Calcium as environmental sensor in plants

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Plants are constantly exposed to a changing and often unfavourable environment which has led to the evolution of adaptive strategies that permit plant cells to sense environmental stimuli and to activate responses that allow avoidance or survival of environmental stresses. A central theme in the current research in stress biology has been the elucidation of signalling pathways and their components controlling the stress-response regulons. Efforts have been directed by several laboratories across the globe to understand how environmental cues are sensed and transduced to the level of gene expression. A number of interacting signal pathways appear to control the activation of stress-responsive genes. Sensing environment by plants must involve a highly precise, specific and finely regulated mechanism in the form of ‘language’. Over the years, search for such a ‘language’ has narrowed down to a divalent cation, calcium (Ca$$^{2+}$$), that acts as a ubiquitous messenger in the responses of a cell to the environment. With the advent of some novel techniques, the measurement of intracellular Ca$$^{2+}$$ concentration has become easy. This has led to unequivocal evidence for involvement of Ca$$^{2+}$$ in several plant responses to the environment. Since development of crops tolerant to abiotic stresses is an important aspect in agricultural research, understanding the involvement of Ca$$^{2+}$$ in facing these challenges becomes imperative and worthwhile. Hence, the present review focuses on some recent aspects of Ca$$^{2+}$$ functioning in relation to some selective abiotic factors.

Calcium (Ca$$^{2+}$$) has many important structural and physiological roles in plants. It is important in maintaining the stability of the cell walls, membranes and membrane-bound proteins, due to its ability to bridge chemical residues among these structures. It is absorbed from the soil solution and transported to various sites in the plants through the xylem, like other minerals. Its mobility is low and its concentration in the cell is kept to the minimum, generally due to active sequestration into cell organelles and rapid chelation. In phloem, Ca$$^{2+}$$ concentrations are minimized by phosphate and other chelators. Recently, it has been found to regulate the phloem P-proteins in legumes. Ca$$^{2+}$$ mediates several plant processes like cytoplasmic streaming, thigmotropism, gravitropism, cell division, cell elongation, cell differentiation, cell polarity, photomorphogenesis, plant defence, stress-responses and stress protection, and the list is growing rapidly. Among all its roles, being a second messenger in several plant processes has attracted major attention and recent research has revolutionized our thinking about the physiology of plant development, as we realize the involvement of Ca$$^{2+}$$ in mediating diverse processes. Now, it has been firmly established as an important component of a diverse array of plant signal transduction pathways. Due to advancement in techniques to precisely measure and monitor expression of intracellular Ca$$^{2+}$$, like use of fluorescent dyes and luminescent protein aequorin, the progress in the search of a role for Ca$$^{2+}$$ in cell signalling has been exciting, and almost every process involving cell-cell communication has been found to depend upon Ca$$^{2+}$$. Among the various plant processes involving Ca$$^{2+}$$, responses of plants to their ever-changing environment draws particular attention. Intracellular Ca$$^{2+}$$ concentration is kept low (100 to 200 nM) and is precisely regulated in order to save the cell from toxicity. Various environmental stimuli affect Ca$$^{2+}$$ channels located at the plasma membrane and organelar membranes to elevate its levels in the cytosol, which may serve to transduce messages and amplify signals by triggering an intracellular cascade of biochemical processes. ATP-dependent Ca$$^{2+}$$-pumps (Figure 1) or H$$^{+}$$ gradient-driven Ca$$^{2+}$$/H$$^{+}$$ antiporters located variously at the plasma and intracellular membranes maintain low cytosolic Ca$$^{2+}$$ by translocating Ca$$^{2+}$$ into the external space and internal pools. Opening of Ca$$^{2+}$$ permeable channels in the plasma or intracellular membranes therefore allows Ca$$^{2+}$$ to move down its concentration gradient into the cytosol, and hence to generate a Ca$$^{2+}$$ signal. Ca$$^{2+}$$ is mobilized from its stores by two major types of Ca$$^{2+}$$-releasing ligands, inositol 1,4,5-triphosphate (IP$_3$) and cyclic ADP-ribose (cADPR). Since different Ca$$^{2+}$$ pools may be accessed in response to external stimuli to generate various types of intracellular responses varying in magnitude and duration (Figure 1), the spatial localization of Ca$$^{2+}$$ influx into the cytosol may contain the key information within the general Ca$$^{2+}$$ signalling response. In fact, Ca$$^{2+}$$ alterations have been found to be highly specific for different environmental variations and have even been observed to be cell- and organelle-specific. For example, the response in the endodermis and pericycle in the presence of salt and osmotic stress is different from other cell types. The stimulus-specific elevations in cytosolic Ca$$^{2+}$$, called Ca$$^{2+}$$ signatures are exhibited in the form of waves or
A temporal and spatial change of Ca\(^{2+}\) and its amplitude seems to determine the Ca\(^{2+}\) signatures precisely and is probably responsible for specificity\(^{27}\). The change in cytosolic Ca\(^{2+}\) concentration as a result of stimulus-sensing can initiate a cascade of downstream events involving binding of Ca\(^{2+}\) with some specific effector proteins, which activate a sequence of reactions leading to gene activation in the nucleus to respond to external stimuli. These proteins are also referred to as Ca\(^{2+}\) sensors and include calmodulin (CaM)\(^{28}\), Ca\(^{2+}\)-dependent protein kinases (CDPKs)\(^{29}\), Ca\(^{2+}\)-regulated phosphatases, annexins and integrins\(^{30}\). The Ca\(^{2+}\) sensors (CaM, CDPK and other Ca\(^{2+}\)-binding proteins) do exhibit tissue-specific expression and are likely to be important in decoding the Ca\(^{2+}\) signals. Detailed description about these proteins is provided elsewhere\(^{7,29,30}\).

