Foliar spraying of melatonin confers cadmium tolerance in *Nicotiana tabacum* L

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**ABSTRACT**

Melatonin is a multifunctional signaling molecule that regulates broad aspects of responses to environmental stresses in plants. Cadmium (Cd) is a persistent soil contaminant that is toxic to all living organisms. Recent reports have uncovered the protective role of melatonin in alleviating Cd phytotoxicity, but little is known about its regulatory mechanisms in plants. In this study, we found that foliar application of melatonin (in particular 100 μmol L^{-1}) remarkably enhanced Cd tolerance of tobacco (*Nicotiana tabacum* L.) leaves, as evidenced by less Cd accumulation and alleviation of growth inhibition and photooxidation, compared with nontreated Cd-stressed plants. The addition of melatonin also controlled oxidative damage of Cd on tobacco through direct scavenging and by enhancing the activities of antioxidative enzymes. Melatonin application promoted Cd sequestration in the cell wall and vacuoles based on the analysis of subcellular distribution of Cd in tobacco cells. Structural equation modeling (SEM) analysis revealed that melatonin-induced Cd tolerance in tobacco leaves was modulated by the expression of Cd-transport genes. Molecular evidence illustrated that modulation of *IRT1*, *Nramp1*, *HMA2*, *HMA4*, and *HMA3* genes caused by melatonin could be responsible for weakening Cd uptake, Cd transportation to xylem, and intensifying Cd sequestration into the root vacuoles.

1. Introduction

Tobacco is a nice model plant for scientific experiments and production of beneficial compounds (Regassa and Chandravanshi, 2016; Feng et al., 2018). Tobacco also produces a large amount of biomass and accumulates relatively more Cd (Rubio et al., 2015). More than 50% of total Cd that has been taken up by tobacco is concentrated in the leaves (Rosén et al., 2012; Liu et al., 2016). Cd accumulation in tobacco leaves could significantly degrade the yield and negatively affect its quality, because Cd disturbs the balance of nitrogen, nicotine, and carbohydrate contents, and makes the tobacco taste worse (Liu et al., 2015). Long-term intake of Cd could exacerbate serious, chronic health problems for smokers, such as kidney damage, bladder, and lung cancer; these problems could also be intensified in nonsmokers through secondhand smoke (Regassa and Chandravanshi, 2016; Zaprianova et al., 2010; Wang et al., 2017). Therefore, increasing Cd tolerance or reducing Cd accumulation in tobacco leaves are ongoing endeavors in production of tobacco products.

Generally, the plasma membrane of plants is the first target of Cd toxicity stress, and Cd stress in plant cells is indicated by the following aspects: it induces reactive oxygen species (ROS) accumulation; alters the permeability of plasma membrane by inhibiting H^+-ATPase; changes the composition and fluidity of membrane lipids; alters plant cell cycle, division etc.; finally damages cell organelles (DalCorso et al., 2010; Rother et al., 2010; Janicka-Russak et al., 2012). Among these, the production of ROS is one of the most common and serious damage to plants exposed to Cd stress, as it causes oxidative damages to plant cells, disrupts both redox homeostasis in cells and ROS-derived DNA oxidation, and even breaks single- and double-stranded DNA (Ahamed et al., 2018). Fortunately these excess ROS can be effectively scavenged by various antioxidative enzymes or antioxidants (Chen et al., 2017). For example, a stable free radical of hydrogen peroxide (H_2O_2) can be scavenged by catalase and peroxidase (Wang et al., 2013). Thus, an effective antioxidative system (the antioxidative enzyme activities and the redox state of primary antioxidants) is critical for plant cells in order to defend against Cd stress. In addition, gene expression patterns change in the uptake, transportation, distribution, and detoxification of Cd (Fässler et al., 2011). For example, Cd uptake...