) and further purification of the extracts was performed by silica gel chromatography. Samples were concentrated to 1 mL and kept in sealed vials at − 20 °C prior to chromatographic analysis. DDTs (*p,p*-DDE, *p,p*-DDT and *p,p*-DDD) were identified and quantified using a gas chromatograph (with autosampler) Shimadzu 17-A gas equipped with a 63Ni Electron Capture Detector (GC-ECD) and a capillary column coated with SPB-5 [(5-phenyl)-methyl polysiloxane, 30 m × 0.25 mm i.d. × 0.25 μm film thickness; Supelco Inc]. The standard solution used for identification and quantification of single compounds were a Standard Pesticide Mixture of organochlorine pesticides from Ultra Scientific (RI, USA and PCB #103 from Accustandard Absolute Standards, INC, CT, USA). Retention times of each compound were confirmed by running solution of single compound from Dr. Ehrenstorfer, Augsburg, Germany, with purity ≥ 96%. Laboratory and instrumental blanks analyzed through the procedure indicate that there were no contaminants or interference on samples during laboratory handling. Single compounds recoveries, calculated by spiking matrix and surrogate recovery, were greater than 90%. Instrumental detection limits (DL) for DDTs were calculated according to Keith et al. (1983) and were < 0.2 ng mL^{-1}, method detection limits were < 0.033 ng g^{-1}. DDTs data for tomato plants were obtained from Mitton et al. (2014).

2.4. Tissue homogenization

Enzyme extraction from tomato and zucchini tissues was done following the method described by Martínez-Domínguez et al. (2008), as modified by Mitton et al. (2014). After grinding in liquid nitrogen, tissues were homogenized (1:2 w/v) in ice-cold buffer [0.1 M sodium phosphate buffer (pH 6.5), 20% glycerol, 14 mM dithiothreitol (DTE), 1 mM phenylmethylsulfonylfluoride (PMSF) and 1 mM (ethane-1,2-diyldinitrilo) tetra acetic acid (EDTA)]. Homogenates were centrifuged at 15,000 × *g* for 20 min (4 °C). Supernatants were collected and stored at − 80 °C for further analysis of protein determination and enzymatic activity using a microplate spectrophotometer (Epoch Bio Tek). All reagents were supplied by Sigma-Aldrich.

2.5. Protein determination

Protein concentration was determined by the Bradford (1976) method with bovine serum albumin (BSA) as standard protein.

2.6. Measurement of glutathione-S-transferase (GST) activity

GST activity was measured using the Habig and Jakoby (1981) methodology. The absorbance at 340 nm generated by the conjugation of 1 mM glutathione (GSH) with 1 mM of 1-chloro-2,4-dinitrobenzene (CDNB) was monitored during 5 min at 25 °C.

2.7. Measurement of glutathione reductase (GR) activity

GR activity was analyzed by the methodology described by Gallagher et al. (1992) using sodium phosphate buffer (200 mM, pH 7.5), oxidized glutathione (GSSG, 10 mM) and NADPH (1 mM). The oxidation of NADPH was monitored at 340 nm during 5 min at 25 °C.

2.8. Measurement of glutathione peroxidase (GPx) activity

GPx activity was based on methodology described by Arun et al. (1999) where the decrease of NADPH at 340 nm was monitored at 25 °C during 5 min. The reaction buffer contained reduced glutathione (GSH, 2 mM), NADPH (0.12 mM). H_2O_2 (2 mM), sodium azide (20 mM) and