Understanding the components of Ca\(^{2+}\) signalling pathways that mediate environmental stress responses is essential to enhance future genetic-engineering strategies for developing stress-tolerant crops. In this context, the recent status of Ca\(^{2+}\) in relation to the following abiotic factors has been reviewed here.

**Gravisensing**

Gravisense is believed to involve the positioning of statoliths, measuring their actual positions by cytoskeleton-mediated changes, which must be transformed into an endocellular signalling pathway\(^{31}\). The actin cytoskeleton is linked with the signalling system of the plasma membrane through its G-proteins\(^{32}\). The actin-binding protein, profilin, was reported to be closely associated with the phosphoinositide signal-transduction system, directly controlling the availability of polyphosphoinositides for second-messenger production\(^{33}\). Inositol 1,4,5-triphosphate (IP\(_{3}\)) is known to be involved in the mobilization of Ca\(^{2+}\) from intracellular stores\(^{34}\), while IP\(_{3}\) receptors have also been identified as Ca\(^{2+}\) channels at the plasma membrane. These IP\(_{3}\) receptors are reported to be connected through F-actin network to intracellular Ca\(^{2+}\) channels\(^{35}\). It was proposed that gravity-induced structural changes in actin-based cytoskeleton activate Ca\(^{2+}\) channels, resulting in quick release of Ca\(^{2+}\) from internal stores. Moreover, F-actin-dependent plasma-membrane adhesion sites (responsible for perception of gravisensing) are closely asso-

