Calcium Signaling of Heat Shock in Crop Plants

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Climate change and the increasing frequency of high temperature (HT) events are significant threats to global crop yields. To address this, a comprehensive understanding of how plants respond to heat shock (HS) is essential. Signaling pathways involving calcium (Ca^{2+}), a versatile second messenger in plants, encode information through temporal and spatial variations in ion concentration. Ca^{2+} is detected by Ca^{2+}-sensing effectors, including channels and binding proteins, which trigger specific cellular responses. At elevated temperatures, the cytosolic concentration of Ca^{2+} in plant cells increases rapidly, making Ca^{2+} signals the earliest response to HS.

crop yield   thermotolerance   signaling

1. Ca^{2+} Signaling and Plant Thermotolerance

The rapid rise in CO_2 levels on Earth is causing global warming, leading to more frequent extreme high-temperature (HT) events. Heat is a major factor in reducing crop yields. It disrupts homeostasis, affects seed germination, and alters (and ultimately stunts) plant growth [1]. Without sufficient adaptation, genetic enhancements, and fertilization, every one degree Celsius increase in global mean temperature could lead to significant reductions in global wheat, rice, and maize yields (averaging 6.0%, 3.2%, and 7.4%, respectively) [2]. Therefore, understanding the molecular mechanisms underlying plant responses to HT stress is vital for improving agricultural production and ensuring future food security.

HT can elevate the intracellular concentration of calcium (Ca^{2+}), a common second messenger in both animal and plant cells. Plants exposed to HT can experience heat shock (HS), which triggers a rise in cytosolic Ca^{2+} and disrupts the oscillations in Ca^{2+} levels [3]. Ca^{2+} plays a critical role in maintaining the normal physiological functions of plant cells and is involved in various physiological processes in plants. Additionally, as a ubiquitous second messenger, Ca^{2+} participates in plant responses to various stressors. Thus, understanding the impact of Ca^{2+} on plant thermotolerance is essential for the development of heat-resistant crops [4][5].

Ca^{2+} is a versatile intracellular signal; information is encoded based on temporal and spatial patterns of Ca^{2+} concentration changes. These patterns are decoded by Ca^{2+}-sensing effectors such as Ca^{2+}-permeable channels and Ca^{2+}-binding proteins to initiate specific cellular responses [5]. The induction of the Ca^{2+} signal represents the most rapid response to elevated temperatures in plants. In one study, the cytosolic Ca^{2+} concentration in wheat peaked within 10–15 min of a sudden temperature increase from 24 to 36 °C before returning gradually to baseline as the HS response (HSR) continued [6].

Ca^{2+} entry into the cytoplasm is facilitated by several families of protein channels, including cyclic nucleotide-gated channels (CNGCs), glutamate (Glu) receptor-like channels (GLRs), annexins, and mechanosensitive (MS) channels. Each of these channel types plays a crucial role in promoting an influx of Ca^{2+}. Furthermore, plants possess various Ca^{2+}-binding proteins that decode and transmit the primary Ca^{2+} signal to elicit specific cellular responses. These proteins include calmodulins (CaMs), CaM-like proteins (CMLs), Ca^{2+}-dependent protein kinases (CDPKs or CPKs), Ca^{2+}- and CaM-dependent protein kinases (CCaMKs), calcineurin B-like proteins (CBLs), and CBL-interacting protein kinases (CIPKs). When these proteins bind Ca^{2+}, they undergo conformational changes that allow them to initiate downstream signaling events via interactions with other proteins or molecules. This mechanism enables plants to translate variations in the intracellular Ca^{2+} concentrations into specific cellular responses, including changes in gene expression, enzyme activity, or ion channel activity [7][8][9].

Recent studies have focused on understanding how plants detect Ca^{2+} increases due to HS, and many studies have identified crucial molecules and signaling pathways involved.

2. Ca^{2+}-Permeable Channels Perceive Elevated Temperatures

Presently, the identity of a definitive thermosensor in plants remains elusive. It is hypothesized that Ca^{2+} channels located in the plasma membrane (PM) are crucial players in perceiving elevated temperatures. These channels could be activated directly or indirectly, leading to increased cytoplasmic Ca^{2+} levels. Consequently, thermosensors may function as Ca^{2+} channels, directly modulating Ca^{2+} signaling in response to external stimuli. Alternatively, they may act as regulatory elements that influence the membrane lipid composition, which is closely linked to Ca^{2+} channel activity. Another possibility is that they operate as GLRs, initiating an influx of Ca^{2+} in response to external stimuli.

