IMPROVEMENTS AND CHALLENGES IN PLANT CALCIUM SIGNALING

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ABSTRACT: Calcium signaling plays a crucial role in plant responses to environmental stimuli, regulating various physiological processes, including growth, development, and stress tolerance. Recent advances in plant biology have significantly improved the understanding of calcium signaling pathways, revealing complex interactions between calcium ions, calmodulins, and other signaling components. This review aims to provide a comprehensive overview of the current state of knowledge on plant calcium signaling, highlighting recent breakthroughs, and discussing the challenges that remain to be addressed. We examine the molecular mechanisms underlying calcium signaling, including the roles of calcium channels, pumps, and exchangers, as well as the downstream signaling cascades that regulate plant responses and the implications of calcium signaling for plant stress tolerance, growth, and development. We also identify key areas for future research, including the need for further elucidation of the complex interactions between calcium signaling and other signaling pathways, and the development of novel strategies for manipulating calcium signaling to enhance plant performance. By combining current knowledge and identifying future directions, this review aim is to provide resources for researchers and practitioners seeking to understand and exploit the complex mechanisms of plant calcium signaling.

[OKO. E & AFOLABI A. E., IBIRONKE H. O. **IMPROVEMENTS AND CHALLENGES IN PLANT CALCIUM SIGNALING,** *J Am Sci* 2025;21(12):1-6]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 01. doi:10.7537/marsjas211225.01

Keywords: Calcium signaling; plant biology; stress tolerance; development; signaling pathways; crop resilience,

INTRODUCTION

Calcium (Ca^{2+}) is a ubiquitous and versatile second messenger in plants, mediating responses to a wide range of environmental and developmental sign (Thor, 2019). Changes in cytosolic Ca^{2+} concentrations serve as a signaling mechanism that translates external and internal stimuli into specific physiological responses (Xiong *et al.,* 2006; Luan *et al.,* 2009; Tang and Luan, 2017; Sharma and Kumar, 2021). Unlike primary messengers such as hormones or light, Ca^{2+} does not carry information directly but modulates cellular processes through transient fluctuations in its concentration, often referred to as “calcium signatures.” These signatures are decoded by calcium binding proteins, such as calmodulins (CaMs), calcium dependent protein kinases (CDPKs), and calcineurin B-like proteins (CBLs), which then activate downstream signaling cascades. Through this mechanism, calcium regulates critical processes including seed germination, root and shoot development, stomatal movement, and responses to biotic and abiotic stresses (Ren et al., 2021; Park and Shin, 2022; Wdowiak et al., 2024). Its universal role as a second messenger highlights its central importance in plant physiology and adaptation

Calcium signaling plays an important role in regulating plant growth, development, and adaptation to environmental stresses. Its importance can be summarized across key physiological processes as Growth and Development, Stress Responses and Cross talk with Other Signaling Pathway, by coordinating these processes, calcium signaling ensures that plants can optimize growth under favorable conditions and survive under stress, highlighting its indispensable role in plant physiology.

The primary purpose of this review is to provide a comprehensive synthesis of current knowledge on calcium signaling in plants, emphasizing its molecular mechanisms, physiological roles, and regulatory networks. By summarizing recent advances, the review highlights key discoveries in calcium channels, pumps, exchangers, and calcium-binding proteins, as well as their downstream signaling cascades. This review identifies major challenges, including the complexity of calcium signaling networks, the redundancy of components, and the technical limitations in monitoring spatiotemporal calcium dynamics. Finally, it outlines future research directions, focusing on integrative approaches, advanced imaging techniques, and potential strategies for manipulating calcium signaling to enhance plant growth, development, and stress resilience. We aims to serve as a valuable resource for researchers, providing insights into both foundational knowledge and emerging trends in plant calcium signaling.