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**Figure 1.** Hypothetical model showing the effect of various stimuli on Ca\(^{2+}\) release channels located on plasma membrane (PM) and organelles. The specificity of different signals in releasing Ca\(^{2+}\) from intracellular stores is also indicated. (See text for details and abbreviations.) The question mark on some arrows indicates unknown mechanisms causing cytosolic Ca\(^{2+}\) elevation.
ated with transmembrane integrins (I) that co-distribute with stretch-activated Ca\textsuperscript{2+} channels (SAC; Figure 1) in tip-growing lower plant cells\textsuperscript{36}. The components of phosphoinositol signalling pathway have been recently investigated in maize nodes after their gravistimulation\textsuperscript{37}. IP\textsubscript{3} increases transiently in the faster-growing lower half within 10 s after gravistimulation, and may act by releasing Ca\textsuperscript{2+} from internal stores. The root tips sense gravity through specialized columna cells having amyloplasts\textsuperscript{38} and transduce the response as a signal to the elongation zone of the root. Ca\textsuperscript{2+} and phosphoinositides are suggested to be involved directly in the transmission of signal between the root tip and elongation zone. A marked asymmetric distribution of Ca\textsuperscript{2+} within 10 min across gravistimulated coleoptile was demonstrated by Slocum and Roux\textsuperscript{39}. A rapid depolarization of gravity-stimulated roots has been observed within 2 s, which may open voltage-gated Ca\textsuperscript{2+} channels (VGC) causing influx of Ca\textsuperscript{2+} into the cytosol, which subsequently may stimulate Ca\textsuperscript{2+} release from intracellular stores\textsuperscript{35}. Changes in cytosolic free Ca\textsuperscript{2+} have been documented for Arabidopsis roots after stimulation by touch\textsuperscript{40} and gravity\textsuperscript{36}, and similar changes were observed for CmM too\textsuperscript{35}. Cyclapiazonic acid, a specific inhibitor of ER- Ca\textsuperscript{2+}-ATPases inhibited the graviresponse of cress roots, but not their growth\textsuperscript{41}. The involvement of CmM in graviresponse had also been demonstrated in Arabidopsis, where wild-type plants showed a three-fold increase in CmM expression in early stages of graviresponse, while a decrease in CmM expression was observed in gravity-insensitive mutants\textsuperscript{42}. In order to observe auxin and Ca\textsuperscript{2+} interaction, Young and Evans\textsuperscript{43} applied Ca\textsuperscript{2+} chelators to roots and found inhibition of auxin redistribution, showing involvement of Ca\textsuperscript{2+} in gravitropic response. They suggested that Ca\textsuperscript{2+} distribution was essential for development of auxin gradient. Friedman et al.\textsuperscript{44} have provided evidence for involvement of Ca\textsuperscript{2+} in controlling gravitropic bending of cut snapdragon (Antirrhinum majus L.) spikes by using Ca\textsuperscript{2+} inhibitor, lanthanum chloride. Gravistimulus may be perceived by mechanosensitive ion channels or voltage-dependent Ca\textsuperscript{2+} channels that affect Ca\textsuperscript{2+} fluxes and alter protein kinase activities, which in turn may regulate the activities of enzymes affecting cell-wall extensibility. This aspect needs further attention to investigate the involvement of Ca\textsuperscript{2+}. Although Ca\textsuperscript{2+} has been found to be the key component in graviresponse, several questions still remain regarding the downstream components of Ca\textsuperscript{2+} signalling.

**Photosensing**

Light-stimulated responses in plants are diverse and involve photoreceptors like phytochrome (PhyA, PhyB, PhyC, PhyD and PhyE forms in Arabidopsis)\textsuperscript{45}, cryptochrome (CRY1, CRY2)\textsuperscript{46} and phototropin (NPH1)\textsuperscript{47}. Phytochrome is one of the key photoreceptors for perceiving red and far-red wavelengths and significant progress has been made in dissecting its signal transduction pathways\textsuperscript{48}. Red light triggers many developmental events requiring Ca\textsuperscript{2+}. Some of these have been studied in the past, including chloroplast rotation in Mougeotia, spore germination and cell expansion in Onoclea, leaflet closure in Mimosa, membrane depolarization in Nitella, activation of NAD kinase and inhibition of mitochondrial ATPase\textsuperscript{49}. Light-mediated responses have been shown to involve changes in free cytosolic Ca\textsuperscript{2+} levels\textsuperscript{50}. A direct link between phytochrome and transient rise in cytosolic free Ca\textsuperscript{2+} in leaf chloroplasts of wheat\textsuperscript{51} as well as in protoplasts of dark-grown wheat seedlings\textsuperscript{52}, was demonstrated. Bowler et al.\textsuperscript{53}, while examining phytochrome-deficient mutants of tomato studied the greening of etioplasts to form fully functional chloroplasts and concluded that there are three separate but interactive signalling pathways operating via G-proteins, cGMP, Ca\textsuperscript{2+} and calmodulin. The development of chloroplasts involved the activation of G-protein and combination of cGMP and Ca\textsuperscript{2+}, and the process was inhibited in the presence of Ca\textsuperscript{2+} and calmodulin alone. Earlier, Neuhaus et al.\textsuperscript{54} obtained similar results using a GUS reporter system which demonstrated that phytochrome phototransduction first involved the activation of two or more G-proteins which activated separate signal transduction pathways, one requiring Ca\textsuperscript{2+} and activated calmodulin, the other being Ca\textsuperscript{2+}-independent. Sopory and Chandok\textsuperscript{55} suggested that phytochrome alters the levels of cGMP or Ca\textsuperscript{2+} by interacting with a G-protein, which could then activate an enzyme such as guanyl cyclase or phospholipase-C which in turn produces IP\textsubscript{3} and DAG, resulting in increase in cytosolic Ca\textsuperscript{2+} and activation of protein kinases (Figure 1). Subsequently, Sanan and Sopory\textsuperscript{56} reported a role of G-proteins and Ca\textsuperscript{2+} in phytochrome signal transduction during primary leaf development in Sorghum bicolor. The leaf development was observed to be arrested at the stage of leaf emergence or leaf expansion by the addition of inhibitors of G-proteins or by Ca\textsuperscript{2+} channel blockers. Reports on modulation of Ca\textsuperscript{2+}/CaM protein kinases by light have been rare. Recently, a novel Ca\textsuperscript{2+}/calmodulin-dependent protein kinase (ZmCCKM), characterized from Zea mays\textsuperscript{57}, was found to be autophosphorylated and regulated its kinase activity towards substrate phosphorylation in response to red light\textsuperscript{58}. In Arabidopsis thaliana, AtSR1, a protein kinase which belongs to the SNF1-related protein kinase subfamily-3 showed accumulation of its transcripts in response to light. It was also observed to interact with six calcineurin-B-like proteins of Arabidopsis and especially with AtCBL2. The physiological meaning of the interaction of AtSR1 and AtCBL2 is not clear, but they presumably function in signal transduction of light\textsuperscript{59}. Using epidermal strips of ‘Argenteum’ mutant of Pisi sativum, Elzenga et al.\textsuperscript{60} provided evidence for Ca\textsuperscript{2+} and CaM involvement in red and blue light signal transduction pathways. Ca\textsuperscript{2+}-channel
blockers inhibited the response to both blue and red light, while Ca²⁺-ionophores, ionomycin and A23187 reduced the light response. Furthermore, the light-induced acidification was inhibited by the calcimodulin antagonists W-7 and trifluoperazine, but not by W-5. These calcimodulin inhibitors completely inhibited the red light-induced acidification, but inhibited the response to blue light by only 60–70%. This differential effect on red and blue light-induced responses indicates a role for Ca²⁺-CaM signalling in both the red and blue light responses, while a second process, independent of Ca²⁺, is activated by blue light.

