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Selenium reduces cadmium uptake into rice suspension cells by regulating the expression of lignin synthesis and cadmium-related genes

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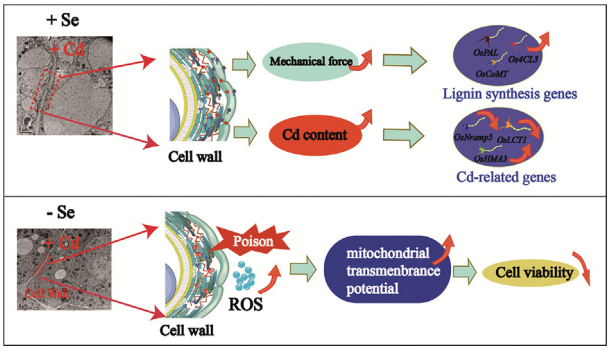
HIGHLIGHTS

- Se could alleviate cadmium toxicity in rice suspension cells.
- Cd was mainly accumulated onto the cell walls by the addition of Se.
- Se improves the mechanical force of the cell walls.
- Se regulates the expression of lignin synthesis and cadmium-related genes.

GRAPHICAL ABSTRACT



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ABSTRACT

Although previous studies have indicated that selenium (Se) can reduce cadmium (Cd) uptake into rice, the mechanism at the cellular level has not been reported. Here, rice suspension cells exposed to Cd treatment in the presence or absence of Se were characterized. Compared with treatment with alone, pretreatment with Se increased the proportion of live cells by 83.1%. The levels of reactive oxygen species and mitochondrial membrane potential in the Se-pretreated rice cells were decreased by 86.6% and 76.0%, respectively. In addition, non-invasive micro-test technology suggested that the mean values of Cd^{2+} influx decreased significantly in the Se-pretreated rice cells in a concentration-dependent manner. The results of inductively coupled plasma-mass spectrometry (ICP-MS) showed that 67.4%–78.8% Cd accumulated onto the cell walls of the pretreated-Se rice cells. The addition of Se increased the lignin content and thickness of the cell walls, leading to an improved mechanical force of the cell walls, as determined by atomic force microscopy (AFM). Furthermore, Se pretreatment decreased the expression of genes involved in Cd uptake (*OsNramp5*) and transport (*OsLCT1*) but activated the expression of genes involved in Cd transport into vacuoles (*OsHMA3*) and lignin synthesis (*OsPAL*, *OsCoMT* and *Os4CL3*). These results indicated that supplying Se alleviates Cd toxicity by regulating the express of lignin synthesis and Cd-related genes. The present findings provide new insights on a plausible explanation of the Se-reduced Cd uptake into rice.

1. Introduction

Cadmium (Cd) is one of the most harmful and widespread pollutants in agricultural soils (Liu et al., 2015). The annual release of Cd into the water and soil reached 22,000 tons by the 1990s (Liu et al., 2007).

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More than 13,330 ha of farmland are reportedly contaminated with Cd in China (Chen et al., 2007). Rice is a staple food crop for southeast Asian countries. Cd is easily taken up by rice in Cd-polluted paddy soil due to the high mobility and hydrophilic properties of Cd (Rizwan et al., 2016). Cd-polluted rice accumulation in humans through the food chain may cause a number of diseases (Templeton and Liu, 2010). For example, a typical type of Cd poisoning caused by environmental Cd exposure is Itai-itai disease in Japan (Ogawa et al., 2004). Therefore, preventing adverse health effects of Cd-polluted soil and alleviating the toxicity of Cd in rice have become important public health problem of food safety.

The supply of different elements can influence the uptake and distribution of Cd and eventually reduce Cd accumulation in rice grains (Shao et al., 2017; Wan et al., 2016). For example, Cao et al. suggested that sulfur supply decreases Cd accumulation in rice grains by enhancing Fe plaque formation on the root surfaces (Cao et al., 2018). Shi et al. found that the application of silicon reduces Cd concentration in rice shoots by 24% (Shi et al., 2005). Silicate effectively reduces the uptake of Cd into the root and shoot of wheat and protoplasts (Greger et al., 2016). We also previously demonstrated that supplying silica hydrosol obviously reduces the Cd concentration in the grains and shoots of rice (Liu et al., 2009). Cd^{2+} can enter rice through Cd transporters or via other cation channels in the plasma membrane (Hart et al., 1998). Several transporters involved in Cd uptake and transport have been identified. A low-affinity cation transporter gene (*OsLCT1*) encodes Cd transport between the grains and phloem (Uraguchi et al., 2011). As a member of the natural resistance-associated macrophage protein (*Nramp*) family, *OsNramp5* plays a role in the transport of Cd from the external solution to root cells (Uraguchi and Fujiwara, 2013). Heavy metal ATPase 3 (*OsHMA3*) is involved in the transport of Cd into the vacuole (Sasaki et al., 2014). Our previous study demonstrated that the silica-induced alleviation of Cd toxicity could be attributed to the down-regulation of Cd uptake and transport gene expression (Cui et al., 2017). In addition, the cell wall is directly in contact with Cd and represents the outermost layer of protection of the cell against Cd toxicity. Shi et al. demonstrated that a new form of organ silicon was present in the cell walls of rice plants and could enhance cell wall stability (Shi et al., 2005).

