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Research article

Alterations in growth, oxidative damage, and metal uptake of five aromatic rice cultivars under lead toxicity

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ABSTRACT

Lead (Pb) affects plant growth and its related physio-biochemical functions negatively. The present study investigated the responses of five different fragrant rice cultivars viz., Meixiangzhan (MXZ-2), Xiangyaxiangzhan (XYXZ), Guixiangzhan (GXZ), Basmati-385 (B-385), and Nongxiang-18 (NX-18) to four different Pb concentrations viz., 0, 400, 800 and 1200 μM. Results depicted that Pb toxicity significantly ($P < 0.05$) reduced the plant height, tillering ability and biomass accumulation by causing oxidative damage to rice plants; nonetheless, a significant variation was found in the sensitivity of rice cultivars to Pb toxicity. Soluble sugars increased significantly only at 1200 μM in GXZ and 800 μM in B-385, whilst the maximum reductions in protein contents were observed at 1200 μM Pb for all rice cultivars. Proline contents were reduced for XYXZ and NX-18 at Pb1200 μM. Activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) as well as reduced glutathione (GSH) and oxidized glutathione (GSSG) showed differential behavior among Pb treatments and rice cultivars. Among rice cultivars, GXZ showed better antioxidative defense system under Pb toxicity compared with all other cultivars. For all rice cultivars, the trend for Pb accumulation was recorded as: roots > stems > leaves. Furthermore, significant but negative correlations among Pb uptake and plant height ($r = -0.79$), tillers per plant ($r = -0.91$) and plant dry biomass ($r = -0.81$) were recorded for all rice cultivars whereas the values of translocation factor (TF) from stems to leaves were higher than roots to stems. In sum, Pb reduced the early growth and caused physio-biochemical changes in all rice cultivars, nonetheless, GXZ proved better able to tolerate Pb stress than all other rice cultivars under study.

1. Introduction

Heavy metal pollution is becoming a serious problem for agricultural lands and a big threat for sustainability of agro-ecosystems (Ashraf et al., 2015; Anjum et al., 2016a, b; Wang et al., 2017). Inevitable industrialization and rapid urbanization are pressing the cultivated lands and deteriorating the soil quality (Wang and Chen, 2009). Transfer of heavy metals to human body by consuming contaminated food products/eatables may cause serious health problems in humans (Hu et al., 2011; Chaney et al., 2004). Lead (Pb) is one of the most hazardous heavy metals and ranked second after arsenic due to its potential toxicity and occurrence worldwide (Agency for Toxic Substances and Disease Registry, 2003).

Pb toxicity reduces plant growth and development by altering physio-biochemical functions (Islam et al., 2007). Exposure of Pb generally leads to excessive production of reactive oxygen species (ROS) that oxidizes numerous biological proteins and may even

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Abbreviations: APX, ascorbate peroxidase; CAT, catalase; DOE, days of exposure; GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde; POD, peroxidase; SOD, superoxide dismutase.

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cause cell death (Clemens, 2006). Cations of Pb react with cell plasma membrane and enhance the production of unsaturated fatty acids in plants (Singh et al., 2010). Cytosolic osmolyte accumulation and/or osmoregulation are important phenomena in plants to protect the cellular structures from ROS. In addition, plants have also developed an efficient enzymatic antioxidative defense system which may directly quench ROS or indirectly by producing non-enzymatic antioxidants (Mittler, 2002; Ali et al., 2014a). Variations in the levels/activities of antioxidants due to Pb-toxicity have been well reported in the previous studies (Mishra and Choudhary, 1998; Ali et al., 2014a); however, maintaining higher levels/activities of these antioxidants often ensure the Pb-tolerance in rice (Verma and Dubey, 2003).

Although toxic levels of Pb in the soil for plants are difficult to establish but soils containing 100–500 ppm of Pb are often recognized as excessive Pb contaminated soils (Kabata-Pendias and Pendias, 1984). Pb transportation from soil to plants is an important factor to decide either a specific Pb concentration is toxic for a plant or not. Different plant species showed differential behavior regarding Pb uptake and transport; however their responses were found concentration dependent and genotype specific (Yoon et al., 2006). Furthermore, the tolerance and sensitivity indices of a specific rice cultivar are associated with the root Pb storage ability and its transportation and/or distribution in above ground plant parts (Rout et al., 2001). Developing rice cultivars with improved Pb storage ability within roots and minimum translocation toward other plant parts may be a better option to grow rice in Pb tainted soils (Ashraf et al., 2015).

Aromatic rice constitutes a small but best quality rice group and globally famous due to its special aroma and unique flavor (Bryant and McClung, 2010). It is mainly produced and consumed in Asian and Middle East countries and is now being exported worldwide; however, expanding Pb contamination in agricultural lands may affect its growth severely. Pb is recently listed as “the chemical of great concern” according to the new European Registration, Evaluation, and Authorization of Chemical Substances (REACH) regulations (Pourrut et al., 2011). Negative effects of Pb toxicity in rice have been studied previously (Verma and Dubey, 2003; Liu et al., 2013); however, studies about Pb toxicity in aromatic rice at early growth stages are very few. Therefore, the present study was conducted to assess the Pb-induced changes in early growth, physio-biochemical responses and Pb translocation in five different fragrant rice cultivars in order to estimate the basis of Pb sensitivity and tolerance in scented rice.

