Science Bulletin 70 (2025) 125–127

Contents lists available at ScienceDirect

Science Bulletin

journal homepage: www.elsevier.com/locate/scib

News & Views

Advancing insights into calcium homeostasis and signaling in plant growth and resilience

Songchong Lu [a], Yan Sun [a], Lichao Ma [b], Sheng Luan [c,*], Guofeng Yang [b,*]

[a] College of Life Sciences, Qingdao Agricultural University, Qingdao 266109, China
[b] Key Laboratory of National Forestry and Grassland Administration on Grassland Resources and Ecology in the Yellow River Delta, College of Grassland Science, Qingdao Agricultural University, Qingdao 266109, China
[c] Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA

Calcium is a crucial macronutrient and functions as a widespread signal in eukaryotes, ranging from yeast, plants to animals. As crucial second messengers, calcium ions (Ca^{2+}) play indispensable roles in plant growth and development, response to external stressors, and signal transduction by modulating downstream cellular responses, including gene expression, metabolic activities, and transport functions [1,2]. Unlike other nutrients, the levels of Ca^{2+} in the extracellular space and in certain intracellular stores are typically 10,000 times higher than resting cytoplasmic Ca^{2+} ($[Ca^{2+}]_{cyt}$) levels. This substantial gradient poses potential risks of $[Ca^{2+}]_{cyt}$ overload and toxicity, and it can also lead to a rapid and transient increase in $[Ca^{2+}]_{cyt}$, which is a key signaling mechanism for plants to respond to environmental cues. To mitigate the cytotoxicity of Ca^{2+} and continuously maintain the potential to generate specific Ca^{2+} signals, it is essential to keep the resting $[Ca^{2+}]_{cyt}$ at a low level of approximately 100 nmol/L[3]. Calcium homeostasis is fundamental for maintaining cellular function and organismal health in plants. Upon perceiving a wide array of environmental stimuli, such as pathogen invasion, changes in temperature, salt stress, or nutrient availability, plants exhibit prompt and temporary increases in $[Ca^{2+}]_{cyt}$, known as 'Ca^{2+} signatures', which act as a versatile signaling hub to orchestrate cellular responses. The formation of these patterns depends on the coordinated actions of various calcium transporters, which mediate calcium influx and efflux across cellular membranes. We summarize the recent studies that uncovered new transporters and/or mechanisms of regulation that shape Ca^{2+} signatures in plants cells and orchestrate effective response to external cues [3–15].

Ca^{2+} transport systems in plants. The cytosolic Ca^{2+} signature is built by diverse membrane-localized Ca^{2+} permeable ion channels and transporters [1,2], each of which plays a crucial role in regulating the dynamic balance of Ca^{2+} within the cell. Influx channels in plants include, but may not be limited to, cyclic nucleotide-gated channels (CNGCs), glutamate receptor like proteins (GLRs), two-pore channel 1 (TPC1), calcium permeable stress-gated channels (CSCs) or hyperosmolarity-induced $[Ca^{2+}]_{cyt}$ channels (OSCAs), WeiTsing (WTS), and the mildew resistance locus O (MLO) family proteins, which facilitate the entry of Ca^{2+} into the cytosol from extracellular or intracellular stores [2]. Notably, an increasing number of NLRs (nucleotide binding and leucine-rich repeat receptor proteins) have been demonstrated to serve as Ca^{2+}-permeable channels, mediating effector-triggered immunity (ETI) [1,2]. Ca^{2+} efflux transporters such as Ca^{2+}-ATPases (ACAs) and Ca^{2+}/H^{+} exchangers (CAXs) mediate the efflux of Ca^{2+} from the cytosol to either the external space or intracellular organelles [2,3]. These efflux transporters are crucial for maintaining cellular homeostasis and orchestrating various physiological responses.

Mechanisms underlying the control of Ca^{2+} influx. When plants are exposed to various stressors ranging from herbivores and microorganisms to abiotic factors like drought and extreme heat, a significant surge in intracellular calcium levels occurs, triggering a cascade of biochemical reactions, known as decoding of calcium signals, essential for the plant's adaptive response [1,2]. The elevation of $[Ca^{2+}]_{cyt}$ is often mediated by calcium influx channels, but the specific Ca^{2+} channels responsible for the Ca^{2+} influx triggered by different external stressors, as well as the mechanisms regulating these channels, have only emerged in recent years. We provide a few examples to illustrate progress in this area.