Selenium (Se) is an essential micronutrient for humans and other animals. Se plays an important role in antioxidant function, immune responses and the alleviation of the toxicity of Cd (Jihen et al., 2009). The biochemical effect of Se on Cd stress has been widely investigated in plants (Khan et al., 2015). For example, Lin et al. reported that exogenous Se could significantly reduce Cd concentration in rice by activating protective mechanisms that can alleviate oxidative stress (Lin et al., 2012). Saidi et al. found that the protective role of Se may be associated with a decrease in lipid peroxidation, an improvement of the scavenging capability for reactive oxide stress, and a decrease in Cd uptake, transport and distribution in plant tissues (Saidi et al., 2014). Other evidence suggests that the addition of Se could enhance the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and maintenance of the integrity of the cell membrane under Cd stress (Filek et al., 2010). Cd and Se are reportedly taken up by different transporters (L.H. Zhang et al., 2014). Huang et al. suggested that Se application can significantly reduce the translocation and accumulation of Cd and that Cd affects Se uptake but not translocation (Huang et al., 2017). Moreover, Se has a strong ability to combine with Cd to form a complex (Shanker et al., 1996). Wan et al. also demonstrated that supplying Se could effectively reduce the translocation of Cd from roots to shoots in rice seedlings (Wan et al., 2016). These studies have indicated that the application of Se-enriched fertilizer represents a simple, practicable and effective strategy to reduce the accumulation of Cd in plants.

Despite Se could effectively reduce the uptake of Cd at the whole-plant level, the underlying mechanisms have not been investigated at the single-cell level. Recently, Si-Cd interactions were investigated at the cellular level in rice suspension cells. The results indicated that the hemicellulose-bound form of Si is present in the cell walls and prevents the cellular uptake of Cd (Ma et al., 2015). The use of individual rice cells avoids the complicated environment of whole plants or tissues and provides a new way to elucidate the mechanisms involved in reduce the uptake of Cd in the rice by the addition of Se. In the present study, a series of experiments were conducted using a combination of plant cell nutritional, molecular biological and physical techniques. These findings will facilitate a better understanding of how Se inhibits Cd uptake and transport at the single-cell level and provide new insights for developing effective fertilizers to reduce the accumulation of Cd in the rice.

2. Materials and methods

2.1. Cell viability assay and the level of reactive oxygen species

Rice suspension cells (*Oryza sativa L. japonica*) were cultivated by the reported method (Liu et al., 2013; Thomas et al., 1989). Rice seeds were washed with water, sterilized using ethanol (75%) and mercuric chloride (0.1%) for 10 min, and then cultured at 28 °C. After incubation of the seeds for 1 month in the dark, the formed calli were transferred to a glass flask, and rice liquid medium was added, followed by incubation of the flask at 28 °C in the dark. The suspension cells in exponential growth phase were collected and transferred to plastic flasks after 5 days. The levels of reactive oxygen species (ROS) were detected by the fluorescence probe DCFH-DA. Sodium selenite (Na_2SeO_3) and Cd^{2+} ($CdCl_2$) were used in the study. Briefly, the rice cells were incubated in the absence or presence of 5 μM Se for 24 h. Then, 40 μM Cd was added to the cell culture medium, and the rice cells were incubated for an additional 24 h in the shaker (pH 5.6). The cells were transferred to a cell culture dish and then stained with 10 μM DCFH-DA at room temperature for 30 min in the dark. Finally, the stained cells were collected and washed three times with ultra-high purity water, re-suspended in phosphate-buffered saline (PBS, pH = 7.2), and immediately detected by flow cytometry (Becton Dickinson, CA, USA). The excitation and emission wavelengths were set at 485 nm and 530 nm, respectively (Saquib et al., 2012). The raw data were analyzed by CELLQuest software (Becton Dickinson, CA, USA). The detailed procedure for cell viability assay and mitochondrial transmembrane potential (MMP) is described in the Supporting information (S1.1–1.2).

2.2. Measurements of Cd^{2+} uptake and subcellular distribution

To detect the uptake of Cd into the rice suspension cells with the pretreatment of Se, Cd^{2+} flux was investigated using noninvasive microtesting (NMT). Details method of Cd^{2+} flux and cell wall extraction are presented in the Supporting information (S1.3–1.4). The Cd contents of the whole cell and cell wall were measured. First, the collected samples were washed thoroughly with ultra-high purity water by centrifugation and then dried. 2 mL of 30% HNO_3 and 3 mL of 2% H_2O_2 were added into a Teflon bottle containing 0.05 g of sample. Heat treatment (80 °C for 3 h, 160 °C for 5 h) was performed in a microwave oven. Finally, the concentration of Cd in the digestate was determined by an Agilent 7700 inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies, CA, USA). Each sample was measured three times (Ma et al., 2015).

2.3. Mechanical properties of the cell wall

Detail experimental procedure of the lignin content is presented in the Supporting information (S1.5). The obtained rice cell walls were suspended in water and placed onto glass slides. Then, the specimens were dried at room temperature and 30% relative humidity. AFM is a powerful tool for the evaluation of the stiffness of cell wall material extracted from plants (Sirghi et al., 2008). In this study, we applied AFM to directly measure the mechanical properties of the cell walls of Se-pretreated or untreated cells. The scheme of the experiment is presented in Fig. 4A. First, we randomly selected the cell wall material