

# Elucidation of early Zinc sensing and signaling events in Arabidopsis, upon Zinc starvation and re-supply



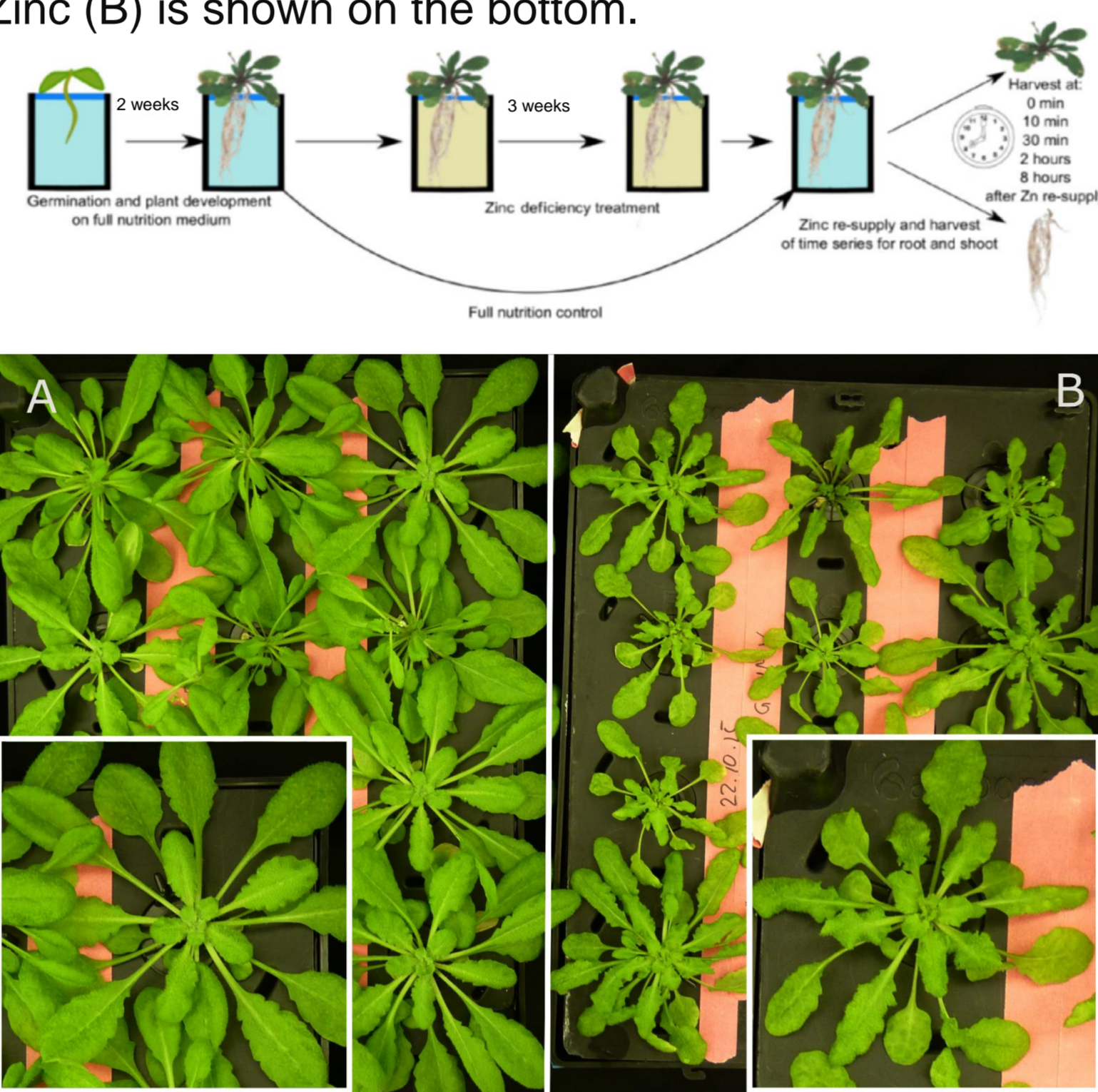
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## Introduction

Zinc (Zn) is an essential micronutrient for many organisms, including plants and humans. Today about 20% of the population is estimated to suffer from Zn deficiency, and relies on plants for their Zn uptake. Thus understanding how plants recognize and take up Zn from the soil can lead to novel approaches of Zn biofortification in cereals and legumes. The approach presented here aims to identify novel mechanisms in Zn sensing and signaling, essentially by kick-starting the Zn signaling cascade on Zn starved plants, with Zn re-supply. Protein response of root and shoot samples was profiled in a time-related manner, and each tissue was subjected to enrichment of the microsomal fraction with the aim to identify the response of lowly abundant membrane proteins.

