Increased camalexin synthesis in Arabidopsis contributes to the Zn excess response

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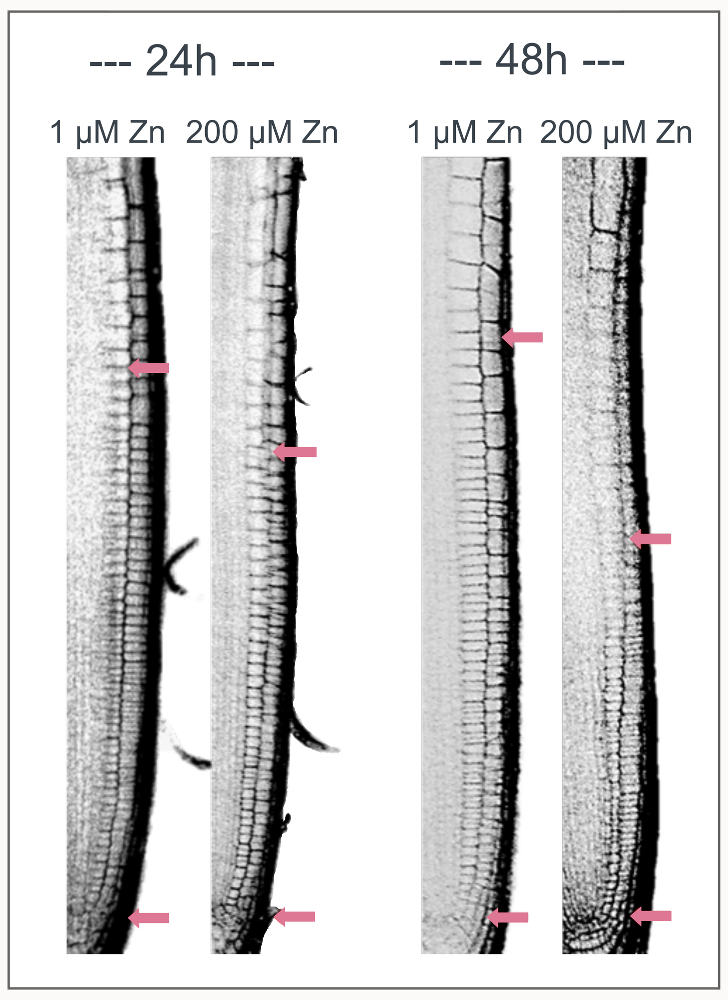
Introduction