Genome sequencing has revealed that plants lack typical animal Ca^{2+} channels such as voltage-dependent Ca^{2+} channels, transient receptor potential channels, purinergic P2X receptor channels, and cysteine loop channels. Instead, they have expanded families of CNGCs, GLRs, annexins, reduced hyperosmolarity-induced $[Ca^{2+}]$ increase channels (OSCAs), "Mid1-complementing activity" channels (MCAs), two-pore channels (TPCs), MS-like channels (MSLs), and Piezo channels (MSPs). **Figure 1** shows several potential candidates among these Ca^{2+}-permeable ion channels.
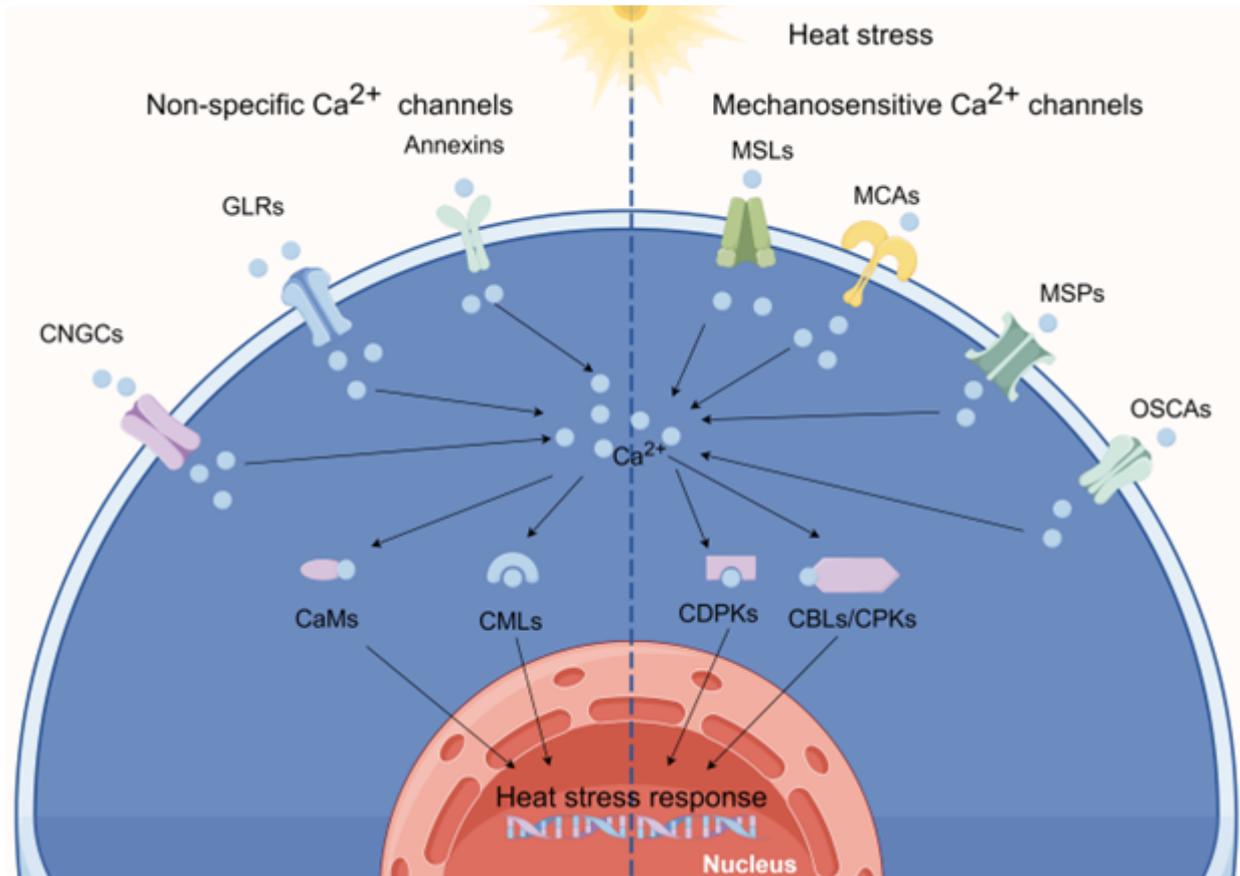
Figure 1. calcium (Ca^{2+}) channels involved in sensing heat. (By Figdraw).