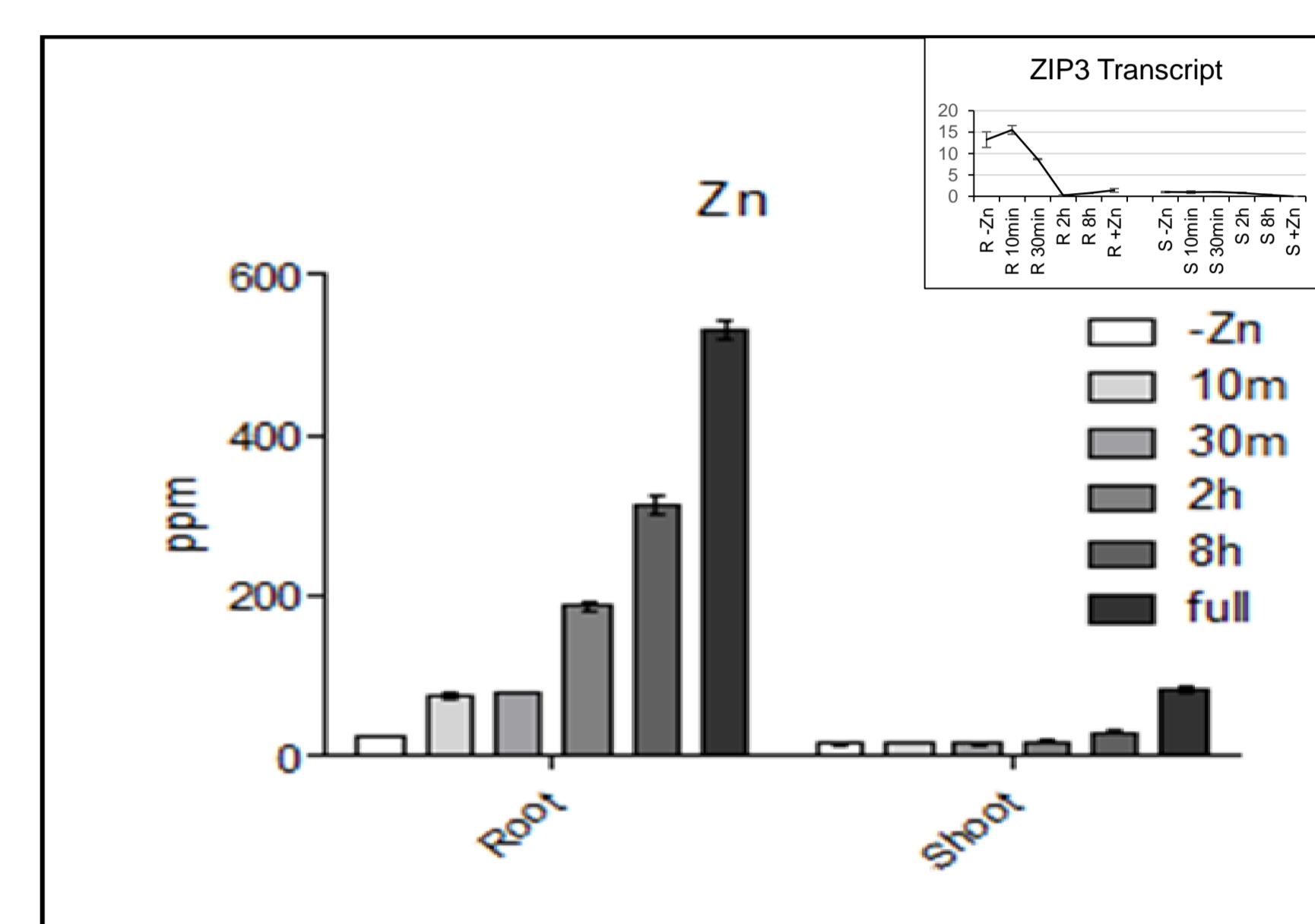
## Experimental design and plant phenotype

Arabidopsis seedlings were grown on full Hoagland solution for 2 weeks, then starved of Zinc for 3 weeks, followed by Zn re-supply to the medium (top). The phenotype of the control plants with Zinc (A) and without Zinc (B) is shown on the bottom.