Blue-light regulates plant growth and development and three photoreceptors, CRY1, CRY2, and NPH1, have been identified⁵⁸,⁴⁹,⁶¹,⁶². The transduction pathways of these receptors are poorly understood. Baum et al.⁵³ used transgenic plants containing aequorin to dissect the involvement of these three receptors in the regulation of intracellular Ca²⁺. Pulses of blue light-induced cytosolic Ca²⁺ transients lasting about 80 s in Arabidopsis and tobacco seedlings. Use of organelle-targeted aequorin showed that Ca²⁺ increases are limited to the cytoplasm. Blue-light treatment of cry1, cry2 and nph1 mutants showed that NPH1, which regulates phototropism, is largely responsible for the Ca²⁺ transients. The special response of the Ca²⁺ transient is similar to that of phototropism, further supporting NPH1 involvement. Christie and Jenkins⁵⁴ examined the induction of chalcone synthase (CHS) gene expression by UV-B and UV-A/blue light in Arabidopsis cell-suspension culture. Both the UV-B and UV-A/blue phototransduction processes involved Ca²⁺, although the elevation of cytosolic Ca²⁺ was observed to be insufficient on its own to stimulate CHS expression. The UV-A/blue-light induction of CHS expression did not appear to involve calmodulin, whereas the UV-B response did involve calmodulin indicating that the signal transduction pathways were at least in part, distinct. The UV-B and UV-A/blue-light signalling pathways are therefore different from the phytochrome signal transduction pathway regulating CHS expression. Long and Jenkins⁵⁵ investigated the effect of UV and blue light in signal transduction processes involved in the induction of CHS and phenylalanine ammonia lyase (PAL) gene expression in Arabidopsis cell-suspension culture. Experiments with electron transport inhibitors indicated that plasma membrane redox activity is involved in both signal transduction pathways and is coupled to the regulation of Ca²⁺ release from an intracellular store, generating a Ca²⁺ signal that is required to induce CHS expression. Although participation of Ca²⁺ and CaM has been established in light-mediated signalling, direct involvement of protein kinases associated with Ca²⁺/CaM is awaited.