A study by Tian et al. [4] opened up this area of research by identifying a CNGC-type channel that is activated during pathogen-associated molecular patterns (PAMPs) -triggered immunity (PTI). They showed that in *Arabidopsis* CNGC2-CNGC4 channel is inhibited by the calcium sensor protein calmodulin (AtCaM7) in the resting state. During pathogen attack, BOTRYTIS-INDUCED KINASE1 (BIK1), a kinase downstream of the pattern-recognition receptor complex, phosphorylates the channel and relieves this inhibition. A similar mechanism may also operate in other plants such as rice [7]. It has been demonstrated that OsCNGC9 is essential for resistance to rice blast and for the generation of the chitin-induced Ca^{2+} signaling, and whose Ca^{2+} channel activity is further enhanced by OsRLCK185-mediated phosphorylation [7]. Additionally, it was reported that OSCA1.3, a Ca^{2+}-permeable channel, may be specifically involved in stomatal immunity [8]. Other candidate channels, such as *Arabidopsis* ACCELERATED CELL DEATH 6 (ACD6), may also be involved in PTI [15].

* Corresponding authors.
E-mail addresses: sluan@berkeley.edu (S. Luan), yanggf@qau.edu.cn (G. Yang).

https://doi.org/10.1016/j.scib.2024.09.031

The other arm of plant immune response, the ETI has also been shown to rely on calcium entry [9–12]. The study by Bi et al. [11] demonstrated that the CNL HOPZ-ACTIVATED RESISTANCE 1 (ZAR1) resistosome forms a Ca^{2+}-permeable channel, through which the influx of Ca^{2+} triggers a cascade of signaling events, including the activation of Ca^{2+}-dependent protein kinases and the production of reactive oxygen species, ultimately leading to defense responses including localized cell death (hypersensitive response). The research of Jacob et al. [9] has identified plant helper NLRs as Ca^{2+}-permeable nonselective cation channels that mediate calcium entry and cell death. Moreover, the newest findings revealed that the helper NLR, NLR-required for cell death protein 4 (NRC4), assembles into a hexameric resistosome that may act as a Ca^{2+} permeable channel, mediating Ca^{2+} entry upon sensing pathogen infection [10]. Together with work on a wheat NLR that also forms a calcium-permeable channel [12], these studies demonstrate how plant immune receptors directly control cytoplasmic Ca^{2+} levels and initiate cell death, a hallmark of effector-triggered immunity.

Recent studies further place calcium signaling as a central hub of plant immunity. Wang et al. [13] identified 'WeiTsing', a calcium-permeable cation-selective channel localized in the endoplasmic reticulum in *Arabidopsis*, which confers broad-spectrum resistance to the devastating clubroot disease caused by *Plasmodiophora brassicae* (Pb). Using a unique screen to identify cell death-suppressing genes, Yu et al. [14] discovered an alternative receptor kinase Back-To-Life 2 (BTL2) that induces strong immunity by activating CNGC20, a typical calcium-permeable channel.

In the context of calcium signaling in systemic response, GLR-type channels have been identified as important players [1]. A recent study [5] identified Ca^{2+}calmodulin-mediated desensitization of GLR channels, crucial in wounding and anti-herbivore defense. Calmodulin (AtCaM7) binds to the C-terminal domain of GLR3.3 in a Ca^{2+}-dependent manner, desensitizing the channel to prevent prolonged Ca^{2+} influx and potential cytotoxicity. A CRISPR-edited GLR3.3 allele with impaired desensitization enhanced plant defense against herbivores without affecting normal growth [5]. This finding may offer a promising avenue for agricultural innovation towards pest control in place of the current heavy use of pesticides.