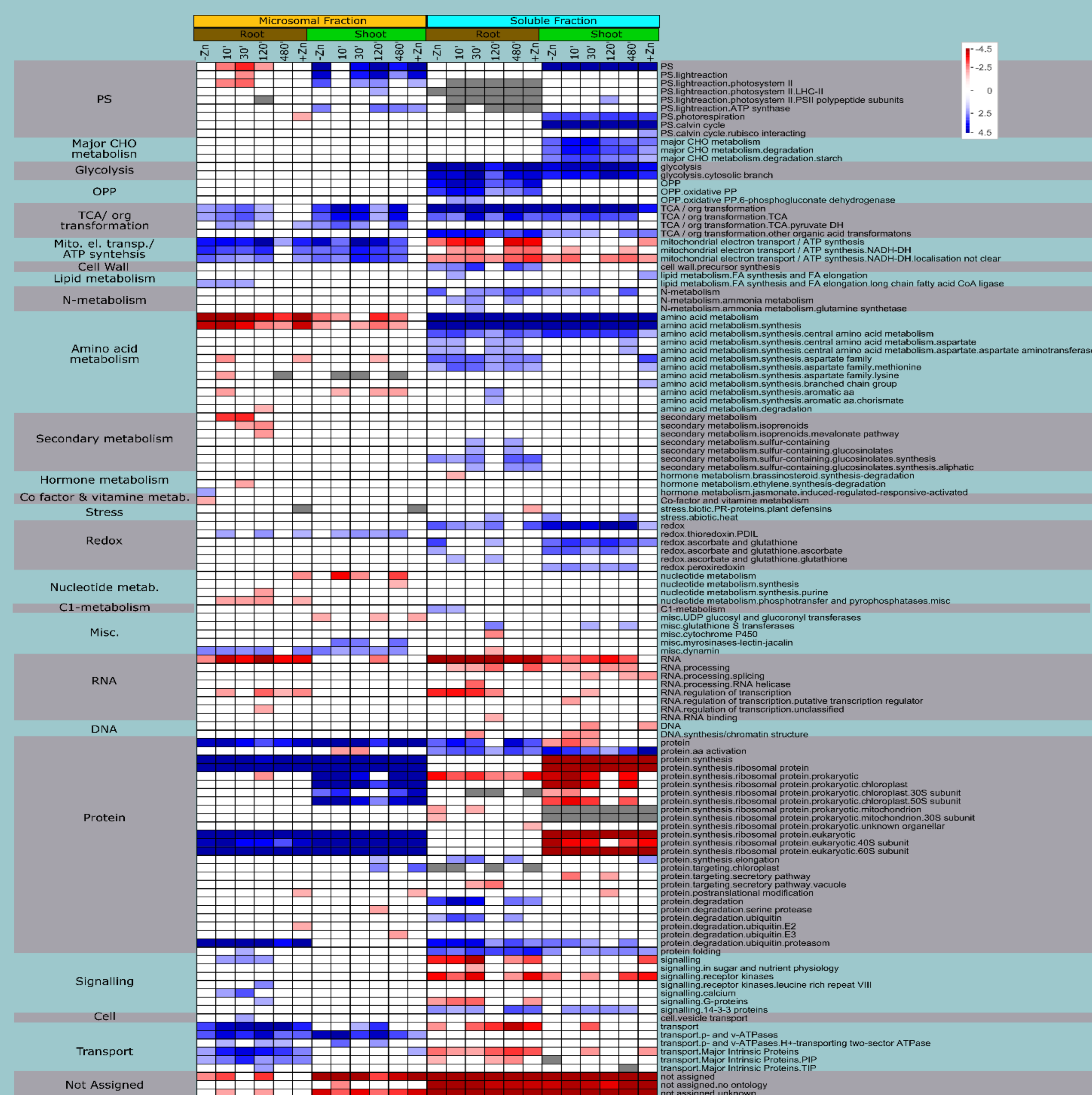
## Metal accumulation through Inductively Coupled Plasma Atomic emission Spectroscopy (ICP-AS)

While in the root Zinc accumulates quickly, with significant differences seen already 10 minutes post Zn re-supply, in the shoot the first significant Zn accumulation is seen 8 hours post re-supply. Appropriately the transcript levels of the Zn transporter ZIP3 in the root increase in the first 10 minutes and then drop as Zn accumulates in the root (insert).