2.1. Heat Sensing via CNGCs

CNGCs are non-specific cation channels that regulate the flow of Ca^{2+} by binding to ligands such as cAMP and cGMP. CNGCs are activated in response to various abiotic and biotic stresses [10][11]. Plant and animal CNGCs belong to the K-selective shaker channel family and share similarities in their amino acid sequences and overall structures [12]. They possess cyclic nucleotide-binding domains and one or more CaM-binding domains at their cytosolic N- and C-termini [13][14].

HS induces PM fluidization, allowing an influx of Ca^{2+} into the cytoplasm. Furthermore, nucleotide cyclases can elevate cAMP and cGMP levels under HS conditions, further promoting the influx of Ca^{2+} and activating associated signaling pathways. CNGCs were initially discovered in barley [15]; subsequent studies identified 20 and 16 CNGCs in the genomes of *Arabidopsis* and rice, respectively [16][17]. Currently, plant CNGCs are known to participate in a range of biological processes [18][19][20][21].

2.2. Heat Sensing via GLRs

In mammals, ionotropic Glu receptor cation channels (iGluRs) respond to the neurotransmitter Glu, initiating Ca^{2+} signal cascades [22]. The first evidence of Glu signaling in plants was uncovered by Lam et al. [23], who identified the *AtGLR* gene family in *Arabidopsis*. Through sequence analysis and comparisons, homologous Glu receptor

variants have been identified in dicot and monocot species [24][25][26]. The *Arabidopsis* genome contains 20 GLRs, while the rice and poplar genomes harbor 13 and 61 GLRs, respectively [27][28]. Plant GLRs display significant sequence and structural homologies with animal iGluRs, including high degrees of amino acid sequence identity in their channel and ligand-binding domains [29].

2.3. Heat Sensing via Annexins

Annexins, an evolutionarily conserved family of proteins present in a variety of organisms, are renowned for their ability to bind to Ca^{2+} and phospholipids. They play pivotal roles in a plethora of cellular processes [30][31]. Annexins exhibit peroxidase and ATPase/GTPase activities and are associated with the regulation of Ca^{2+} channels [32]. Their functions encompass a wide range of intracellular and extracellular phenomena, including vesicular trafficking, organization of the membrane–cytoskeleton, exocytosis, endocytosis, phagocytosis, ion channel regulation, and apoptosis [33]. The genomes of *Arabidopsis*, rice, and wheat contain 8, 10, and 25 annexin genes, respectively [34][35][36]. Annexins feature a conserved protein core domain capable of binding both Ca^{2+} and phospholipids alongside an N-terminal domain that varies in sequence and length among different annexins [37].

In plants, annexins participate in environmental stress responses, and they play roles in growth, development, and signaling [38]. Some plant annexins regulate the level of free cytosolic Ca^{2+}, and certain annexins can form Ca^{2+}-permeable channels in lipid bilayers or vesicles [39]. Moreover, plant annexins may possess peroxidase activity or ATPase/GTPase activity, each contributing to functional specificity [40]. Annexins play a broad regulatory role in diverse biochemical and cellular processes, including Ca^{2+} channel regulation, and in plant growth, development, and biotic and abiotic environmental stress responses [40].

2.4. Heat Sensing via OSCAs

Recently discovered MS Ca^{2+} channels called OSCAs have the ability to detect and respond to changes in osmotic pressure, regardless of whether it originates externally or internally. OSCAs are essential for regulating the flow of Ca^{2+} in plants; thus, they play a crucial role in plant growth and adaptation to environmental stress. OSCA1, the first OSCA identified in *Arabidopsis thaliana*, is responsible for raising cytosolic Ca^{2+} levels by triggering an influx of Ca^{2+}. In fact, this channel is responsible for the increase in intracellular Ca^{2+} triggered by multiple stimuli in plants [41]. In total, 15, 11, 12, and 62 *OSCA* genes have been identified in *Arabidopsis* [41], rice [42], maize [43], and cotton [44], respectively.

2.5. The Functions of MSLs, MCAs, and MSPs in Plants following HT Treatment

In addition to OSCAs, plants possess several other types of MS ion channels, including MSLs, MCAs, and MSPs. These channels are permeable to Ca^{2+} and play crucial roles in enabling plants to respond to mechanical stimuli and changes in osmotic pressure.

MSLs are MS ion channels found in both bacteria and plants. In *Arabidopsis*, MSL2 and MSL3 are essential to protect plastids against bursting inside leaf epidermal cells during growth under conditions of high osmotic pressure

[45]. MSL8 responds to PM distortion during pollen grain rehydration and germination [46]. Additionally, MSL10 and MSL9 exhibit MS ion channel activity in root protoplasts [47]. In rice, most *OsMSL* genes are expressed during reproductive growth, suggesting their involvement in plant growth, development, and stress responses [48].