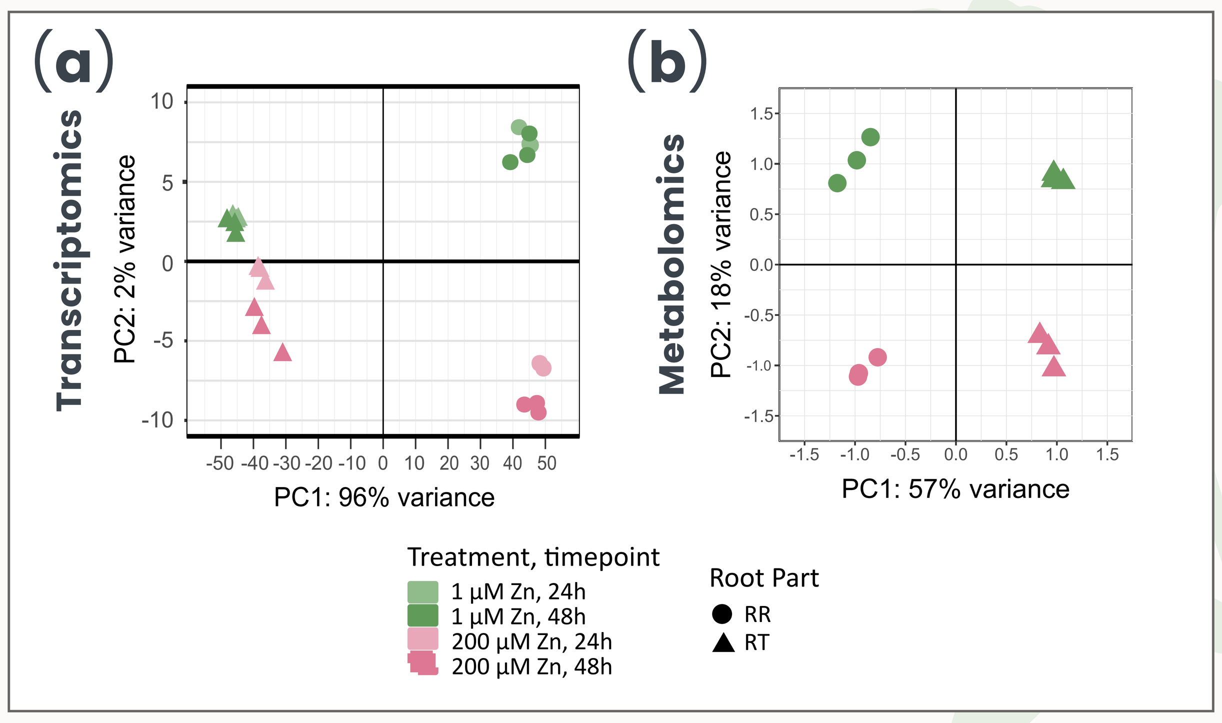
In plants, the **root system** is crucial for nutrient uptake and translocation. Various traits such as **root hair** length and density, **vacuolar storage**, or even **apoplastic barriers** regulate nutrient absorption and transport. In Arabidopsis thaliana, excess of the essential micronutrient **zinc (Zn)** is toxic, disrupting root growth, altering metal homeostasis - particularly causing iron (Fe) deficiency - and affecting root architecture. While the impact of Zn on differentiated roots is documented, its effects on **root tips** (RT) remain less understood. This study uses a **multi-omics** approach to explore the **specific responses** to Zn excess in RT and the **remaining root system** (RR), highlighting the involvement of ***PAD3*** in the stress response.

Root tip architecture



Zn excess was applied to 7-day-old Col-0 seedlings for 24 to 48h. The **treatment reduces** the distance between the tip of the root and the root hairs, the number cortical root apical meristem (RAM) cells, **RAM size** (arrows) and total **root length**.