Mechanosensing

The response to various mechanical stimuli like touch, wind and injuries due to biotic interference is an important factor by which a living plant adapts itself to these stresses⁶⁶. Mechanical signals are important both as environmental and endogenous developmental cues in plants. During the past few years, Knight and coworkers⁶⁷, and Knight and Grattan⁶⁸ have been able to measure short-duration transient rise in cytosolic Ca²⁺ in plant cells by developing recombinant tobacco plants that express the coelenterate Ca²⁺-indicator, aequorin. Stimulation by wind resulted in an immediate increase in aequorin luminescence⁶⁹, probably reflecting increased cytosolic Ca²⁺. Some other mechanical stimuli that result in transient increase in Ca²⁺, like wounding, also induce a rise in Ca²⁺ in small, isolated groups of cells some distance from the wound, as well as at the wound site itself⁷⁰. Little is known about the signalling events and components that link perception of mechanical signals to gene expression in plants. Ca²⁺ has been identified previously as being potentially involved, and a role for ethylene has also been suggested. In Arabidopsis, the expression of some specific genes, like TCH3 was found to be upregulated by mechanical stimuli⁷¹. A role of ethylene, besides Ca²⁺, has been suggested from studies using Arabidopsis mutants ein6, which indicated the requirement of EIN6 protein; but its role in mechanically induced TCH3 expression appears to be independent of ethylene. However, EIN6 and protein kinase activity operated downstream of Ca²⁺ to mediate mechanically stimulated TCH3 expression. The mechanism(s) whereby a plant perceives mechanical stimuli remains almost unresolved. These stimuli, working on membrane systems at cell level, play an important role not only in thigmomorphogenesis but also in various responses to environmental factors, including water, temperature and gravity. This has led to the exploitation of a mechano-sensor or a mechano-receptor at membrane level that perceives these stimuli. The Ca²⁺ elevation appears to result from movement of the tissue probably due to mechanosensory Ca²⁺ channels that detect flexing and compression of cells. Ca²⁺ channels in plasma membranes may be implicated as Ca²⁺ sensors and evidence favours the organelar channels in this context⁷². The stretch-activated Ca²⁺ channel (Figure 1) was observed to cluster in response to mechanical stimulus and make a close association with plant integrin-like proteins⁷³. Integrins, a type of transmembrane glycoproteins, have been established to act as mechanosensors in animals⁷⁴ and their possible action in plants is being explored⁷⁵. It appears that a plant has mechanosensors in both the plasma membrane and the intracellular membrane. In future studies, it will be necessary to prove this hypothesis as well as to elucidate the mechanism whereby such mechanosensors can induce multiple responses of a plant to the environment.

Drought sensing

Among the various abiotic stresses, signal transduction of water stress has invited more attention. Molecules func-
tioning as osmosensors and abscisic acid (ABA) receptors have not been identified in higher plants as yet and based upon knowledge of osmosensors in yeast and bacteria, cloning of homologues of the two component histidine kinase as an osmosensor is in progress in higher plants. ABA occupies a central place in almost all the stresses causing dehydration of cells and its signal transduction has been the subject of recent interest [2,7]. ABA signalling [4] has been found to be dependent or independent of Ca++. Ca++ involvement in water-stress response has been shown by identification of stress- and ABA-inducible mRNAs that code for a Ca++-binding membrane protein in rice [5] and for a phosphatidylinositol-specific phospholipase-C in Arabidopsis [70]. ABA raised the levels of cytosolic Ca++ in hypocotyls and roots of parsley and coleoptiles of corn [77]. The link between ABA and Ca++ was further proved in a study on maize leaf protoplasts, where a barley HVA promoter was fused to reporter genes that could be activated by applied ABA or by various stress conditions [78]. The expression of the reporter gene could also be induced by treating the protoplasts with 1 mM Ca++ and Ca++ ionophores, or by overexpressing constitutively active forms of the normally Ca++-dependent protein kinases from Arabidopsis ATCDPK1 and ATCDPK1a, even in the absence of these stimuli. Stomatal response to water stress has been extensively studied to investigate the signal transduction pathways in guard cell [79]. Stress-induced ABA is perceived at receptors located in the plasma membrane of guard cells or in the cytosol. ABA elevates the cytosolic Ca++ (ref. 74), but the origin of Ca++ required to increase its cellular level is unclear [80]. Evidence has been presented for influx of Ca++ across plasma membrane [81] or its release from intracellular stores mediated by IP3 and/or cADPR [72]. ABA activates the S-type anion channels through increase in Ca++ levels and inhibits the inward-rectifying K+ channels at the same time [82]. Evidence for Ca++-independent signalling pathways directing stomatal control also exists, and involves rise in pH by ABA [83]. The involvement of kinases and phosphatases has been proved by inhibitor studies in stomatal regulation in Vicia faba and Commelina [84]. Both Ca++-dependent protein kinases [85] and phosphatases [86] have been inferred to be potential candidates in modulating the activity of various ion channels in guard cells, but their precise roles in ABA-signalling have not been clearly established at present. In a recent study on a facultative CAM plant, Mesembryanthemum crystallinum, Cushman [87] observed that water stress can induce the CAM pathway and Ca++ plays a central role in the signalling events following stress perception [88]. The functional role of stress-induced CDPKs (McCPK1) from M. crystallinum by defining proteins with which they interact, such as two-component pseudo-response regulators [89], is currently under investigation and will lead to a better understanding of this adaptation.