2. UNDERSTANDING PLANT CALCIUM SIGNALING COMPONENTS

Calcium (Ca^{2+}) signaling is a central regulatory system that enables plants to perceive and respond to environmental and developmental cues (Tong *et al.*, 2025). Over the past decade, substantial progress has been made in elucidating the molecular components of Ca^{2+} signaling, including the channels, transporters, and sensors that generate, decode, and transmit Ca^{2+} signals. These advances have transformed our understanding from descriptive observations to mechanistic insights, linking Ca^{2+} dynamics with specific physiological responses. Recent studies have expanded knowledge of Ca^{2+} influx pathways, particularly plasma membrane channels such as cyclic nucleotide-gated channels (CNGCs), glutamate receptor like channels (GLRs), and mechanosensitive channels. These proteins regulate stimulus specific Ca^{2+} influx in response to stress, hormones, and mechanical stimuli. Organelle based Ca^{2+} transport systems, including vacuolar exchangers (CAX), two pore channels (TPC1), and ER localized Ca^{2+} ATPases, have been shown to fine tune cytosolic Ca^{2+} concentrations and shape distinct spatial and temporal Ca^{2+} signatures. Mitochondrial and chloroplast Ca^{2+} fluxes further coordinate energy metabolism and photosynthetic performance during stress adaptation. On the decoding side, major progress has been achieved in understanding the role of Ca^{2+} sensors. Calmodulins (CaMs) and CaM-like proteins (CMLs) act as universal integrators, modulating enzymes, transcription factors, and ion transporters. The CBL CIPK network provides modular signaling complexes that link Ca^{2+} perception to ion homeostasis and stress responses, while calcium-dependent protein kinases (CDPKs) directly convert Ca^{2+} oscillations into phosphorylation events that regulate growth, immunity, and metabolism (Luan 2009; Mao *et al.*, 2016). Technological innovations in imaging and biosensors have been instrumental in mapping Ca^{2+} signatures. Genetically encoded Ca^{2+} indicators (GECIs) and advanced microscopy now allow real-time, subcellular visualization of Ca^{2+} dynamics (oh *et al.*, 2019). These studies have revealed that both amplitude and frequency of Ca^{2+} oscillations encode specific developmental and stress signals, highlighting the concept of a "Ca^{2+} code." Combined with computational modeling, these findings provide predictive insights into how Ca^{2+} networks integrate multiple environmental and endogenous cues.

Table 1: Key Component of Plant Calcium Signaling

Components Class	Examples	Primary Function	Biological Role
Ca^{2+} Channels	CNGCs, GLRs, OSCAs, TPC1	Mediate Ca^{2+} influx across membrane	Stress perception, growth signaling
Ca^{2+} Pumps	ACAs, ECAs	Actively remove Ca^{2+} from cytosol	Maintain Ca^{2+} homeostasis
Ca^{2+} Exchangers	CAX family	Ca^{2+}/H^+ antiport transport	Vacuolar Ca^{2+} sequestration
Ca^{2+} Sensors	CaM, CMLs, CBLs	Bind Ca^{2+} to trigger downstream signaling	Decoding Ca^{2+} signatures

Ca^{2+}-Dependent Kinases	CDPKs, CIPKs	| Phosphorylation-based signal transduction	Stress tolerance, hormonal responses
Regulatory Proteins	Annexins, calcineurin-like proteins	Modulate Ca^{2+} signaling pathways	Abiotic and biotic stress adaptation

