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Proteomic and enzymatic response of poplar to cadmium stress

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ABSTRACT

This study highlights proteomic and enzymatic changes in roots and leaves of actively growing poplar plants upon a cadmium stress exposure. Proteomic changes in response to a short-term (14 days), as well as a longer term (56 days) treatment are observed between the different organs. In leaves, stress-related proteins, like heat shock proteins, proteinases and pathogenesis-related proteins increased in abundance. A response similar to a hypersensitive response upon plant–pathogen interaction seemed to be induced. Concerning roots it appeared that the metabolic impact of cadmium was more deleterious than in leaves. This is evidenced by the early increase in abundance of many typical stress-related proteins like heat shock proteins, or glutathione-S-transferases, while most proteins from the primary metabolism (glycolysis, tricarboxylic acid cycle, nitrogen metabolism, sulfur metabolism) were severely decreased in abundance. Additionally the impact of cadmium on the glutathione metabolism could be assessed by activity assays of several important enzymes. Cadmium treatment had an inhibitory effect on glutathione reductase and ascorbate peroxidase in leaves, but not in roots. Conversely, glutathione-S-transferase showed a higher activity (and abundance) in roots but not in leaves.

1. Introduction

Plants carry out the necessary functions essential for growth and development via specialized organs. Whereas roots are responsible for water and nutrient absorption, leaves carry out light harvesting and carbon fixation. These different organs are interdependent, as the non-photosynthetic roots require the assimilated carbon from the source leaves. This high level of specialization results in fundamental morphological, physiological and metabolic differences, which impact on all the molecular levels. Comparison of roots and leaves in a systematic manner, particularly at the protein level has been infrequent [1]. Environmental constraints have an effect on the whole plant, affecting all organs and also resulting in a coordinated response. Nonetheless most studies on molecular changes in stressed plants focus on particular organs.

Heavy metals constitute an important worrying environmental pollution primarily caused by industrial activities. Among heavy metals, cadmium is of particular concern due to its widespread occurrence and its high toxicity. This metal has

Abbreviations: APX, ascorbate peroxidase; CDNB, 1-chloro-2,4-dinitrobenzene; GR, glutathione reductase; PR, pathogenesis-related protein; PSI, photosystem I; PSII, photosystem II; ROS, reactive oxygen species; RuBisCO, Ribulose-1,5-bisphosphate carboxylase/oxygenase; SOD, superoxid dismutase; TCA cycle, tricarboxylic acid cycle.

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also a very high global emission rate into soils, by industrial activities, and also by application of contaminated sewage sludge and phosphate fertilizer onto agricultural soils [2]. Cadmium may enter plants via the root system. Several transporter families have been identified as playing a potential role in the uptake, among them members of the ZIP family of metal transporters (ZRT1/IRT1-like proteins) [3], calcium channels [4] or members of the Nramp family [5], which are transporters normally responsible for the uptake of Zn^{2+}, Fe^{2+}, Ca^{2+} or Mn^{2+}. Cadmium ions are taken up as "opportunistic hitchhikers" by ion transporter/channels with a low specificity [6].

Once transported across the plasma membrane inside the roots, heavy metal ions may be translocated, in some cases, to the aerial part of the plant via the xylem. Besides, compared to other metals (i.e. As) cadmium has high propensity to accumulate also in other organs than the roots, like stems and leaves. [6]. This ability of plants to translocate significant quantities of cadmium to aerial parts, has promoted the idea to use plants to clean up polluted soils, a process called phytoremediation [7]. Several species from *Salicaceae* family show a promising potential for uptake and translocation of heavy metals to the aerial parts. Specifically, trees from the genus *Populus* have already been used in several field studies for phytoremediation of heavy metal contaminated soils [8–12]. Poplar trees are fast growing plants, able to produce a high biomass in a short growing period. They can be vegetatively propagated and serve as a non-food crop, for the pulp and paper industry as well as renewable energy source [13].

