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Effect of lead on physiological and antioxidant responses in two *Vigna unguiculata* cultivars differing in Pb-accumulation

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HIGHLIGHTS

- Pb is distinctly accumulated in *Vigna unguiculata* cultivars.
- CAT and APX revealed peculiar involvement in the detoxification process.
- SV showed a better tolerance than SET to the stress caused by Pb accumulation.

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ABSTRACT

Lead (Pb) is one of the most toxic anthropogenic pollutants, occurring widely in both terrestrial and aquatic ecosystems, where it impairs plant growth and development. In this work, the effect of 0.5 mM EDTA-Pb was evaluated in two *Vigna unguiculata* cultivars (SV and SET), with the aim of detecting genotype/cultivar dependent changes in the physiological and anti-oxidant responses (CAT and APX) of a leguminous plant. The data showed that SV accumulated more Pb in roots while SET accumulated more in leaves, indicating differential regulation in Pb-translocation/accumulation. Lead affected the growth of SV less severely than SET, mainly associated with reduced inhibition in photosynthetic parameters. Furthermore, CAT and APX activities increased or were sustained at elevated levels in both cultivars in response to lead. However, gene expression analyses revealed that *CAT1* was the main lead responsive gene in SET while *CAT2* was more responsive in SV. *APX1* was higher expressed in tissues with higher Pb-accumulation while *APX2* was ubiquitously responsive to lead in both cultivars. Taken together, these results reveal differential ability of *V. unguiculata* cultivars in Pb-accumulation in different tissues affecting distinctly physiological and anti-oxidant responses. In addition, the existence of cultivars with predominant Pb-accumulation in aerial tissues invokes a need for studies to identify pollution-safe cultivars of leguminous plants to ensure food safety.

1. Introduction

Lead (Pb) is naturally present in small amounts in soils as a trace constituent of common rock-forming and readily weatherable minerals from the Earth's crust (Saifullah et al., 2009). However, in recent decades, anthropogenic activities have accelerated the release of various metals, including lead, into the environment, creating potential hazards to ecosystems and human health (Ashraf et al., 2011; Lantzy and Mackenzie, 1979; Nriago, 1979). Lead is not degradable in soil and has a toxic impact on plants even at low concentrations (Fahr et al., 2013). This toxicity is revealed in

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Abbreviations

Pb	Lead
APX	Ascorbate peroxidase
CAT	Catalase
ROS	Reactive oxygen species
SET	Setentão
SV	Sempre Verde
HNO_3	Nitric acid
HCl	hydrochloric acid
HF	hydrofluoric acid

alterations to metabolic pathways such as photosynthesis, respiration, and other processes involving the imbalance of specific cellular enzyme activities (Dixit et al., 2001; Erdei et al., 2002; Ruley et al., 2004). Plant responses to lead toxicity depend on the genotype and physiological characteristics, thus there are different defense strategies against its harmful effects (Gupta et al., 2013). The first strategy is to avoid metal entry into the cell by excluding it or binding it to the cell wall. However, lead mostly penetrates into the plant through its roots, remaining there or being translocated to shoots.

Once the metal has been taken up, one plant defense mechanism consists of an enzymatic antioxidant system to overcome ROS production induced by metals (Mishra et al., 2006a). Among the antioxidant enzymes, catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.11) are the key enzymes to detoxify H_2O_2. Both enzymes have several isoforms that are expressed in different cellular organelles, and the number of genes is species dependent. CAT has a high reaction rate but a low affinity for H_2O_2 whereas APX has a high affinity for H_2O_2 rendering this enzyme as a special candidate in the management of ROS during stress (Gill and Tuteja, 2010).

