



Regulation of cadmium-induced responses in *Arabidopsis thaliana*: A possible role of miRNAs

Smeets K, Donckers K, Remans T, Opdenakker K, Ruytinx J, Vangronsveld J, Cuypers A
Hasselt University, Agoralaan Building D, B-3590 Diepenbeek, Belgium

Introduction

As a highly toxic metal, knowledge about molecular mechanisms of cadmium-induced responses is rather scarce. Cadmium does appear to provoke several oxidative stress effects similar to other metals. Therefore, in order to unravel the complexity of signals involved in heavy metal stress responses, oxidative stress related genes were measured in combination with known complementary miRNAs.

ROS-production

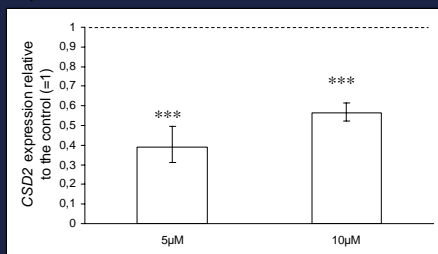
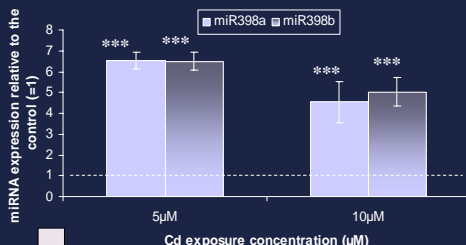
I. Induction of lipoxygenases and/or NADPH oxidases

AND/OR

II. Inhibition of the antioxidative defence system

VIA miRNA?

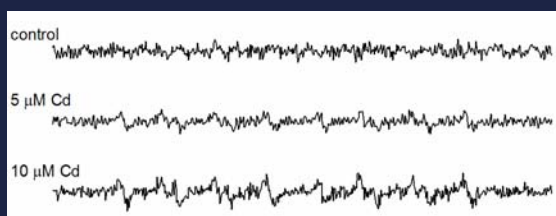
miR398 expression under cadmium stress



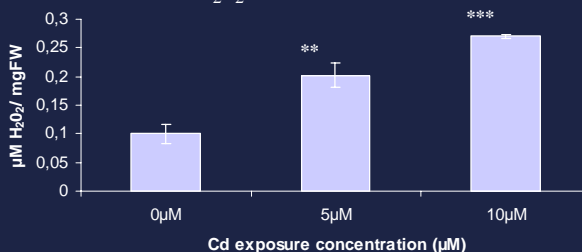
CSD2 inhibition under cadmium stress

OXIDATIVE STRESS

EPR measurements: $O_2^{\cdot-}$ under cadmium stress



H_2O_2 under cadmium stress



Methods

- Arabidopsis thaliana* ecotype Columbia:
 - Hydroponics, 3 weeks
 - 24 hr 5 or 10 µM $CdSO_4$
- Gene expression: quantitative RT-PCR (Taqman chemistry)
- miRNA expression: quantitative RT-PCR (Applied biosystems)
- ROS: EPR measurements or fluorospectrophotometric assays
- The datasets were analyzed using linear mixed models: ***: $p > 0.01$ and **: $p > 0.05$

ROS-scavenging

Transcriptional upregulation of antioxidative mechanisms

ROS-scavenging enzymes: Relative gene expression in LEAVES (control = 1)

Gene	Localization	5µM	10µM
<i>MSD1</i>	Mitochondrion	1,37±0,07	1,20±0,13
<i>FSD1</i>	Plastid	1,37±0,39	0,85±0,54
<i>APX1</i>	Cytoplasm	1,46±0,17	3,02±0,29
<i>CAT1</i>	Peroxisome	1,71±0,25	2,94±0,36
<i>GPX2</i>	Cytoplasm	1,93±0,12	2,13±0,17
<i>GRI</i>	Cytoplasm	2,08±0,14	2,15±0,13

Discussion

Cadmium is a non-redox active metal but also induces oxidative stress, even at low concentrations. Since cadmium itself is not redox-active, the increased ROS levels are likely to be induced via indirect mechanisms. Preliminary research already showed the importance of ROS-producing enzymes such as NADPH oxidases and lipoxygenases within the cadmium toxicity. Other indirect measurements by which cadmium induces oxidative stress are disruption of the electron transport chain or inhibition of the antioxidative defence system. Our results show a significant downregulation of the *CSD1* and *CSD2* expression under cadmium toxicity. Furthermore, miR398 expression is significantly upregulated under the same conditions, which suggests that *CSD1* and *CSD2* expression is 'fine-tuned' by miR398-directed mRNA cleavage under cadmium toxicity. Sunkar¹ and colleagues already showed a downregulation of miR398 under iron and copper stress, hence our results indicate a possible cadmium specific stress mechanism. It is becoming increasingly evident that signalling mechanisms in plants often do not operate alone, therefore other regulatory mechanisms within the cadmium toxicity remain to be elucidated.