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Parameter determination involved in phytotoxicity and transport of cadmium in rice seedlings

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ABSTRACT

Hydroponic experiments were conducted with rice seedlings to determine parameters involved in phytotoxicity and transport of Cadmium (Cd). Results showed that a linear decrease in relative growth rate and water use efficiency was observed in rice seedlings with increasing Cd concentrations. More severe inhibitory effects on both parameters were found at the 4-d treatment. The effective concentrations (EC) were estimated using the Levenberg–Marquardt Algorithm. The EC values obtained from the relative growth rate were always smaller than these from water use efficiency, indicating that the former was more sensitive to change of Cd than the latter. Phyto-transport of Cd was apparent, but the distribution of Cd in different parts of rice seedlings was different and roots were the dominant sink for Cd accumulation ($p < 0.05$). Although the translocation of Cd into shoots was detectable, more Cd was detected in shoots at the 4-d treatments than that at the 2-d treatments ($p < 0.05$). The partial correlation analysis suggested that the total accumulation rate of Cd was highly related to the doses of Cd supplied ($p < 0.05$). In conclusion, Cd is problematic at relatively low concentrations for rice seedlings, and inhibitory effects are highly dependent on the selected response endpoints and the duration of exposure periods.

Introduction

The main source of cadmium (Cd) into the environment is anthropogenic (Žaltauskaitė and Sodienė, 2014), which has imposed a long-term risk on environmental and human health (Wong et al., 2002). In mainland China, the rapid industrial development and population expansion, coupled with the lack of pollution controls, has resulted in a substantial increase in heavy metal contamination of agricultural soils (Liu et al., 2005). It has been estimated that around 200,000 hm^2 of these agricultural soils polluted with Cd, which has become principal concerns for agriculture activities (Hu, 2004). For instance, the mean Cd concentration of 2.1 mg kg^{-1} in agricultural soils has been reported in Guangdong Province, China (Li et al., 2003; Yu et al., 2006a, b). Soils are environmental matrices that can transfer Cd to plants. It has been reported that more than 1.46×10^8 kg agricultural products was contaminated with Cd yearly and the average content of Cd in rice grains was detected to be 0.45 mg kg^{-1} (Li et al., 2003; Chen et al., 2013), which exceeds the maximum allowable limit (0.2 mg kg^{-1}) of the National Food Hygiene Standard of China (Yu et al., 2006a, b). Therefore, the total annual economic damage of agricultural products due to Cd contamination was estimated to be at least 20 billion of CNY in China (Chen et al., 2013).

Cd is a non-essential element for plants and therefore phytotoxic, even at low concentrations. It can interfere with photosynthetic and respiratory activities, mineral nutrition, enzymatic activities, membrane functions, and hormone balance (Chien and Kao, 2000; Liu et al., 2003). Indeed, a number of toxic symptoms such as reduction/inhibition of plant growth and transpiration, chlorosis in young leaves, induction/inhibition of antioxidant enzymes, disturbance of nutrient balance, altered stomatal function, wilting of tops and root injury have been observed frequently as endpoints for phytotoxicity determination (Carrier et al., 2003; Scoccianti et al., 2006). The uptake and translocation of Cd in different species of plants has been intensively reported, but the majority with specific emphasis on screening hyperaccumulator species. Recently, a growing public concern regarding the food safety issue of agricultural crops produced from the Cd-contaminated soils has been received considerable attention in China. Rice is the staple food for much of the world, second only to

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wheat in its importance as a food cereal in the human diet, especially in Asia (Yu et al., 2006a). Here, interests have been generated in using rice seedlings as testing materials to determine parameters involved in phytotoxicity and transport of Cd in plants.

Toxic effects of chemicals on plants can be measured in different ways. Trapp et al. (2000) specially designed an acute hydroponic phytotoxicity for chemicals using growth, transpiration and water use efficiency to evaluate the impacts of pollutants to plants. Stress reduces water use efficiency (Larcher, 1995), and change in water use efficiency can be used as an indicator for chemical stresses to the trees on a sublethal level (Trapp et al., 2000). We aimed to quantify the effective concentrations (EC) based on the two response parameters relative growth rate and water use efficiency of rice seedlings exposed to Cd hydroponically, using the Levenberg–Marquardt Algorithm at different time intervals. Additional experiments were also conducted to clarify the distribution and translocation of Cd in rice seedlings.