The latest research by Pei et al. [6] has identified a pair of OSCA-type channels in plant response to changing water availability. The identification of OSCA2.1 and OSCA2.2 as hypo-osmosensitive channels provides valuable insights into the molecular mechanisms underlying membrane response to cell turgor change. The researchers showed that these OSCA proteins are activated under hypo-osmotic stress in bacteria, suggesting their role as osmosensors. The *osca2.1*/*osca2.2* double mutant displayed reduced rate in pollen germination. This defect correlated with an impaired cytosolic Ca^{2+} spiking in pollen grains upon hydration, indicating that OSCA2.1/2.2 play an essential role in hydration-induced calcium oscillation during pollen germination (Fig. 1).

Regulatory mechanisms of calcium efflux. While recent research has primarily focused on the significance of Ca^{2+} influx in triggering Ca^{2+} signals, effective efflux mechanisms for removing surplus Ca^{2+} from the cytosol are equally crucial for terminating these signals, thus forming a signaling initiation and termination cycle. It has long been suggested and supported by increasing experimental evidence that Ca^{2+}/H^+ exchangers (CAXs, antiporters) and Ca^{2+}-ATPases (ACAs) are accountable for transporting Ca^{2+} from the cytosol to either the extracellular space or intracellular stores [2]. For instance, vacuolar CAX1 and CAX3 have been identified as crucial components for plant adaptation to varying external Ca^{2+} conditions, likely achieved through the sequestration of excess Ca^{2+} into the vacuolar lumen [3]. At the plasma membrane, ACA8 and ACA10, in association with the Ca^{2+}-dependent phospholipid-binding protein BONZA1 (BON1), are implicated in regulating $[Ca^{2+}]_{cyt}$ levels [2]. Other ACAs localized to the ER (eg., ECAs) and vacuole membranes also participate in maintaining cytosolic Ca^{2+} homeostasis under various conditions [2]. While all plant ACAs contain a calmodulin (CaM)-binding domain and may be activated in a Ca^{2+}/CaM-dependent manner in response to elevated $[Ca^{2+}]$cyt levels [2], the activation mechanism of CAX family members in response to elevated $[Ca^{2+}]$cyt remains unclear.

The recent study by Wang et al. [3] presents a comprehensive examination on how plants manage calcium (Ca^{2+}) levels to balance growth and defense mechanisms. The notable contribution from the study by Wang et al. is the identification and characterization of two distinct signaling pathways that regulate vacuolar Ca^{2+}/H^+ exchangers (CAXs) to maintain cytosolic Ca^{2+} homeostasis. The first pathway involves Calcineurin B-like (CBL) proteins and CBL-interacting protein kinases (CIPKs). This pathway responds primarily to elevated external Ca^{2+}levels. The CBL-CIPK complex, sensing and activated by elevated cytosolic calcium, derepresses CAXs by phosphorylating a serine cluster within their auto-inhibitory domains. This phosphorylation event triggers the transport of excess Ca^{2+} into the vacuole, thus preventing cytosolic Ca^{2+} toxicity. The second pathway is associated with the plant's immune response and involves the FLS2-BAK1 receptor complex and kinases such as BIK1 and PBL1 [3]. This pathway is activated by pathogen-associated molecular patterns (PAMPs) and, like the CBL-CIPK pathway, phosphorylates the same serine cluster on CAXs through BIK1 and PBL1 in a calcium-independent manner, modulating Ca^{2+} signals that are crucial for initiating a moderate immune response in pattern-triggered immunity. This dual mechanisms of CAX activation underscores the versatility and importance of these exchangers in plant physiology. Furthermore, Wang et al. effectively illustrate how plants integrate calcium signaling to maintain growth and defense balance. Simply put, cytosolic calcium levels serve as an indicator for growth or defense: sustained elevation of calcium is an "alarm system" to trigger defense response and possibly cell death, whereas low resting levels is favorable for growth (Fig. 1). The plant's ability to swiftly and accurately modulate Ca^{2+} influx and efflux is critical to avoid toxicity and to utilize Ca^{2+} as a second messenger in signaling pathways to support adaptation in response to a variety of environmental conditions [2]. Future work should be directed to understanding the interplay between ACAs and CAXs in regulating plant fitness and immunity under specific conditions. Notably, vacuolar sequestration mediated by CAXs, but not by ACAs, is highlighted as a crucial mechanism for $[Ca^{2+}]_{cyt}$ homeostasis under external Ca^{2+} stress [3]. However, the coordination between CAXs and ACAs in controlling plant growth and immunity remains ambiguous and necessitates additional research. In addition, CAXs may collaborate with influx channels like CNGC2/4 to maintain normal plant growth in soil, as disruptions in either CNGC2/4 or CAX1/3 lead to growth inhibition and autoimmunity [3].