While the direct involvement of these channels in the HSR has not been extensively studied, their potential role in such responses warrants further investigation.

3. Ca^{2+}-Binding Protein Involvement in the HSR

Under HS conditions, the intracellular Ca^{2+} concentration in plants increases, primarily due to an influx of Ca^{2+} from outside the cell or the release of Ca^{2+} from intracellular stores [49][50]. This rise serves as a pivotal "signal" that triggers a complex signal transduction cascade. This signal, in conjunction with downstream effector proteins, including CaMs, CMLs, CBLs, and CDPKs/CPKs, serves as a molecular code that must be interpreted by the cell. Together, these proteins create a sophisticated signaling network that transmits stress signals and orchestrates a multitude of responses in plants [51].

3.1. CaMs in HS Signaling

CaMs, highly conserved sensor proteins containing EF-hand motifs, are found in various plant organelles. In total, 9, 5, and 8 *CaM* genes have been identified in *Arabidopsis* [52][53], rice [54], and maize [55], respectively. CaMs are ubiquitously expressed in all eukaryotic cells and possess a dumbbell-shaped structure. They are multifunctional, consisting of two globular lobes at the N- and C-termini connected by a flexible central linker. Each lobe contains a pair of EF-hand motifs capable of binding Ca^{2+} ions with positive cooperativity [56]. The EF-hand motif in CaM is characterized by a helix–loop–helix (D-X-D) conformation, consisting of 12 amino acid residues, totaling 36 amino acid residues across both motifs. In the D-X-D motif, the 14th and 16th positions are fixed, while the 15th position can be occupied by any amino acid [57]. These motifs are involved in interactions with a diverse array of downstream target proteins, including ion channels, pumps, antiporters, kinases, phosphatases, transcription factors, and enzymes involved in metabolic pathways. *Arabidopsis* CaM isoforms CaM1/4, CaM2/3/5, CaM6, and CaM7 were found to bind to CNGC6 to varying degrees, and this binding was dependent on the presence of Ca^{2+} and IQ6, an atypical isoleucine–glutamine motif in CNGC6. Knockout of *CaM2*, *CaM3*, *CaM5*, and *CaM7* genes led to a marked increase in PM inward Ca^{2+} current under HS conditions; however, knockout of *CaM1*, *CaM4*, and *CaM6* genes had no significant effect on PM Ca^{2+} current [58]. In some instances, these motifs function as transcription factors, recognizing and binding to target genes, thereby modulating stress responses in plants.

3.2. CMLs in HS Signaling

CMLs differ from CaMs in their length and the number of EF-hand motifs they possess. While CaMs typically contain four EF-hand motifs, CMLs can have varying numbers, ranging from one to six. CMLs are termed "CaM-like" due to their 15% amino acid identity with CaM. These proteins play a crucial role in physiological responses to

various stresses, including salinity, drought, heat, and cold. In *Arabidopsis*, 50 CMLs have been identified, compared with 32 in rice [59].

3.3. CDPKs in HS Signaling

CDPKs, or CPKs, belong to a class of protein kinases that possess four C-terminal EF-hand motifs, which enable them to sense changes in intracellular Ca^{2+} levels. CDPKs are effector proteins that play pivotal roles in regulating a wide range of physiological processes, including environmental stress responses, in various plant cell types [60]. CDPKs consist of several domains, including a variable N-terminal domain, a serine/threonine protein kinase domain, an autoinhibitory junction domain (JD), and a C-terminal CML regulatory domain (CaMLD) connected by a tether. The CaMLD is composed of four EF-hand Ca^{2+}-binding motifs. At low Ca^{2+} concentrations, the C-lobe of a CDPK already contains Ca^{2+} and interacts with the JD, stabilizing its conformation. The JD forms a helical structure that blocks substrate access by being buried within the active site of the kinase domain due to an intramolecular interaction. When the concentration of Ca^{2+} rises, both the N-lobe and C-lobe of the CaMLD interact with the JD, leading to a substantial conformational change that releases the active site [61].

The CDPK family is diverse, with multiple members and isoforms across plant species. Recent whole-genome expression analyses have shed light on the transcriptional regulation of CDPKs in response to various stresses, including HS, in important crop species [62][63][64][65][66][67]. These findings provide valuable insight into the functional roles of CDPKs and their potential utility in enhancing the thermotolerance of crops.