## General overview of the proteomics dataset

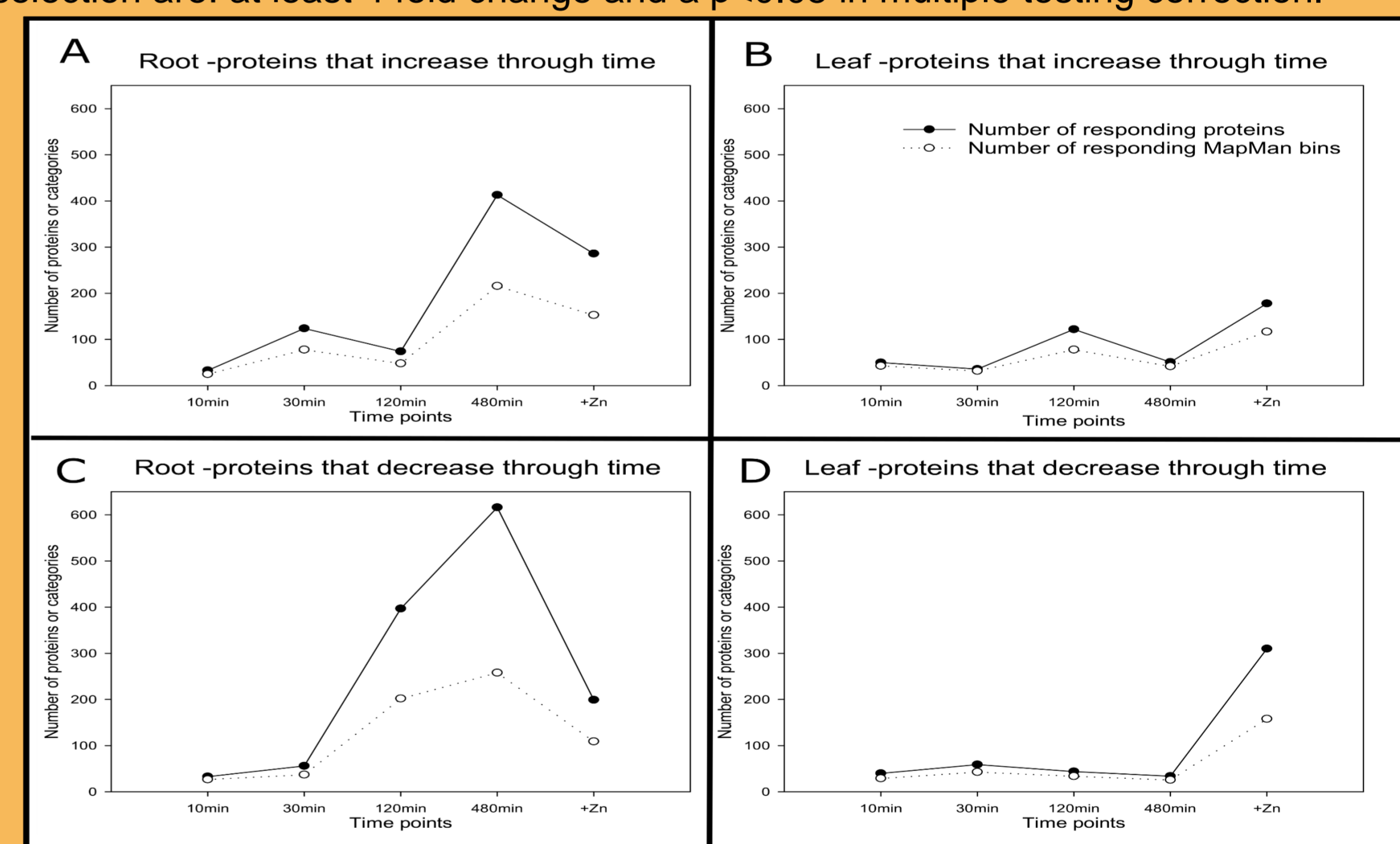
Over and underrepresented categories for each time point are presented using MapMan (Usadel et al., 2009). The protein levels were log<sub>2</sub> transformed and sorted into MapMan bins, the proteins above a cut-off of 1 were subjected to a bin-wise Wilcoxon test and the Benjamini-Hochberg multiple testing correction. Data is presented using PageMan (Usadel et al., 2006), over represented categories (blue); under-represented categories (red).