Understanding the molecular pathways for Ca^{2+} regulation not only advances fundamental plant biology but also provides new strategies for crop improvement. By manipulating these pathways, it might be possible to enhance plant growth and immunity simultaneously. For example, genetic engineering approaches could be used to develop crops with modified CAX activity, optimizing their ability to manage Ca^{2+} levels under stress conditions such as high salinity or pathogen attack.

In summary, stimulus-specific patterns of Ca^{2+} change, known as 'Ca^{2+} signatures', play a crucial role in various physiological processes [2]. Plants have evolved sophisticated mechanisms to

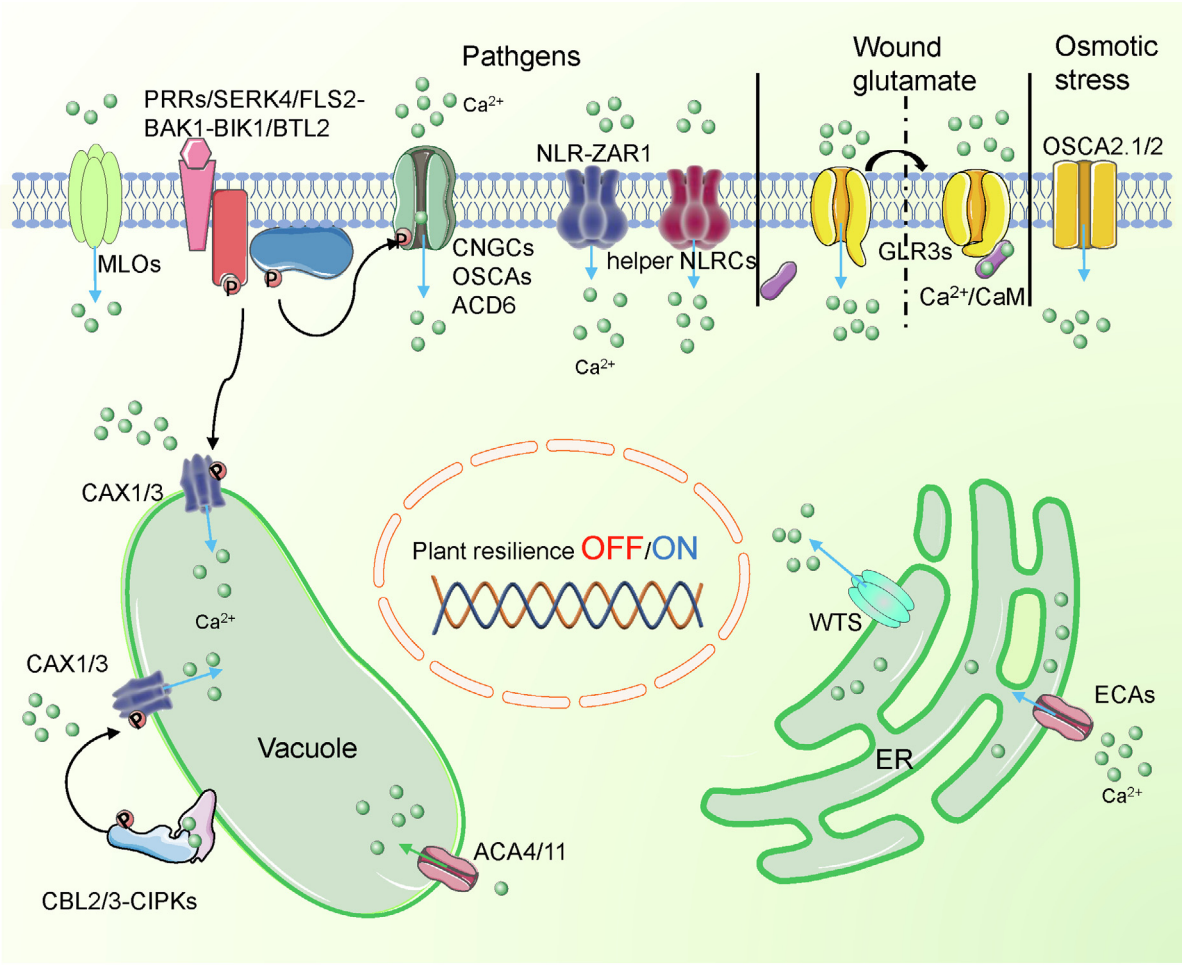

Fig. 1. Recent advances in calcium homeostasis and signaling in plant cells.