3.4. CBLs and CIPKs in HS Signaling

CBLs comprise a distinct family of Ca^{2+} sensors in plants with essential roles in Ca^{2+} signaling pathways. Specifically, they interact with and modulate the activity of CIPKs. CBLs bear resemblance to the calcineurin B-subunit in yeast and neuronal Ca^{2+} sensors in animals [68]. CBLs possess four EF-hands, which are Ca^{2+}-binding motifs found in various proteins. EF-hands are structural domains characterized by a D-X-D structure, with the loop region serving as the Ca^{2+}-binding site. These domains allow proteins to function as Ca^{2+} sensors that can respond to changing levels of intracellular Ca^{2+}. In the case of CBLs, the four EF-hand domains facilitate the capture of Ca^{2+} ions, enabling them to play pivotal roles in numerous plant physiological processes [69]. Notably, CBLs lack intrinsic kinase activity. To transmit signals, they must form complexes with CIPKs, which are serine/threonine kinases found in plants. CIPKs possess a functional kinase domain but remain in an inactive state because of the autoinhibition caused by an interaction between the kinase domain and regulatory domain. An inhibitory motif known as NAF/FISL blocks the active site in CIPKs, preventing substrate binding and subsequent phosphorylation. This autoinhibitory mechanism keeps CIPKs in an inactive state until they are activated by binding to a CBL. Once active, the CIPK can regulate downstream proteins. This activation process is crucial for the proper functioning of the CBL–CIPK signaling pathway in plants [70]. When plants encounter stress, such as HS, the intracellular concentration of Ca^{2+} rises. This increase enables Ca^{2+} ions to bind to the EF-hand motifs in CBLs. This binding promotes the interaction of CBLs with the NAF/FISL element in CIPKs. Consequently, the CIPKs become active and can participate in stress response pathways by phosphorylating downstream proteins.

4. Ca^{2+} Signaling Networks Mediate the Plant HSR

Ca^{2+} are ubiquitous second messengers in eukaryotes, participating in a wide array of signaling pathways and responses to various environmental conditions. As mentioned above, when plants experience HS, the cytosolic Ca^{2+} concentration rises because of an influx of Ca^{2+} facilitated by membrane-localized Ca^{2+} permeable cation channels. These ions subsequently bind to Ca^{2+}-binding proteins, initiating signal transmission to their respective downstream pathways. In recent years, substantial progress has been made in understanding the mechanisms related to thermotolerance in plants.

The HSR signaling pathways in plants include the Ca^{2+} dependent, ROS, NO, HSF-HSP, HSF-independent, hydrogen sulfide (H$_2$S), and unfolded protein response (UPR) pathways, etc. [4][71]. There are interactions and crossovers between different HS pathways. For example, the Ca^{2+} signaling may intersect with the ROS, NO, and HSF-HSP pathways, forming a complex signaling network [4]. Additionally, in the HSF-independent pathway, Ca^{2+} might contribute to the activation of certain transcription factors independent of HSF [72][73]. The H$_2$S pathway introduces a novel dimension, suggesting that Ca^{2+} may modulate signaling events in conjunction with H$_2$S [74][75]. The association of Ca^{2+} with endoplasmic reticulum (ER) stress responses suggests a potential link between Ca^{2+} and UPR [76][77]. These propositions underscore the versatility of Ca^{2+} signaling and its potential contributions to diverse HSR pathways in plants. Future research is needed to experimentally validate these hypotheses and enhance our understanding of the intricate molecular mechanisms governing plant responses to HS.