## Main findings

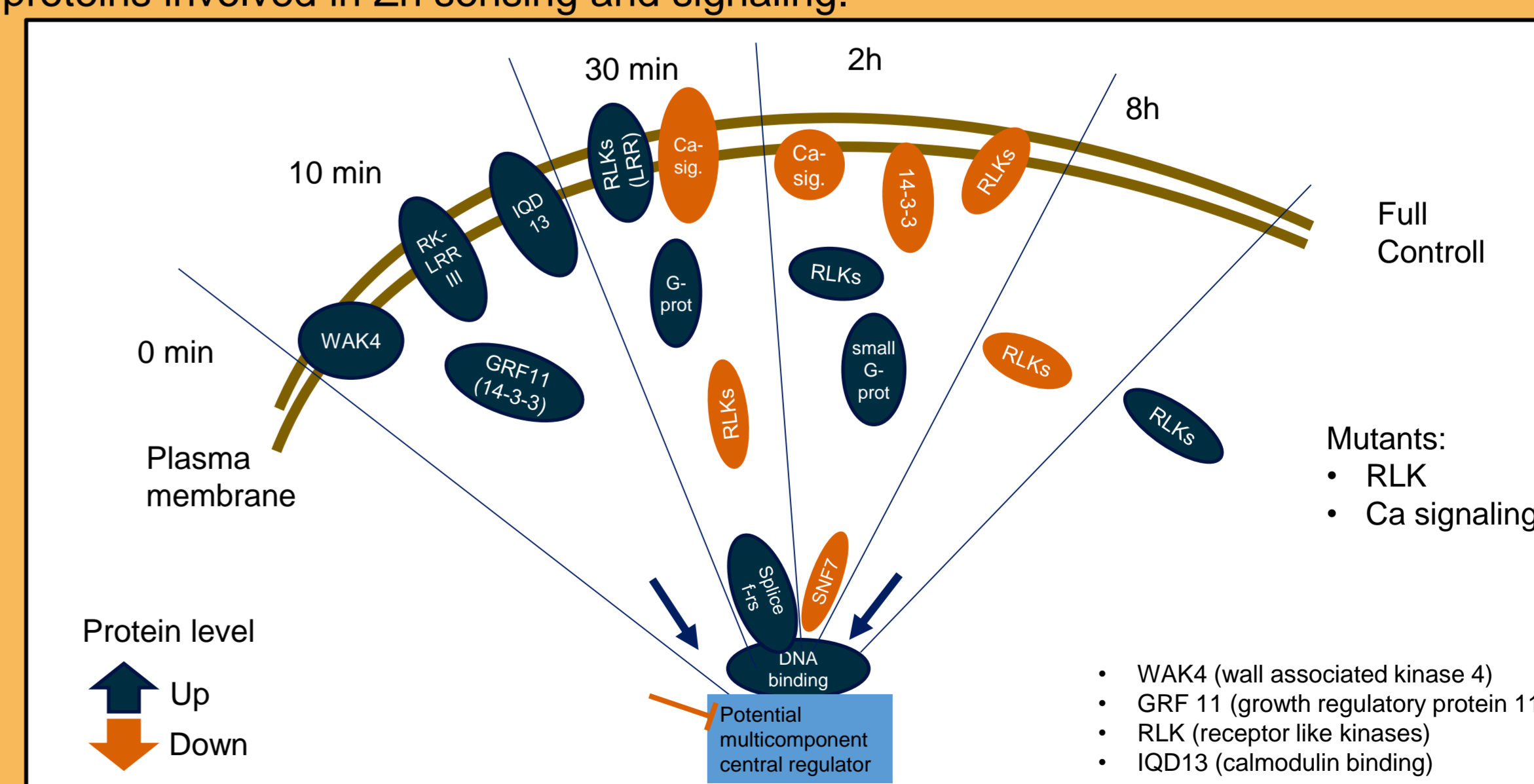
### 1- Gradual increase in the systemic response after Zn re-supply

A representation of the number of responding proteins for the root and shoot datasets, and the number of unique MapMan (sub)bins that are represented at each time-point, demonstrates the gradual involvement in a larger (systemic) response through the time series. In agreement with the delayed Zn accumulation in the shoot the number of responding proteins in the shoot is always lower than in the root. The criteria for protein selection are: at least 4 fold change and a  $p < 0.05$  in multiple testing correction.



### 2- First ideas about Zn signaling cascade

Investigating the dynamic response of the proteins from the MapMan 'Signalling' bin, as well as other regulators functional categories we propose a preliminary list of proteins involved in Zn sensing and signaling.



**Outlook:** After a detailed analysis of the dataset 75 proteins were selected and 130 T-DNA insertion lines are tested under Zn deficiency and excess. Novel candidates involved in Zn sensing and signaling are/will be identified and targeted molecular analysis will be carried out. Furthermore, "in planta" confirmed candidates will be tested in the model grass *Brachypodium*, in order to provide potential breeding targets for crop plants like wheat and barley.