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From ion transport to stress resilience: insights from CAX mutant trichomes

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The distribution of ions across tissues and within cells is crucial for maintaining nutritional needs and driving biochemical processes during plant growth (Conn and Gilliam, 2010). Vacuoles within plant cells are important for storing excess mineral elements until required for nutrition, and at the same time they compartmentalize toxic ions that are inadvertently taken up by the cells. Cation/H⁺ Exchangers (CAX) transporters play a critical role in ion homeostasis by moving calcium (and other cations) from the cytoplasm into vacuoles. During the last few decades, numerous genetic and biochemical studies have elucidated the functions of individual CAX proteins in ion balance, nutrient (or calcium) storage, cellular pH and sequestration of heavy metals (Pittman and Hirschi, 2024). Because CAX transporters can control cytosolic calcium levels, studies have also identified how they modulate calcium signaling during various abiotic and biotic stress responses (Pittman and Hirschi, 2024). Together, these insights are not only valuable for enhancing agricultural productivity but also for their potential applications in improving the nutrient content of crops and remediating polluted soils.

The study of plant CAX mutants can provide insights into how plants respond to ion imbalances and reveal broader adaptation and compensatory mechanisms. In this issue, a new study by Guo et al., (2024) examined plant responses to altered ion homeostasis using trichomes of Arabidopsis CAX mutants as a model system. Trichomes are highly specialized, single- or multicellular, self-contained structures that are easy to visualize and isolate, thereby allowing elemental stress responses to be studied within a defined environment. Additionally, their role in environmental adaptation and defense against pathogens and herbivores makes them ideal systems to explore how ion partitioning contributes to environmental adaptation and protection. This study specifically investigated how CAX mutations affect ion accumulation in trichomes and how trichomes in turn respond to this elevated elemental stress. Using synchrotron X-ray fluorescence microscopy for elemental imaging, the authors found that deletion of a single CAX transporter, CAX1, primarily influenced calcium accumulation, with minor effects on chlorine. In quadruple mutants, where CAX1-4 were deleted, calcium, manganese, chloride, and to a lesser extent, potassium levels were elevated. Interestingly,

in contrast to previous studies focusing on leaf and root tissue (Cheng et al., 2005), this ion imbalance did not affect trichome density and morphology but triggered a broad range of internal changes in transcript and protein abundances, suggesting the presence of physiological adaptation mechanisms to maintain normal trichome growth and development under elemental stress. Concomitantly, the study also served as proof of concept that this system indeed can aid as a model to understand how plants respond to and manage elemental stress.

The authors employed an elegant approach to study protein alterations in response to elemental changes by performing proteomic analysis on isolated trichomes from CAX mutants. Notably, the trichome purification procedure was validated by the enrichment of trichome-specific transcripts and the absence or depletion of leaf-specific transcripts. Moreover, at the protein level, processes related to fundamental metabolism, including primary metabolic functions and photosynthesis-related activities, were depleted in trichomes compared to leaf cells, further confirming the robustness of their experimental setup. A comparative proteomic analysis of trichomes isolated from CAX mutants and wild-type plants revealed a broad range of adaptive responses, which the authors discuss in the context of elemental adaptation as well as their role in general stress responses. Interestingly, the proteomic changes observed in CAX mutants point to an adaptive mechanism in which trichomes prioritize energy-intensive processes, such as proton pumping at the plasma membrane and the tonoplast, as well as water transport, to maintain cellular function during disrupted ion transport. This is also reflected in the observed increase in proteins involved in carbon and energy metabolism in CAX mutants. Broader stress-response mechanisms were also noted, including redox regulation, protein folding, and endocytosis. Thus, trichomes of CAX mutants, and especially the single *CAX1* mutants, appear to compensate for disrupted ion homeostasis not through changes in ion transport mechanisms but via other compensatory responses, including specific metabolic adjustments and broader stress-related adaptations (**Fig. 1**). Furthermore, changes were observed in proteins involved in fatty acid metabolic processes and plasmodesmal signaling, likely reflecting altered membrane and symplastic permeability (**Fig. 1**). Overall, the authors conclude that tolerance to ion imbalance is not primarily driven by primary transport mechanisms, but rather by a complex interplay between water and proton pumping, metabolic reprogramming, membrane trafficking, and intercellular communication. However, they note that with more advanced proteomic analyses, additional proteins—especially low-abundant ion transporter proteins—are likely to be identified. Additionally, given the membrane-bound nature of CAX and other ion transporters, specific protocols to enrich membrane proteins could improve their detection. This is also supported by a complementary transcriptome analysis, which highlights altered transcript levels of calcium-binding and transport mechanisms that were not identified in the proteomic study.