Several potential downstream pathways of Ca^{2+} in response to HS in plants are shown in **Figure 2**.
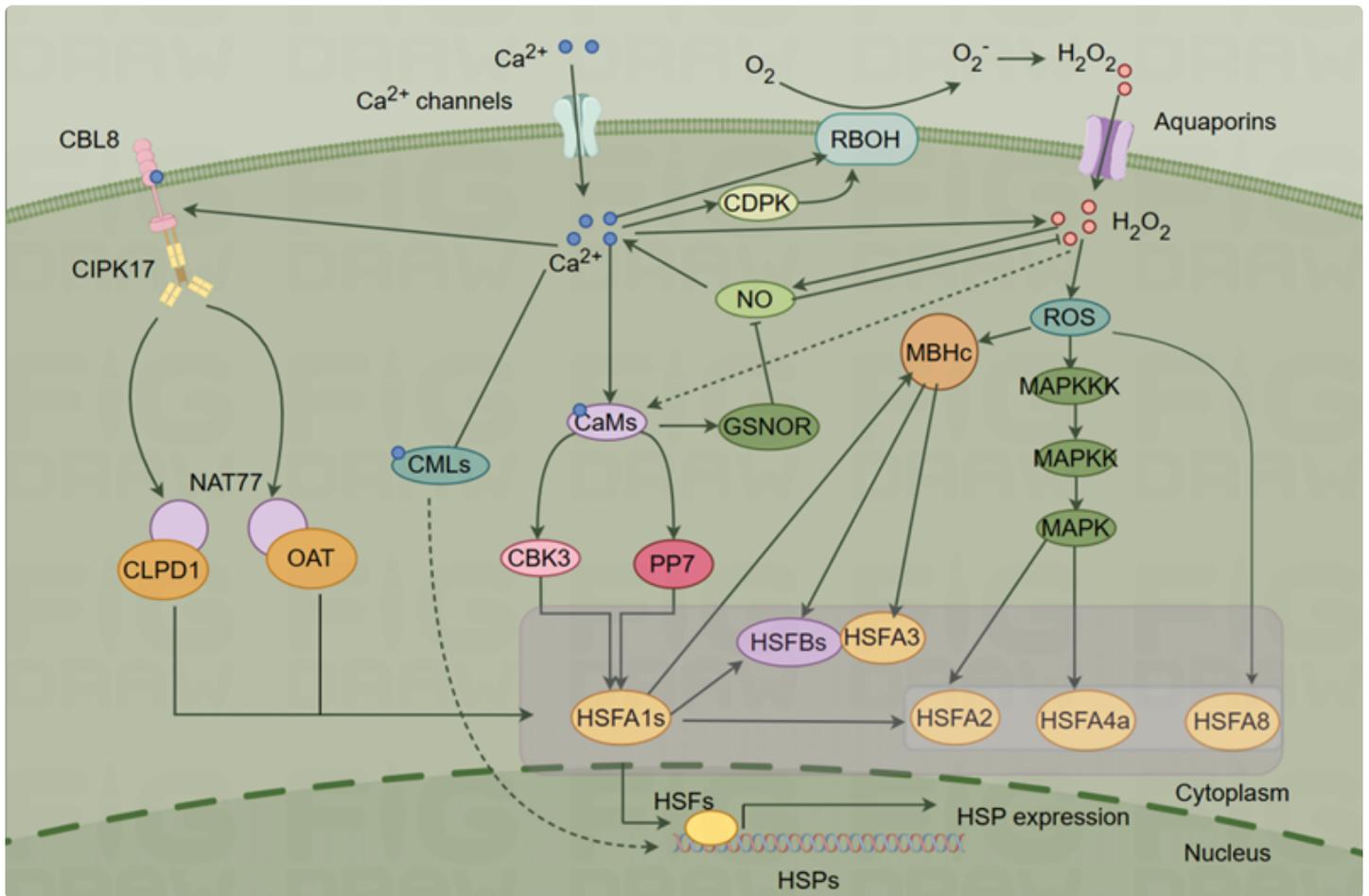
Figure 2. Ca^{2+} signaling pathway under heat shock (HS) (By Figdraw).

5. Conclusions and Perspectives

In addition, the complex interplay of signaling pathways, including Ca^{2+}, NO, ROS, and others, deserves further investigation. Based on the above-mentioned research gaps, future research should prioritize the following areas: (1) Integration of existing pathways. There is a need to integrate various fragmented pathways into a unified primary Ca^{2+} signaling pathway associated with HS. This endeavor could result in a more comprehensive understanding of the role of Ca^{2+} signaling in heat resistance. (2) Identification of new components. Ongoing efforts should focus on identifying new components that sense Ca^{2+} signals induced by HS. This continuing exploration will contribute to an improved understanding of the HSR pathway in plants. (3) Ca^{2+} crosstalk. Understanding the crosstalk between Ca^{2+}-mediated HSR and other stress-signaling pathways is essential. This knowledge can shed light on the broader regulatory network that governs plant responses to multiple stressors.

A deeper understanding of the molecular mechanisms may enable the identification of key genes and pathways so as to provide targets for genetic engineering. Moreover, marker-assisted selection, next-generation molecular breeding, precision breeding, and genome editing techniques represent powerful tools to enhance the efficiency of plant breeding programs. These methods will allow breeders to select plants with desirable traits more accurately

and quickly and speed up the development of crops better suited to elevated temperatures. These improvements will be conducive to promoting sustainable agriculture.

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