Salt sensing

Salinization is a major environmental stress limiting crop production [90]. Salt stress disrupts ion homeostasis in plants, resulting in excess toxic Na+ in the cytoplasm and a deficiency of essential ions such as K+ (ref. 90). Various ion transporters function to limit Na+ entry into and exit out of plant cells, to regulate Na+ compartmentation in the vacuole, and to selectively import K+ over Na+ into plant cells [91]. When the plants are subjected to salt stress, some of the ion transporters need to be activated or to have their activities enhanced, whereas others (e.g. Na+ influx transporters) may need to have their activities suppressed. In addition, the transcript levels of many of the transporters are increased or decreased in response to salt stress [90]. A signalling pathway for regulation of ion homeostasis and salt tolerance has emerged following the recent cloning and biochemical characterization of several SOS (salt overly sensitive) pathway genes and gene products from Arabidopsis (Figure 1). The initial receptor for Na+ sensing remains to be identified. SOS1 encodes a plasma membrane-localized Na+/H+ antiporter [92]. Thus, the biochemical and physiological function of SOS1 is to remove Na+ from the cytoplasm and export it to the extracellular space or the root medium. SOS1 is indicated to be Na+ sensor and has been suggested to have a long cytosolic tail, besides having transmembrane domains [92]. The role of SOS1 in K+ acquisition may be indirect and could possibly arise through H+ coupling with H+-K+ co-transporters [93]. The SOS2 gene encodes a serine/threonine protein kinase with an amino-terminal catalytic domain and a carboxy-terminal regulatory domain [93]. SOS2 has been found to negatively regulate the expression of AtPLC1 (Arabidopsis phospholipase-C) [94]. The SOS3 gene encodes a myristoylated Ca++-binding protein [95] with a sequence similarity to the regulatory B-subunit of calcineurin (a Ca2+-dependent protein phosphatase-2B) and neuronal Ca++ sensors [96]. SOS3 does not appear to function through a protein phosphatase. SOS3 physically interacts with and activates a protein kinase encoded by SOS2 (ref. 97). The sequence of signal events begins with elevation of Ca++ in response to salt stress and its binding with SOS3, which leads to interaction with SOS2 and activation of the kinases. The components of the SOS pathway, either SOS3 or upstream elements, might be associated with an osmotically responsive channel through which Ca++ influx could initiate the signalling. The SOS3–SOS2 kinase complex regulates the transcript levels of SOS1 and activates the Na+/H+ antiporter activity, as observed in isolated plasma membrane vesicles under salt stress [98]. It is yet to be ascertained whether or not SOS3–SOS2 directly regulates the activities of SOS1 and other transporters through phosphorylation. The SOS3/SOS2/SOS1 interaction seems to be operative only in plants and differs from yeast that has calcineurin as a downstream target of Ca2+ signalling.
though a similarity exists between this calcineurin and SOS3. Among the SOS signal-pathway outputs are transport systems that facilitate ion homeostasis. Ca\(^{2+}\) appears to have at least two roles in salt tolerance, a pivotal signalling function in the salt-stress response leading to adaptation and a direct inhibitory effect on a Na\(^+\) entry system.