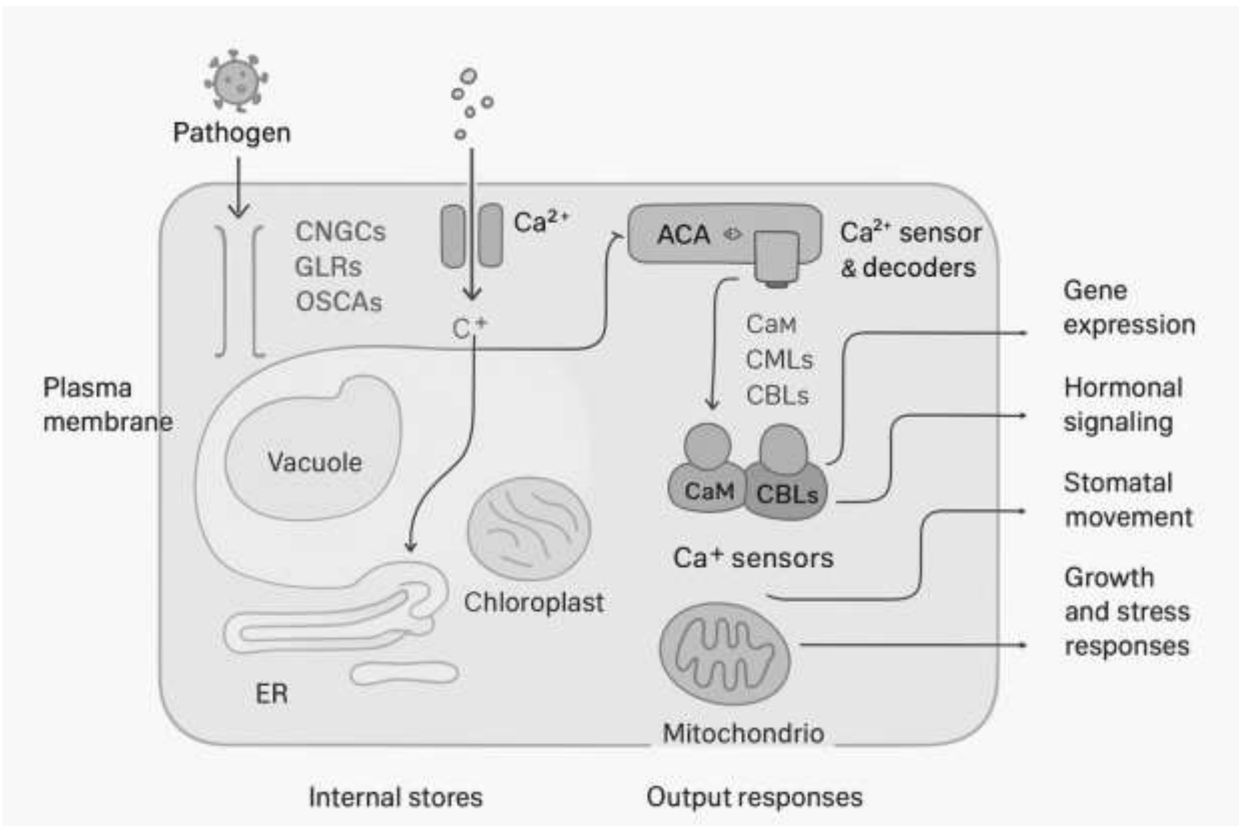

Figure 1: Calcium Signaling Overview Diagram

3. INTEGRATION OF CALCIUM SIGNALING WITH OTHER REGULATORY NETWORKS

Calcium signaling in plants functions as a central hub, integrating multiple regulatory pathways to coordinate growth, development, and stress responses (Negi *et al.*, 2023). Rather than acting in isolation, Ca^{2+} signals interact extensively with hormonal networks, reactive oxygen species (ROS), and other secondary messengers, creating complex signaling modules that allow plants to respond with specificity and precision. Hormones such as abscisic acid (ABA), auxin, ethylene, and salicylic acid (SA) are closely linked to Ca^{2+} dynamics (Ding *et al.*, 2025). During drought, ABA triggers Ca^{2+} influx in guard cells, which activates calcium dependent protein kinases (CDPKs) and results in stomatal closure to minimize water loss (Wei *et al.*, 2025). Auxin induced Ca^{2+} fluxes regulate root growth and architecture, demonstrating that Ca^{2+} can both relay hormonal signals and feedback to modulate hormone biosynthesis, forming tightly controlled regulatory loops (Jan *et al.*, 2024). Ca^{2+} also interacts with ROS to amplify stress signals. ROS production can trigger Ca^{2+} influx, while Ca^{2+} activates ROS generating enzymes, creating positive feedback loops that propagate systemic stress signals. These Ca^{2+} ROS waves coordinate responses across tissues, enabling plants to mount rapid and synchronized defense mechanisms. Ca^{2+} signaling crosstalk with secondary messengers such as nitric oxide (NO) and cyclic nucleotides, regulating programmed cell death, immune responses, and metabolic adaptation under stress.