maintaining Ca^{2+} homeostasis. By understanding the sophisticated mechanisms of regulating $[Ca^{2+}]_{cyt}$ homeostasis, we can unlock novel strategies to bolster plant resistance to biotic stressors such as pests and pathogens, as well as abiotic stressors like salt, drought and high temperatures, while preserving or even improving crop yields. Exploring calcium signaling pathways and using gene-editing tools (such as CRISPR) presents promising opportunities for innovative approaches in agricultural technology, which could reduce the use of fertilizers and pesticides and ensure food security worldwide.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

We regretfully extend our apologies to the authors whose valuable work could not be cited owing to space limitations. This work was supported by the National Natural Science Foundation of China (31701063), the Talents of High-Level Scientific Research Foundation of Qingdao Agricultural University (6631120074 and 6651118004), and Shandong Forage Research System (SDAIT-23-01). Research in Sheng Luan group was supported by the National Science Foundation (2344945).

References

[1] Jiang Y, Ding P. Calcium signaling in plant immunity: a spatiotemporally controlled symphony. Trends Plant Sci 2023;28:74–89.
[2] Luan S, Wang C. Calcium signaling mechanisms across kingdoms. Annu Rev Cell Dev Biol 2021;37:311–40.
[3] Wang C, Tang RJ, Kou S, et al. Mechanisms of calcium homeostasis orchestrate plant growth and immunity. Nature 2024;627:382–8.
[4] Tian W, Hou C, Ren Z, et al. A calmodulin-gated calcium channel links pathogen patterns to plant immunity. Nature 2019;572:131–5.
[5] Yan C, Gao Q, Yang M, et al. Ca^{2+}/calmodulin-mediated desensitization of glutamate receptors shapes plant systemic wound signalling and anti-herbivore defence. Nat Plants 2024;10:145–60.
[6] Pei S, Tao Q, Li W, et al. Osmosensor-mediated control of Ca^{2+} spiking in pollen germination. Nature 2024;629:1118–25.
[7] Wang J, Liu X, Zhang A, et al. A cyclic nucleotide-gated channel mediates cytoplasmic calcium elevation and disease resistance in rice. Cell Res 2019;29:820–31.
[8] Thor K, Jiang S, Michard E, et al. The calcium-permeable channel OSCA1.3 regulates plant stomatal immunity. Nature 2020;585:569–73.
[9] Jacob P, Kim NH, Wu F, et al. Plant "helper" immune receptors are Ca^{2+}-permeable nonselective cation channels. Science 2021;373:420–5.
[10] Liu F, Yang Z, Wang C, et al. Activation of the helper NRC4 immune receptor forms a hexameric resistosome. Cell 2024;187:4877–89.
[11] Bi G, Su M, Li N, et al. The ZAR1 resistosome is a calcium-permeable channel triggering plant immune signaling. Cell 2021;184:3528–41.
[12] Förderer A, Li E, Lawson AW, et al. A wheat resistosome defines common principles of immune receptor channels. Nature 2022;610:532–9.
[13] Wang W, Qin L, Zhang W, et al. WeiTsing, a pericycle-expressed ion channel, safeguards the stele to confer clubroot resistance. Cell 2023;186:2656–71.
[14] Yu X, Xie Y, Luo D, et al. A phospho-switch constrains BTL2-mediated phytocytokine signaling in plant immunity. Cell 2023;186:2329–44.
[15] Chen J, Li L, Kim JH, et al. Small proteins modulate ion-channel-like ACD6 to regulate immunity in Arabidopsis thaliana. Mol Cell 2023;83:4386–97.