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Antioxidant responses in soybean and alfalfa plants grown in DDTs contaminated soils: Useful variables for selecting plants for soil phytoremediation?

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ABSTRACT

Phytoremediation is a low-cost alternative technology based on the use of plants to remove pollutants from the environment. Persistent organic pollutants such as DDTs with a long half-life in soils are attractive candidates for remediation. This study aimed to determine the potential of antioxidant response use in the evaluation of plants' tolerance for selecting species in phytoremediation purposes. Alfalfa and soybean plants were grown in DDT contaminated soils. After 60 days, growth, protein content, antioxidant capacity, GST activity, concentration of proteic and non-proteic thiol groups, chlorophyll content and carotenoid content were measured in plant tissues. Results showed no effect on alfalfa or soybean photosynthetic pigments but different responses in the protein content, antioxidant capacity, GST activity and thiol groups on roots, stems and leaves, indicating that DDTs affected both species. Soybean showed higher susceptibility than alfalfa plants due to the lower antioxidant capacity and GST activity in leaves, in spite of having the lowest DDT accumulation. This study provides new insights into the role of oxidative stress as an important component of the plant's response to DDT exposure.

1. Introduction

The organochlorine pesticide dichlorodiphenyltrichloroethane (DDT) and its metabolites dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) belong to persistent organic pollutants (POPs), regulated by the Stockholm Convention and characterized by a long half-life, bioaccumulative behavior and ability to produce chronic adverse effects on humans and animals. DDT was widely used throughout the world to control arthropod disease-vectors and agricultural pests before it was banned. Consequently, the residues of DDT and metabolites are widely distributed in different environmental compartments [1,2]. Nowadays, DDT is still in use for malaria control in developing countries [3]. Considering the physico-chemical properties of DDTs (DDT + DDD + DDE) and their bioaccumulation potential, phytoremediation is a likely tool to clean soils contaminated by DDTs. This technique is defined as the use of green plants to remove pollutants from the environment or to render them harmless [4]. It has been well-demonstrated that some crops incorporate organochlorine pesticides from soil, depending on plant species, soil type and involved insecticide [5,6]. In this sense, previous studies showed that soybean and alfalfa plants grown in DDT polluted soils (500 ng g^{-1} dry weight) bioconcentrate pesticides in roots reaching values of 830 and 1120 ng g^{-1} dry weight of DDTs, respectively [7].

However, the extent of phytoremediation success is conditioned by two main factors: the pollutant availability that would have a direct consequence on the soil-root transfer [8], and the toxicity, that might limit the plant growth affecting uptake and translocation processes. Moreover, each plant species will also influence those processes by modifying the soil–root environment with root exudates and specific rhizospheric interactions as well as having different levels of tolerance towards the contaminants [9]. Identification and selection of suitable plants for pollutant removal from the environment require a broad knowledge of the physiological and biochemical features of the different plant species. Edwards [11] defined the xenome as "the biosystem responsible for the detection, transport and metabolism of xenobiotics within the plant tissues". Pollutants induce plant stress because they may elicit toxic effects by disrupting membrane integrity or metabolic pathways, making it necessary to safely sequester, extrude or detoxify the plants rapidly through biotransformation. During severe and persistent stress conditions, reactive oxygen species (ROS) accumulate

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causing several damages including membrane and protein modifications if they are not detoxified by cell mechanisms. The oxidative stress in several plant species is indicated by an enhancement of lipid peroxidation, protein oxidation and activation of the antioxidant system [11].

However, plants cells are equipped with both non-enzymatic antioxidants and enzymatic ROS scavengers to protect themselves from oxidative damage [13]. In classical oxidative stress studies, the variation of levels or activities of individual antioxidants is used to indicate ROS mediated toxicity. Particularly, GSTs are a family of very abundant and ubiquitous enzymes present in aerobic organisms that catalyze the conjugation of GSH to a wide variety of hydrophobic and electrophilic compounds to form less- or non-toxic derivatives [16]. This conjugation reaction is involved in the detoxification and processing of various xenobiotics, which after glutathionylation are rapidly transported to the vacuole [17]. The role of glutathione-S-transferase (GST) during various stress conditions in plants has been reported [15].

The redox state of thiol (SH) groups affects the activity and structure of many enzymes, receptors and transcription factors, and organisms maintain it in proteins and low-molecular-mass thiols with complex regulatory machinery [18]. Oxidation of cysteine SH groups can cause intermolecular protein cross-linking and enzyme inactivation, leading eventually to cell death. The protein S-thiolation is a process in which protein-SH groups form mixed disulfide with low-molecular-mass thiols such as GSH [19]. Moreover, it represents a post-translational modification that possesses an antioxidant role in the protection against irreversible oxidation, or may alternatively serve in a regulatory role, analogous to other post-translational modifications such as protein phosphorylation [20]. The measurements of a limited number of antioxidants do not consider that the antioxidant systems can act in a cooperative way [14]. Therefore, a more holistic determination of total antioxidant capacity will provide a better understanding of an organism's resistance to toxicity caused by ROS. Additionally, the determination of pigment concentration [12] has also been employed as a marker to assess plant damage by pollutant exposure.