2. Materials and methods

A pot experiment was conducted during April–May 2015 in rain-protected wire-house at the Experimental Research Farm, College of Agriculture, South China Agricultural University, Guangzhou (23°09′ N, 113°22′ E and 11 m above the sea level), China. This region has a humid subtropical, monsoon type of climate characterized by warm winters and hot summers with yearly average temperature range lies between 21 and 29 °C with 77–89% relative humidity (Li et al., 2016; Mo et al., 2016).

2.1. Experimental soil and plant material

For pot filling, the upper soil layer (0–30 cm) was collected from an uncontaminated paddy field. The experimental soil was sandy-loam containing 22.83 g kg^{-1} organic matter, 1.195 g kg^{-1} total N, 0.958 g kg^{-1} total P, 18.41 g kg^{-1} total K, 90.34 mg kg^{-1} available N, 9.30 mg kg^{-1} available P, 131.32 mg kg^{-1} available K, and 38.07 mg Pb kg^{-1} of soil. The pH of the soil was 5.79 (moderately acidic). Each pot (32 cm in diameter and 29 cm in height) contained 9 kg of soil.

Seeds of five different aromatic rice cultivars i.e., Meixiangzhan (MXZ-2), Xiangyaxiangzhan (XYXZ), Guixiangzhan (GXZ), Basmati-385 (B-385), and Nongxiang-18 (NX-18) were obtained from College of Agriculture, South China Agricultural University Guangzhou, China. These rice cultivars are well recognized fragrant rice cultivars and widely grown in South China. Before sowing, seeds were soaked in water for 24 h and allowed to germinate in dark chamber at 28–30°C for next 24 h. Well germinated and undamaged seeds were sown during 2nd week of March for rice nursery. One month old rice seedlings were transplanted to soil-filled plastic pots with 3–4 seedlings per hill and 5 hills per pot. A water layer of 2–3 cm was maintained in all the pots till harvest.

2.2. Pb-treatment application

For Pb-contamination, Pb $(NO_3)_2$ was dissolved in deionized water to make 400, 800 and 1200 μM Pb concentrations. Three weeks before nursery transplantation, the respective solutions were poured into the pots carefully and mixed uniformly. The uncontaminated pots (0 μM of Pb) were recognized as control. These Pb concentrations are quite higher than the actual Pb levels in agricultural soils, just to get a clear understanding of the distribution of Pb within different plant parts and to assess tolerance ability of the studied rice cultivars in relation to physio-biochemical traits. Furthermore, the choice of these Pb concentrations represents low, moderate and high Pb level.

2.3. Data collection and measurements

All plants were sampled after 30 days of exposure (DOE) to different Pb concentrations. A meter scale was used to measure the plant height at 30 DOE, while total number of tillers were counted of each plant in triplicate and averaged. For Pb-determination and dry biomass accumulation, plants were separated into roots, stems and leaves and placed in oven at 80 °C till constant weight. All plant parts were weighed with a digital electrical balance (BSA224S, Sartorius, Japan) and added up to record plant aboveground dry biomass. For biochemical analyses, sampled plants were washed thoroughly with tap water and then two times with distilled water, and leaves were carefully separated and stored at −80 °C.

2.3.1. Biochemical assays

The malondialdehyde (MDA) content was measured according to Campos et al. (2003). The absorbance of the reaction solutions was read at 532 nm, 600 nm, and 450 nm. The MDA content of the reaction solutions was calculated as follows:

$$\text{MDA content}\ \ (\mu\text{mol L} - 1) = 6.45(\text{OD532}{-}\text{OD600}){-}0.56\text{OD450}$$

The final MDA contents were expressed as μmol g^{-1} fresh weight (FW). To determine hydrogen peroxide (H_2O_2), fresh leaf samples (0.2 g) were homogenized with 1 ml of 0.1% trichloroacetic acid (TCA) and the homogenate was centrifuged at 8000 *rpm* for 15 min (Velikova et al., 2000). The reaction mixture contained 0.5 ml of the potassium phosphate buffer (pH 7.0), 1 ml of 1 M potassium iodide (KI) and 0.5 ml aliquot of the supernatant. The absorbance was read at 390 nm and the final contents were expressed as μmol g^{-1} FW. For the estimation of electrolyte leakage (EL), fresh leaf samples (0.2 g) were washed with distilled water and placed in closed vials containing 10 ml deionized water and incubated at 25 °C for 6 h and first electrical conductivity (EC_1) was recorded. Then samples were incubated at 90 °C for 2 h and second EC (EC_2) was recorded after cooling down at 25 °C. Electrolyte leakage (EL) was determined as the percent ratio of EC_1 and EC_2.