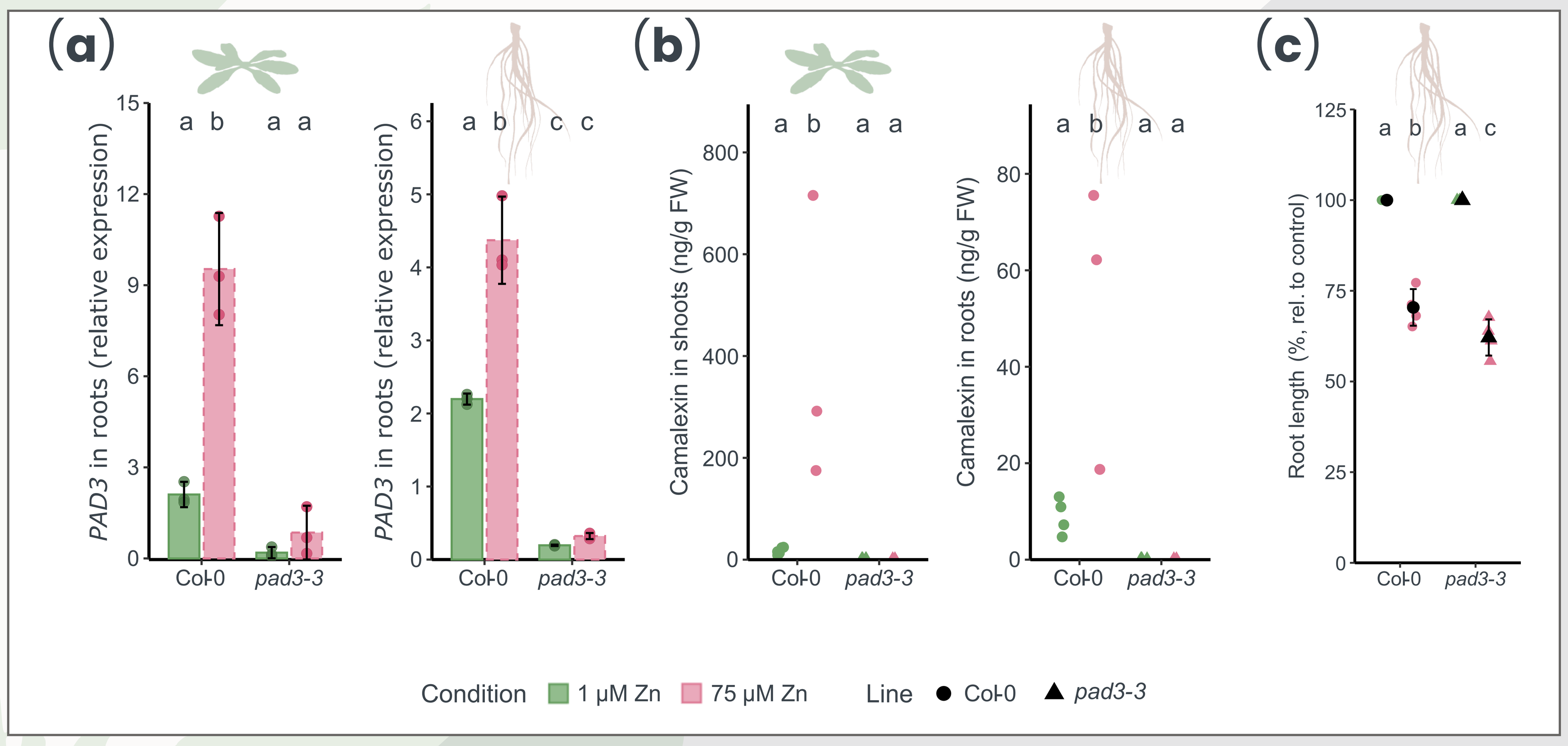
Transcriptomics and metabolomics



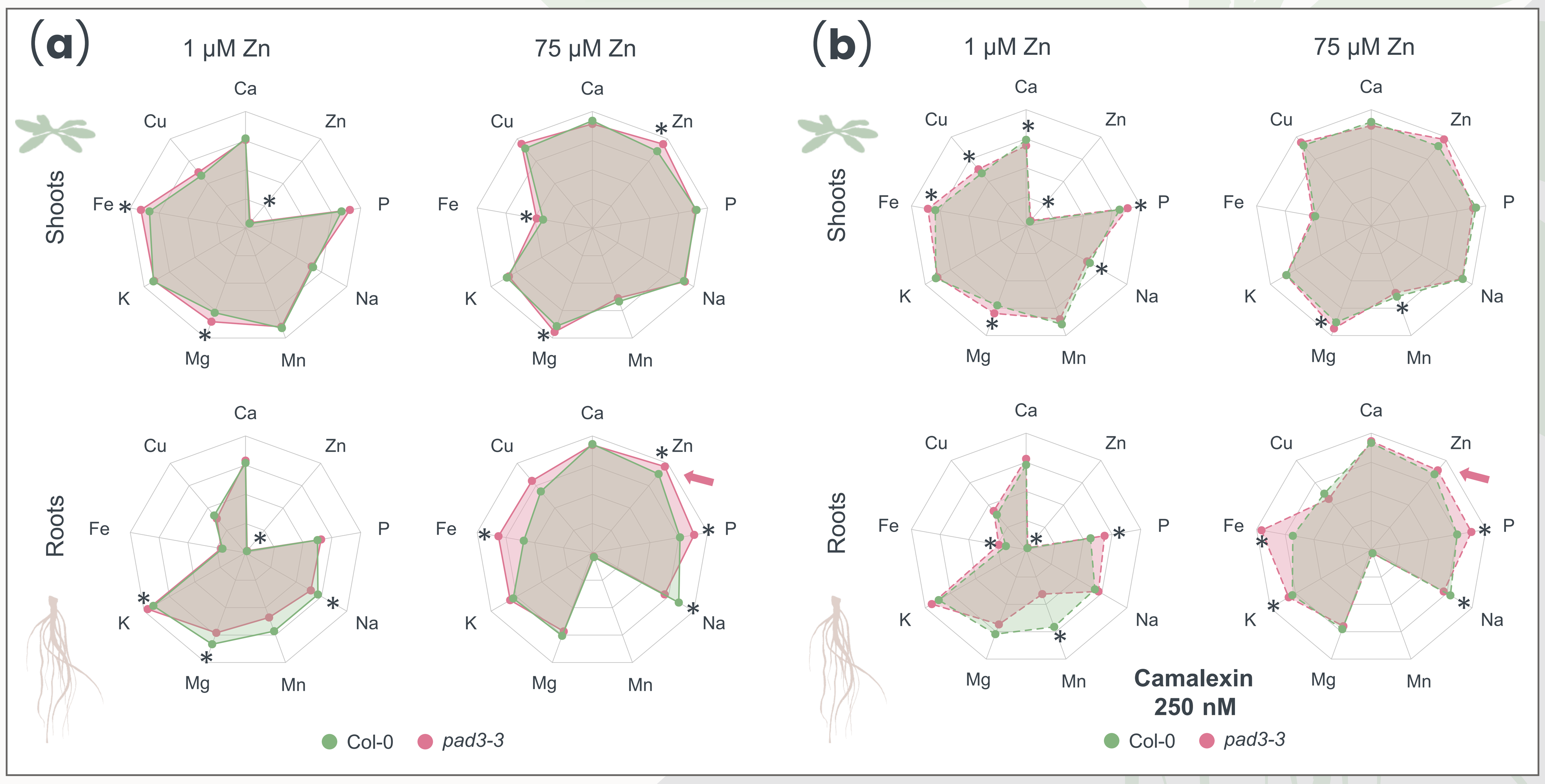
Bulk **RNA-seq** on the RT (2-3 mm) and RR of 7-day-old seedlings treated with Zn for 24 to 48h showed that **root part** had a stronger influence on the **transcriptome** than treatment or time (**a**). **GO analysis** revealed stress response and homeostasis gene regulation in both root parts, with **RT specifically regulating genes** related to root development, ROS, hypoxia, and specialized metabolism. **Metabolomics** of 48h-treated seedlings showed that the root part had less influence, and **treatment** had more influence on the metabolome compared to the transcriptome (**b**).

PAD3 and camalexin

PAD3, encoding the final enzyme in camalexin synthesis, was strongly up-regulated in the RT under Zn excess. **Camalexin**, known for its role in leaf defense against pathogens, encouraged us to study the role of this gene under Zn excess. Consistent with RNA-seq data, Col-0 seedlings exposed to mild Zn excess (75 µM for 10 days) showed **increased *PAD3* expression**, particularly in shoots (**a**), leading to **higher camalexin** levels in both roots and shoots (**b**). Additionally, Zn excess more significantly impacted primary **root growth** in ***pad3-3* KO mutant** than in Col-0 (**c**).



Ionomics of *pad3-3*



Finally, we investigated the **ionomic response** of the ***pad3-3*** to Zn excess. Compared to Col-0, the mutant showed significant changes in **Zn, Fe**, and **Mg** concentrations in both roots and shoots, with notably **higher Zn** levels (**a**). Adding camalexin (250 nM) to the medium altered metal homeostasis in both Col-0 and the mutant (**b**). Under camalexin treatment and Zn excess, the mutant accumulated **Zn at levels comparable** to the wild type (**b**).

Conclusion

This study reveals **distinct molecular mechanisms** between the **root tip** and the **remaining root system**, highlighting how the root tip is **protected from stress** to preserve meristematic function. We also suggest that the phytoalexin camalexin may play a role in this protection, likely through its **redox activity** or potential direct binding to Zn. This study offers insight into the **diverse responses** to Zn excess within the root system.

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