Materials and methods

Test chemicals and experiment design

Plant materials and exposure regime were identical to our previous work (Yu and Zhang, 2013). Fifteen-day-old rice seedlings (*Oryza sativa* L. cv. XZX 45) with similar height and weight were transplanted to a pre-treatment solution containing 1 mM $CaCl_2$ + 2 mM MES-Tris buffer (pH 6.0) for 4 h to clear the ions from cell wall space (Ebbs et al., 2008), and then ten rice seedlings were transferred into a 50 ml Erlenmeyer flask filled with 50 ml modified ISO 8692 nutrient solution (Yu and Zhang, 2013) with addition of 10 μM Fe-EDTA. The plants were first conditioned for 24 h to allow adaptation to the new environmental conditions. The flasks were all wrapped with aluminum foil up to the flask mouth to prevent escape of water, and to inhibit potential growth of algae inside. All flasks were housed in a plant growth chamber with constant temperature of 25 ± 0.5 °C and a relative humidity of 60 ± 2% under continuous artificial light (illumination intensity: 20,000 lux). Then, the nutrient solution in each flask was replaced by respective spiked solution, except control.

Cadmium nitrate ($Cd(NO_3)_2$, CAS No.10325-94-7, 99.5% purity) were purchased from Sinopharm Chemical Reagent Co., Ltd. Shanghai, PR China. Eight different concentrations were used. Mean measured initial concentrations of Cd in treatments spiked with $Cd(NO_3)_2$: 0, 0.64(SD:0.13), 1.29(SD:0.36), 2.57(SD:0.29), 5.14(SD:0.62), 10.28(SD:1.21), 12.85(SD:1.09) and 15.42(SD:1.27) mg Cd/l. Each treatment concentration was conducted in four independent biological replicates. Two test series (48-h and 96-h exposure period) were conducted.

Chemical analysis

Plant materials from treated and non-treated rice seedlings were collected at the termination of experiments and rinsed with deionized water. Plant materials were dried at 90 °C for 48 h and mixed with 10 ml of 4:1HNO_3–$HClO_4$ solution for overnight. Then, samples were placed in a digestion block and heated for 2 h at 200 °C until the digested liquid was clear. The cooled residue was dissolved in 2 ml of 1% HNO_3 and deionized water was added up to 50 ml of total volume (Banks et al., 2006). The content of Cd in different parts of plant materials was analyzed by inductively-coupled plasma atomic emission spectrometry (ICP-AES). The initial concentrations of Cd in spiked solutions were also determined.

Relative growth rate

Rice seedlings were weighed prior to application and at termination of exposure. The relative growth rate (RGR, %) was calculated using the formula

$$\mathrm{RGR} = \frac{M_{(F)} - M_{(I)}}{M_{(I)}} \times 100$$

where $M_{(I)}$ and $M_{(F)}$ are the initial and final weight (g) of rice seedlings, respectively.

Water use efficiency

Water use efficiency (WUE) (mg biomass/ml water) is the ratio between produced biomass and water transpired (Trapp et al., 2000).

$$\mathrm{WUE} = \frac{M_{(F)} - M_{(I)}}{V_{\mathrm{transpired}}}$$

where $M_{(I)}$ and $M_{(F)}$ are the initial and final weight (mg) of rice seedlings. $V_{\mathrm{transpired}}$ is the transpiration (ml water) of seedlings calculated by the weight loss of the plant-flask system.

Inhibition rate

Percent inhibition rate (IR, %) on each parameter was calculated using the equation (Trapp et al., 2000) with modification.

$$IR_{(C,t)} = \left(1 - \frac{{}^{1}/_{n}\sum_{i=1}^{n}\mu_{(C,t)}}{{}^{1}/_{m}\sum_{j=1}^{m}\mu_{(O,t)}}\right) \times 100$$

where *C* is concentration (mg Cd/l), *t* is time period (d), μ is different measured parameter, *i* is replicate 1, 2,…, *n* and *j* is control 1, 2,…, *m*.

Effective concentration

Effective concentration (EC) was determined by measurements of acute toxicity using different parameters of treated plants. The EC values at the respective time intervals were estimated by Levenberg–Marquardt Algorithm with 95% confidence intervals using Logistic Model of Origin v. 9.0, which is a commonly used program designed for logistic dose response in Chemistry.

$$f(x) = A_2 + \frac{A_1 - A_2}{1 + \left(\frac{x}{x_0}\right)^P}$$

where A_1 is the initial value, A_2 is the final value, X_0 is the central value for EC of the does-response curve, and *P* is the slope of dose-response curve; *f(x)* is the function of chemical concentration *x*, here it refers to percent inhibition rate for each selected parameter.

Total accumulation rate

The total accumulation rate (TAR, μg Cd/gDW/d) was calculated from final mass accumulated in different parts of plant materials using the formula (Zhu et al., 1999) with slight modification