Advances in imaging, biosensors, and computational modeling have revealed that plants encode information in Ca^{2+} signatures through variations in amplitude, frequency, and spatial distribution. These combinatorial codes allow simultaneous integration of multiple signals, supporting highly nuanced physiological responses. The integration of Ca^{2+} with other signaling networks highlights its role as a master regulator, coordinating environmental perception and adaptive responses, while also highlighting the complexity that poses challenges for fully deciphering and manipulating these pathways for crop improvement.

4. FUNCTIONAL ROLES OF CALCIUM SIGNALING IN PLANT DEVELOPMENT AND STRESS TOLERANCE

Calcium signaling is important in regulating a broad spectrum of plant physiological processes (Hamouzová *et al.*, 2023; Wdowiak *et al.*, 2024), linking environmental perception to adaptive responses. Its role extends from fundamental developmental processes to complex stress tolerance mechanisms, highlighting the versatility of Ca^{2+} as a signaling molecule. In plant development, Ca^{2+} orchestrates key processes such as cell division, elongation, differentiation, and organ patterning. Ca^{2+} gradients in growing pollen tubes guide directional growth, ensuring successful fertilization, while root tip Ca^{2+} oscillations regulate meristem activity and root architecture. During embryogenesis and leaf development, spatiotemporal Ca^{2+} signatures modulate hormone-mediated signaling pathways, coordinating tissue differentiation and organ morphogenesis. Calcium dependent protein kinases (CDPKs) and calmodulin-like proteins (CMLs) translate these oscillatory signals into precise transcriptional and metabolic outputs, ensuring robust developmental outcomes even under fluctuating environmental conditions. Calcium signaling is equally critical for plant responses to abiotic and biotic stresses. Under drought, salinity, or extreme temperature (Naz *et al.*, 2024), rapid cytosolic Ca^{2+} elevations trigger downstream signaling cascades that activate stress responsive genes, osmoprotectant synthesis, and antioxidant defenses. Similarly, in pathogen interactions, Ca^{2+} mediates the activation of defense responses, including the hypersensitive response and systemic acquired resistance. The interplay between Ca^{2+} and secondary messengers such as reactive oxygen species (ROS) and nitric oxide (NO) amplifies these responses, enabling coordinated systemic signaling across tissues. Moreover, the specificity of Ca^{2+} signaling lies in the unique temporal and spatial patterns commonly referred to as Ca^{2+} signatures generated in response to distinct stimuli. These signatures encode information that determines the amplitude, timing, and nature of the physiological response, allowing plants to fine tune their growth and defense strategies. Technological advances in live-cell imaging, genetically encoded Ca^{2+} sensors, and computational modeling have significantly enhanced our understanding of how these signatures operate at cellular and whole-plant levels. Calcium signaling serves as an integrative and versatile regulator, linking environmental cues to developmental programming and stress adaptation. Its ability to encode and decode complex information through dynamic Ca^{2+} patterns underscores its potential as a target for improving crop resilience and productivity under changing environmental conditions.

5. CHALLENGES IN PLANT CALCIUM SIGNALING RESEARCH

Despite remarkable progress in understanding plant calcium (Ca^{2+}) signaling, several challenges continue to limit our ability to fully exploit this pathway for crop improvement. One major difficulty lies in the complexity of Ca^{2+} signatures. Plants encode information in the amplitude, frequency, duration, and spatial localization of Ca^{2+} transients, but these signals often overlap between different stimuli, making it challenging to assign specific physiological outcomes to individual Ca^{2+} patterns. Another key challenge is the spatiotemporal regulation of Ca^{2+} signals. While advanced imaging techniques have improved visualization of cytosolic Ca^{2+}, organellar dynamics in mitochondria, chloroplasts, and the endoplasmic reticulum remain poorly characterized. The integration of Ca^{2+} signals across tissues and developmental stages is not fully understood, limiting our capacity to predict whole plant responses. The crosstalk between Ca^{2+} and other signaling pathways, including hormones, ROS, and secondary messengers, adds another layer of complexity. Although these interactions are essential for better understanding of responses, they complicate mechanistic studies and raise difficulties in isolating the effects of specific Ca^{2+} components.

