

THE CELL WALL AS SHIELD AGAINST CADMIUM TOXICITY: PROTEOME CHANGES IN ALFALFA STEMS EXPOSED TO CADMIUM

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Introduction

Due to industrial activities such as mining, industrial production and the agricultural use of metal-containing fertilizers, environmental pollution with toxic metals has become a serious issue. Among others cadmium (Cd) is of great concern due to its widespread occurrence and high toxicity (Satarug et al., 2003). Plants growing on Cd-polluted soil suffer from growth inhibition, chlorosis, and essential physiological and biochemical processes are impacted. Further, metal accumulation in plant tissues introduces Cd into the animal and human food chain. Being a protective barrier, the cell wall acts as the primary defense against environmental threats. Alterations in cell wall structure were reported when plants are exposed to Cd (Krzesłowska, 2011; Parrotta et al., 2015).

We choose alfalfa (*Medicago sativa*) stems as a model to study the detailed impact Cd exposure has on the cell wall structure. The presented work is a proteomics study combining extraction protocols for untargeted and cell wall-targeted proteins. Proteomics has previously been used to study the impact of metal exposure on plant physiology (Kieffer et al., 2009). With the focus on cell wall proteins new insight will be obtained on how the cell wall acts as a protective barrier. Structural cell wall alteration might make fiber crops cultivated on heavy-metal polluted soil more or less suitable for specific non-food applications.

Methods

Extraction of the cell wall proteins from alfalfa stems was done according to an in-house developed protocol for sequential protein extraction resulting in three fraction of which each contains a specific group of cell wall localized proteins with a different affinity to the cell wall matrix (Printz et al., 2015). The soluble proteins were extracted using an optimized SDS/Phenol extraction protocol used in most currently ongoing plant proteome studies (Wang et al., 2003). 2-D DIGE was used to separate the proteins and determine their abundance in each fraction. This method enables accurate analysis of different protein abundance between conditions (control and Cd).

Results

In the cell wall fractions a much higher number of protein spots show a significant change in their abundance compared to the soluble fraction. In both fractions an abundance change of ferritin appears, a protein related to metal homeostasis and stress response. In the cell wall fraction cysteine-rich secretory protein (CAP), which is known to be involved in stress response, and proteins involved in the cell wall remodeling were identified: polygalacturonase non-catalytic subunit, polygalacturonase inhibitors, xylogucanase-specific endoglucanase inhibitor and lignin biosynthetic peroxidase. While the polygalacturonase non-catalytic subunit is less abundant in Cd-exposed plants all other proteins involved in cell wall remodeling show a higher abundance in the Cd-treated samples.

Conclusion

So far the current results show abundance changes in a high number of cell wall and soluble proteins. Cell wall remodeling enzymes were either found down-regulated (polygalacturonase non-catalytic subunit) or up-regulated (polygalacturonase inhibitors xyloglucanase-specific endoglucanase inhibitor) supporting the hypothesis that Cd exposure influences the cell wall structure. Identifications and analyses addressing the regulation are still ongoing.

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