**Thermosensing**

The understanding of temperature sensing and signalling is also rudimentary\(^9\). Identity of a low temperature (LT) sensor or receptor linked with elevating cytosolic Ca\(^{2+}\) concentration is not known. Ding and Pickard\(^10\) proposed that sensor-receptor for LT may be a Ca\(^{2+}\) channel in the membrane. Based upon these studies, it was hypothesized that proteins such as annexins, which are Ca\(^{2+}\) and phospholipid-binding proteins, capable of forming Ca\(^{2+}\) channels in vitro, may be involved in the LT signal-transduction pathway\(^10\). Recently, two new isoforms of wheat annexin protein with molecular mass of 39 and 22.5 kDa have been identified by Breton et al.\(^1\), and the level of both proteins increased rapidly in response to LT. It has been suggested that a change in membrane fluidity in response to temperature be used as a biological thermometer by *Synechocystis*. Suzuki et al.\(^12\) identified Hik33 and Hik19 as two histidine kinases, necessary for low temperature induction of *desB* (one of the three fatty acid desaturase genes in *Synechocystis*). The inactivation of these kinases results in a reduction of transcription of several low temperature-inducible genes\(^13\). Subsequent experiments by these researchers led to the identification of a gene, *Rer1* (response regulator) that encodes for a putative protein having domains typical of a response element in a two-component regulator. The sequence of events begins with change in membrane fluidity which activates Hik33 that gets autophosphorylated and transfers the phosphate group to Hik19 (Figure 1) and finally to Rer1 and induces the expression of *desB*. Low temperature alters membrane fluidity\(^14\) that leads to cold acclimation through changes in cytoskeleton organization and the induction of Ca\(^{2+}\) fluxes into the cells\(^15\). Whether these types of sensors are also operational in plants remains to be determined. Investigations on the chilling-sensitive species, *Nicotiana plumbaginifolia*, demonstrated that cold shock can induce a large transient rise in cytosolic Ca\(^{2+}\) (ref. 106) and pharmacological studies suggest the possible involvement of membrane fluidity, cytoskeleton arrangement and protein phosphorylation in plant cold responses\(^17\). Murata and Los\(^18\) suggested that a primary signal might be the change in membrane fluidity, which is one of the most rapid effects of temperature on the plasma membrane. The mechanisms by which reduction in membrane fluidity would lead to gene activation are not understood. A relationship between the membrane fluidity and Ca\(^{2+}\)-channel activity at low temperature was proposed\(^10\), but so far, no such mediator has been detected. However, a clear correlation between temperature and Ca\(^{2+}\) influx into the cells has been demonstrated in *Arabidopsis*, suggesting that a temperature-modulated Ca\(^{2+}\) channel could indeed be involved in temperature sensing. Mechanosensitive Ca\(^{2+}\) channels exhibiting temperature-dependent modulation have been identified in plants and they might be involved in low-temperature sensing\(^19\). Further evidence for the role of plasma-membrane fluidity in cold sensing has come from experiments in alfalfa and *Brassica*\(^12\), where chemical agents were used to modulate membrane fluidity. Membrane fluidization during cold treatment inhibited the induction of cold-inducible genes and the development of freezing tolerance. The effects of gene expression were apparently mediated by reorganization of cytoskeleton and a following Ca\(^{2+}\) influx channel activity at low temperature. The presence of low temperature-induced Ca\(^{2+}\)-dependent protein kinases (CDPKs) in *Arabidopsis*\(^10\) and alfalfa\(^10\), and the fact that their inhibition prevents cold acclimation\(^11\) supports the connection between changes in cytosolic Ca\(^{2+}\) levels and protein phosphorylation. ABA has been implicated in several stress responses and is involved in low-temperature response too that leads to its transient increase\(^11\), which in turn triggers downstream events leading to target gene expression. Ca\(^{2+}\) is suggested to act as a signal transducer, which is released through cADPR-controlled release from intracellular stores\(^13\). Both protein kinases and phosphatases have been involved in these events, as observed in phosphatase mutants\(^14\). Calmodulin acts as a signalling component during cold induction of *COR* (cold on regulated) genes (*LTI78* and *KIN1/2*) in *Arabidopsis*\(^19\) and *COR* genes require Ca\(^{2+}\) for expression\(^9\). A recent study by Townley and Knight\(^15\) has shown that overexpression of calmodulin in plants causes inhibition of *COR* gene expression, indicating that CaM may negatively regulate the *COR* gene expression. Thus, the possibility is raised that calmodulin might also act as a negative agent with respect to *COR* gene expression. In this case, in addition to positive Ca\(^{2+}\)-dependent pathways leading to increased *COR* gene expression at low temperature\(^10,10\), there would also be negative Ca\(^{2+}\)-dependent pathways involving calmodulin. The mechanism of action of CaM under such circumstances may involve activation of Ca\(^{2+}\)-ATPases, causing Ca\(^{2+}\) efflux and reducing its intracellular concentration\(^16\). This observation is of immense importance in signal engineering for developing LT-tolerant plants.