CAT and APX activities have been evaluated in different tissues and species revealing variable responses to lead stress. For CAT, increases in leaves and roots were observed in leguminous plants as *Macrotyloma uniflorum* and *Cicer arietinum* (Reddy et al., 2005). However, in rice this activity decreased in both tissues under lead stress (Verma and Dubey, 2003). In some species CAT response was dependent of Pb content, i. e., in wheat roots, CAT activity increased under 1.5 mM and decreased under 3 mM Pb (Lamhamdi et al., 2011). Similar results were found to *Jatropha curcas* leaves (Shu et al., 2012). High Pb content also inhibited CAT activity in *Vicia faba* roots. In Arabidopsis, CAT activity in leaves was higher than in roots and seeds (Mhamdi et al., 2012) and this activity appeared linked to high expression levels of *CAT2* gene in leaves under oxidative stress (Mhamdi et al., 2012). For APX, variable activities were observed in *Ceratophyllum demerssum*, which APX activity increased and decreased under lower and higher Pb concentrations, respectively (Mishra et al., 2006b). However, in *Talinum triangulare* roots APX increases were detected under different $Pb(NO_3)_2$ concentrations reaching the highest values at 0.5 mM (Kumar et al., 2013). In the leguminous *Vicia faba*, APX activity increases were also observed under Pb stress (Shahid et al., 2014).

Few studies on Pb effect are still available for leguminous plants and the responses of different genotypes/cultivars to this metal remain to be elucidated. Thus, in the present work, two cultivars of *Vigna unguiculata* (L.) Walp. (cowpea), Sempre Verde (SV) and Setentão (SET) were evaluated to gain insight on genotype/cultivar dependent lead effects revealed by their physiological and antioxidant responses.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of two cowpea (*Vigna unguiculata* L. Walp.) cultivars, Setentão (SET) and Sempre Verde (SV), were provided by the seed bank of the Center for Agricultural Sciences, Federal University of Ceara, Brazil. They were germinated in vermiculite under sterile conditions, and daily irrigated with distilled water for seven days. The seedlings were then transferred to plastic containers (12 L) for hydroponic cultivation, in half strength Hoagland solution (Hoagland and Arnon, 1950). After seven days of growth in the hydroponic system these plants were supplemented or not with 0.5 mM EDTA-Pb. Plants were harvested for analysis seven days after treatment. The whole experiment was kept in a greenhouse at 28.3 ± 0.62 °C, 75.0 ± 1.9% humidity and 1200 μmol s-1 m-2 photosynthetically active radiation (PAR). It is important to highlight that preliminary experiments were conducted with $Pb(NO_3)_2$ and EDTA-Pb to determine the best Pb source and concentration to be applied to *V. unguiculata* cultivars.

2.2. Plant growth and physiological parameters

Roots were dried in a forced air circulation oven at 60 °C for five days and weighed to determine the root dry mass (RDM). Leaf area was determined using a Li − 3100 Area Meter (Li-cor Inc. Lincoln, Nebraska EUA). The physiological parameters: photosynthetic rate (A), stomatal conductance (gs), internal CO_2 concentration (Ci), electron transport (ETR) and efficiency in PSII (ϕ) were analyzed in the untreated and treated groups using a portable infrared gas analyzer (IRGA, ADC System − Hoddesdon, UK).

2.3. Lead content

Six dried plants from each treatment (control and EDTA-Pb) were pulverized with a mortar and pestle and the powders were divided to form two biological replicates. Each sample (0.2 g) was then incubated with 5.0 mL of a mixture of 65% HNO_3 and 37% HCl (3:1 v/v) for 12 h at room temperature (25 °C), and the digestion was performed in a digestion block (Tecnal TE 015, Brazil) at 120 °C for 3 h. After cooling, 4 mL of 40% HF was added and the mixture was further digested for 3 h. The samples were allowed to cool to room temperature and deionized water was added to a final volume of 25 mL. Lead content was determined by inductively coupled plasma optical emission spectrometry (ICP OES, Perkin Elmer 4300 DV). The lead content was calculated on a dry weight basis for the roots and leaves.

2.4. Enzyme assays

Leaves and roots from control and treated plants (150 mg) frozen in liquid nitrogen were ground to a fine powder and separately homogenized in 1.5 mL of cold 100 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA for catalase (CAT) and 0.1 mM EDTA and 1.0 mM ascorbate for ascorbate peroxidase (APX) assays (Nakano and Asada, 1981). The homogenate was centrifuged at 12,000*g* for 30 min at 4 °C and the supernatant was used as the crude extract for the enzymatic activity assays. Preliminary experiments were performed and the influence of protease inhibitors on the activity was less than 1%; thus, no inhibitor was added to the extraction medium. An aliquot was used to determine the total soluble protein content according to Bradford (1976), using bovine serum albumin as standard.

CAT (EC 1.11.1.6) activity was measured as described by Havir and McHale (1987) with minor modifications. Decrease in H_2O_2