Genetic redundancy and functional overlap among Ca^{2+} channels, pumps, and sensors hinder functional characterization. Many Ca^{2+}-related genes exhibit overlapping roles, and knockouts often produce subtle or compensatory effects, making it difficult to assign clear biological functions. Translating findings from model species to crops introduces further complications, as orthologous Ca^{2+} components may differ in regulation or specificity. Addressing these challenges requires the integration of high resolution imaging, functional genomics, computational modeling, and cross species validation to achieve a systems level understanding of Ca^{2+} signaling.

6. PERSPECTIVES AND OPPORTUNITIES

The emerging understanding of plant calcium signaling offers several promising avenues for research and biotechnological applications. Technological innovations will continue to be crucial, particularly the development of next-generation genetically encoded Ca^{2+} indicators and advanced microscopy, which can resolve sub cellular and tissue level dynamics in real time. These tools will enable precise mapping of Ca^{2+} signatures under physiological and stress conditions. Synthetic biology and gene editing approaches offer opportunities to manipulate Ca^{2+} channels, sensors, and signaling modules with high specificity. CRISPR-Cas and other genome editing tools allow targeted modulation of Ca^{2+} components, potentially enhancing stress tolerance, growth, and yield without triggering adverse pleiotropic effects. Designing synthetic Ca^{2+} circuits could also enable customizable responses to environmental cues.

Systems level and computational modeling approaches are becoming increasingly important. Machine learning and predictive modeling can decode complex Ca^{2+} patterns, integrate multi-signal networks, and identify key regulatory nodes for targeted manipulation. This integrative approach will facilitate the translation of fundamental insights into practical crop improvement strategies. Also understanding the interplay between Ca^{2+} signaling and other stress responsive pathways will be important for developing crops resilient to multiple stresses simultaneously. Exploiting the modularity and combinatorial nature of Ca^{2+} signaling could allow the understanding of adaptive responses in diverse environmental conditions. The future of plant calcium signaling research lies in combining molecular, computational, and biotechnological innovations to enhance plant performance in a changing climate.

7. CONCLUSION

Calcium signaling is a central regulatory hub in plants, coordinating responses to environmental cues, developmental processes, and stress conditions. Over the past decade, significant advances have improved our understanding of the molecular components, including Ca^{2+} channels, pumps, exchangers, and diverse sensor proteins, as well as the decoding mechanisms that translate Ca^{2+} signatures into physiological outcomes. Technological innovations, such as genetically encoded Ca^{2+} indicators, high resolution imaging, and computational modeling, have enabled unprecedented insight into the spatiotemporal dynamics and complexity of Ca^{2+} signaling networks. Despite these advances, challenges remain. The intricate nature of Ca^{2+} signatures, crosstalk with other signaling pathways, genetic redundancy, and limited organelle- and tissue level resolution continue to constrain a full mechanistic understanding. These obstacles highlight the need for integrative approaches that combine molecular, computational, and systems biology techniques to decipher the Ca^{2+} code and predict plant responses under complex environmental conditions. Looking forward, targeted manipulation of Ca^{2+} signaling through gene editing, synthetic biology, and precision breeding offers considerable potential for enhancing plant growth, development, and resilience. By harnessing the modularity and flexibility of Ca^{2+} networks, future research can contribute to the development of crops capable of thriving under increasingly variable climates. Overall, continued exploration of plant calcium signaling promises both fundamental insights and practical applications, positioning it as a key frontier in plant biology and agricultural innovation.

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