In conclusion, Guo et al.'s study sheds light on the complex interactions between ion transport and protein regulation in plant trichomes, opening new research avenues for improving stress resilience in plants. For example, the proteins identified in this study could be further explored for their activity and functional significance in ion homeostasis and plant adaptation mechanisms. Therefore, further research should explore how these proteomic changes translate to metabolic and physiological adaptations in other tissues or at different stages of development. Equally, it would be valuable to investigate whether similar proteomic changes occur in different types of trichomes or in plant species other than *Arabidopsis*, as this could reveal whether the findings are broadly applicable. Finally, as chlorine has been rarely studied in this context, it would be interesting to further explore its role in response to changes in elemental concentrations in trichomes. Investigating the

mechanisms by which chlorine contributes to stress adaptation in trichomes could uncover new strategies for improving ion and water balance in crops, especially in environments prone to drought or salinity stress. Overall, proteome studies of CAX mutant trichomes, similar to other CAX proteome profiling studies, provide a glimpse into how ion imbalances can reshape cellular functions with the potential to guide future research in plant nutrition, stress resilience and adaptation.

Disclosures

The author has no conflicts of interest to declare.

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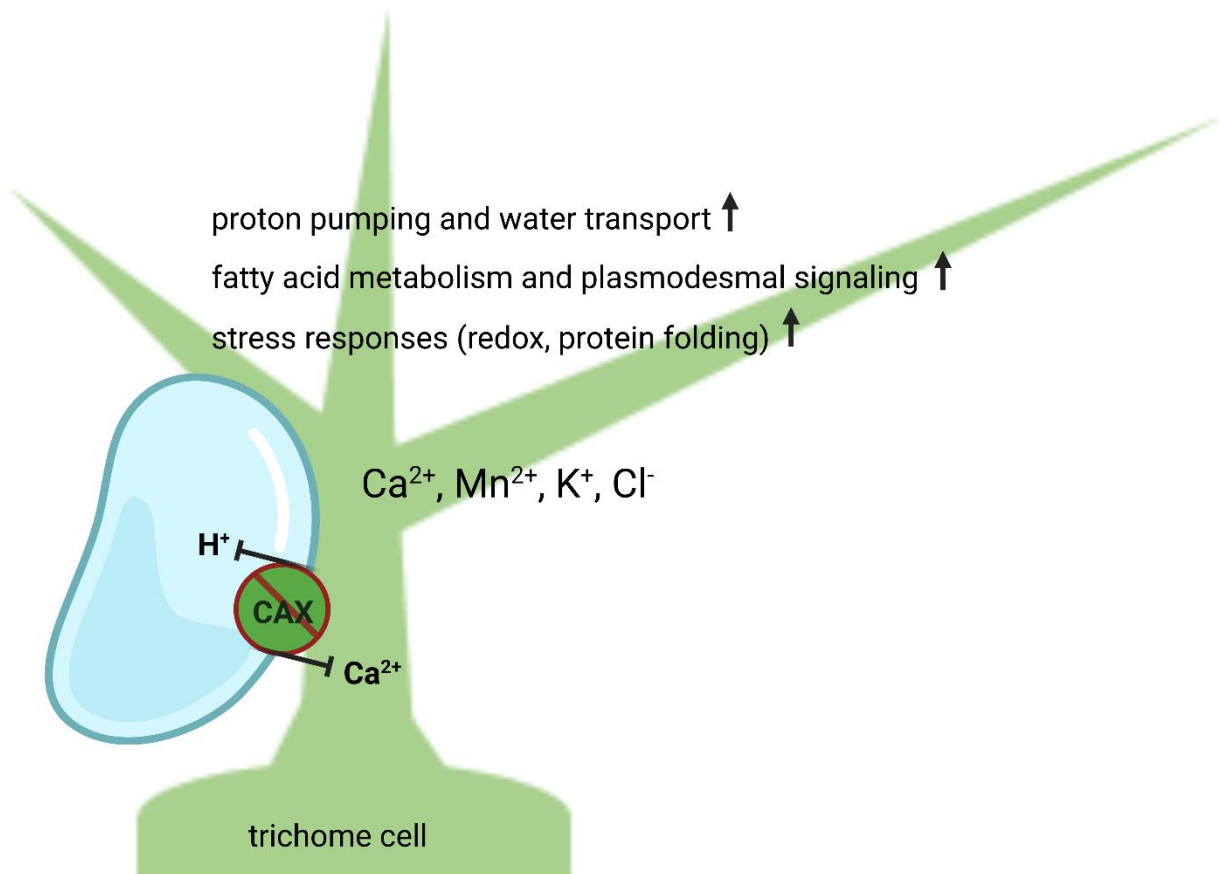


Fig. 1. Trichomes of CAX mutants as a model system to study ion stress responses. Trichomes in CAX mutants, which are deficient in elemental accumulation in the vacuole, accumulate Ca, Mn, K and Cl. These elemental imbalances trigger proteomic changes associated with processes such as proton pumping and water transport, fatty acid metabolism, plasmodesmal signaling and broader stress response mechanisms. (Created in BioRender.com.)