The present study investigates the GST activity, total antioxidant capacity, and the concentration of proteic and non-proteic thiol groups as useful biomarkers for selecting plant species to remediate soils contaminated with DDTs.

2. Materials and methods

2.1. Plant growth

Plants were grown in rectangular pots of 6000 cm^3 filled with 1000 g of dry polluted soil (455.3 and 63.5 ng g^{-1} dry weight of DDE and DDT, respectively), obtained from a typical apple and peach field settled in Villa Regina city in the Upper Valley of the Rio Negro basin, Argentina (S 39°04.9′14″, W 67°02.9′59″) [21].

Seeds of *Glycine max* "soybean" (5) and *Medicago sativa* "alfalfa" (50) were placed in three separate pots and kept in a greenhouse at a temperature of 10–26 °C under natural sunlight (light:dark cycle 14:10 h). Planted control pots with non-polluted soil were also established. All pots were weeded on demand and watered weekly with tap water.

2.2. Plant sampling

Soybean and alfalfa plants were destructively harvested at 60 days after germination (appearance of the first true leaves). Roots, stems and leaves were separated and washed to remove attached soil particles. For soybean plants, roots, stems and leaves were obtained, while for alfalfa plants, due to the small size of each individual, the aerial tissues (stems + leaves) were pooled. Samples from each pot were composited and individually analyzed. All samples were kept in a freezer at −80 °C until biochemical analysis.

2.3. Tissue homogenization

For measurements of protein content, total antioxidant capacity, GST activity and proteic and non-proteic sulfhydryl groups, roots and aerial tissues were homogenization following the method described by Martinez-Dominguez et al. [22], with some modifications. Briefly, the tissues were prepared in liquid nitrogen and homogenized (1:2 *w*/*v*) in ice-cold 0.1 M sodium phosphate buffer containing 20% glycerol, 14 mM dithrothreitol (DTE), 1 mM phenylmethylsulfonylfluoride (PMSF) and 1 mM (ethane-1,2-diyldinitrilo) tetra acetic acid (EDTA) and adjusted pH to 6.5. All reagents were obtained from Sigma-Aldrich. Homogenates were centrifuged at 15,000 × *g* for 20 min (4 °C) and the supernatants were collected and stored at −80 °C for later use.

2.4. Protein determination

Protein concentration was assayed with bovine serum albumin (BSA; Sigma-Aldrich) as standard protein according to the Bradford method [23].

2.5. Determination of antioxidant capacity

Antioxidant capacity was assayed according to the method described by Amado et al. [24] which is based on the detection of ROS by fluorometry (ex/em: 485/520 nm). The assay was performed with some modifications of Vianna [25], which allows their use in samples with low protein content. Peroxyl radicals were generated in the analyzed samples by thermal decomposition at 37 °C of 2,2′-azobis(2-methylpropionamidine) dihydrochloride (ABAP, Sigma-Aldrich), resulting in the emission of a fluorescent signal caused by the reaction between ROS and 2′,7′-dichlorodihydrofluorescein (H_2DCF) probe, that resulted in the previous cleavage of 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA, Invitrogen) by alkaline hydrolysis for 30 min. The blanks were prepared with the buffer of homogenization and with and without ABAP or probe addition. The reaction buffer, containing 30 mM HEPES (pH 7.2), 200 mM KCl, and 1 mM $MgCl_2$, was added to the samples. Then, ABAP (10 mM) was added to three wells of each sample, while the same volume of ultrapure water (Milli-Q) was added to the three remaining wells. Immediately before the microplate reading, the hydrolyzed probe was added to the wells at a final concentration of 40 μM and lectures were performed in a fluorescence microplate reader (Victor2 D, Perkin Elmer, Waltham, MA, USA). The oxidation of non-fluorescent H_2DCF by the ROS generated by thermal decomposition of ABAP into a fluorescent compound (DCF) was detected at 485 (excitation) and 520 (emission) wavelengths (nm), every 5 min for 30 min.

Total fluorescence production was calculated according to Eq. (1), and the results were expressed in percentage of antioxidant capacity (%AC).

$$\%AC = (\Delta Blank - \Delta Sample)/\Delta Blank \times 100 \quad (1)$$

ΔBlank = NF Blank with ABAP − NF Blank without ABAP; ΔSample = NF Sample with ABAP − NF Sample without ABAP; NF (Net fluorescence) = AF with H2DCF − AF without H2DCF; AF = average fluorescence, calculated from each triplicate.

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

GST activity was based on methodology described by Habig and Jakoby [26] where the absorbance generated by the conjugation of 1 mM glutathione (GSH, Sigma-Aldrich) with 1 mM of 1-chloro-2,4-dinitrobenzene (CDNB, Sigma-Aldrich) was monitored at 340 nm during 1 min at 25 °C.