Once inside the plants, most transition metals will not be present in their hydrated form in the cells but bound to ligands. Cadmium has a high affinity for thiolates; X-ray absorption spectroscopy studies also indicate that Cd is coordinated with sulfur ligands [14]. Indeed, in response to a metal stress (particularly cadmium), an accumulation of glutathione and glutathione-derived peptides called phytochelatins can be observed in many plant species [15]. These Cys-rich compounds act as cytosolic binding partners. Phytochelatins in particular, with a general structure of $(\gamma\text{-Glu-Cys})_n$-Gly ($n=2$–11) are able to chelate and sequester cadmium ions in the cytosol or vacuole [16]. The depletion of glutathione (GSH) by chelation to cadmium, (and incorporation into phytochelatins) is the most direct effect of cadmium on the oxidative balance in cells, and it has been proposed that this depletion of GSH is one of the main causes for oxidative stress and reactive oxygen species (ROS) generation induced by cadmium [6,16]. Glutathione, indeed together with ascorbate, takes part in the Halliwell–Asada pathway for detoxification of ROS [17]. Apart from oxidative stress, cadmium also induces other toxicity symptoms in plants, among them whole plant and cell growth inhibition. From a metabolic point of view, cadmium has also an inhibitory effect on photosynthesis, as well by interfering with different steps in the Calvin cycle, as by acting on PSII or plastoquinone, or by interfering with RuBisCO activation [18].

To study abiotic stress exposure in plants, several molecular techniques have been established in the last year, trying to tackle the response mechanisms in a global view. Among these techniques, differential proteomic analysis is an essential tool. Numerous proteomic studies have been carried out in plants facing abiotic stress conditions, like cold or ozone exposure [19,20]. Studies on heavy metal responses have also seen the emergence of proteomic analysis as a tool. Within the recent findings on the effects of metal ions, and cadmium in particular several proteomic studies have been published [21–24]. Among the different organisms that were studied, one can find mostly model organisms like rice, *Arabidopsis thaliana*, but also *Populus tremula*, a model tree. Results of these analyses show the importance of transporter proteins in roots, the effect on primary metabolism, most noticeably the importance of the sulfur assimilation and metabolism in roots, as well as phytochelatin and glutathione synthesis [21–24].

The present work focuses on proteomic responses in roots of cadmium exposed poplar plants, and complements preceding work focusing on proteomic changes in leaves in poplar upon cadmium exposure [22]. This study compares and discusses the observed proteomic changes in the roots with results obtained in leaves. To complement the proteomic results, several enzymatic activity assays were also carried out, to gain further insight in the mechanism behind cadmium stress response.

2. Material and methods

2.1. *Plant material, growth conditions and cadmium treatment*

Poplar clones (*Populus tremula* L.) were multiplied *in vitro* in controlled growth chambers as previously described [22]. Rooting of *in vitro* plants was followed by transfer and acclimation to hydroponic culture in a modified 1/4-strength Hoagland's solution [22]. When plants reached the desired size (22–24 leaves) they were divided in 2 sets. The first one acted as control while in the second set, the nutritive solution was enriched with $CdSO_4$ up to a final concentration of 20 μM. Sampling started at day 3 and was also carried out on days 7 and 14 for the short-term treatment. For the longer term treatment sampling started on day 14, and was also done on days 28 and 56. Leaves from the same foliar stage were used for proteomic analysis (300 mg FW) at each sampling date (always leaf number five counted from the apex, corresponding to the last foliar stage of still expanding leaves). For roots about 500 mg FW were used. For enzymatic activity assays, all the other leaves from a plant were used starting with the youngest foliar stages and finishing when reaching 3 g of FW. Immediately upon cutting, leaves and roots were frozen in liquid nitrogen for further analysis.

2.2. *Soluble protein extraction and labelling*

Protein extraction was performed as described in [22] with slight modifications. In brief, a whole leaf (about 300 mg of FW) was crushed in liquid nitrogen and then mixed with extraction buffer (20% TCA and 0.1% w/v DTT in ice-cold acetone) up to 25 ml and kept overnight at −20 °C. The same protocol was carried out for roots, where approximately 500 mg of FW were crushed in liquid nitrogen. After centrifugation for 45 min at 35 000 g and 4 °C, the pellets were washed three times with ice-cold acetone containing 0.1% w/v DTT before freeze-drying.