**Time sensing**

Plants show many rhythmic phenomena like leaflet and petal movement, stomatal conductance, photosynthetic
rate, ion flux and gene expression\textsuperscript{117}. The biological clock has been suggested to be comprised of three components. The first component (the entrainment pathway) couples the second component (the autonomous regulator) to the environmental periodicity and determines the phase of the free-running rhythm. The third component (the output pathway directed by the oscillator) gives rise to the overt biological rhythm\textsuperscript{118}. Johnson et al.\textsuperscript{119} working on transgenic tobacco and Arabidopsis plants (having aequorin), observed circadian oscillations in free cytosolic Ca\textsuperscript{2+} that can be phase-shifted by light–dark signals. When aequorin was targeted to the chloroplast, Ca\textsuperscript{2+} rhythms were likewise observed after transfer of seedlings of these plants to constant darkness. Circadian oscillations in free Ca\textsuperscript{2+} concentrations can be expected to control many Ca\textsuperscript{2+}-dependent enzymes and processes accounting for circadian outputs. In an attempt to find out whether the plants possess a master clock directing a multitude of diverse rhythmic outputs or whether multiple circadian oscillators exist, either within single cells or distinct morphological structures, Wood et al.\textsuperscript{118} used transgenic plants of tobacco (Nicotiana plumbaginifolia) containing aequorin to investigate the involvement of cytosolic or nuclear Ca\textsuperscript{2+} in circadian rhythms. These plants showed luminescence, thereby directly indicating Ca\textsuperscript{2+} oscillations when challenged with environmental stimuli. Measurement of cytosolic and nuclear Ca\textsuperscript{2+} showed that circadian variations in the cytoplasm are not expressed in the nucleus. The Ca\textsuperscript{2+} rhythmicities of cells and tissues oscillated with distinct differences in phase and this might represent different underlying cellular control mechanisms. Circadian changes in Ca\textsuperscript{2+} are likely to emanate from a number of different cell or tissue locations within the plant and different tissues and cells generate different rhythmic patterns in Ca\textsuperscript{2+} (ref. 118). Further, experiments have indicated that circadian variation in Ca\textsuperscript{2+}-regulated genes is not transcriptionally controlled by nuclear Ca\textsuperscript{2+}, although a cytoplasmic transduction mechanism operating through Ca\textsuperscript{2+} was not precluded. There may be multiple copies of independent oscillators in different plant tissues and expressing circadian controls through regulation of Ca\textsuperscript{2+} rhythmicities.

CAM pathway is controlled by a circadian rhythm and Taybi et al.\textsuperscript{120} working on this metabolism have recently cloned and characterized a CDPK-related protein kinase responsible for phosphorylating phosphoenolpyruvate carboxylase (PEPC) kinase, a key component of the CO\textsubscript{2} pump. In CAM plants, nocturnal phosphorylation renders PEPC considerably less sensitive to inhibition by negative effectors, but both more active and more sensitive to activation by positive effectors. Expression of PEPC kinase is controlled by a circadian oscillator that largely restricts its own mRNA and protein expression to the night. Circadian control of PEPC kinase provides one of the key regulatory steps in controlling the competing actions of PEPC and ribulose 1,5-bisphosphate carboxylase/oxygenase. The role of calcium in entrainment and resetting the clock requires to be examined.

**Engineering Ca\textsuperscript{2+} expression**

Efforts are being made to manipulate signalling components to develop stress-tolerant plants\textsuperscript{121}. It may be possible by regulating signal pathways that control tolerance effectors or modulate effector activity or efficacy. Ca\textsuperscript{2+} expression is also being modulated by targeting Ca\textsuperscript{2+} transporters and protein kinases to alter their stress sensitivity. An enzyme glyoxylase I, which catalyses the conversion of toxic methylglyoxal to a nontoxic metabolite, is expressed in response to NaCl, mannitol or abscisic acid\textsuperscript{122}. Glyoxylase I from Brassica juncea (BjGly I) binds CaM-sepharose and its activity is stimulated by Ca/CaM\textsuperscript{123}. Leaf discs from transgenic plants expressing BjGly I showed tolerance to methylglyoxal and salt compared to control antisense and wild-type leaf discs, indicating that BjGly I plays a role in conferring tolerance to salt stress in plants\textsuperscript{123}. Another example is of inducing salt tolerance by affecting SOS signalling pathway, which, as described above, is Ca\textsuperscript{2+}-activated and maintains Na\textsuperscript{+} and K\textsuperscript{+} homeostasis. SOS2 kinase requires Ca\textsuperscript{2+} for activation and its kinase activity is essential for salt-tolerance determinant function\textsuperscript{124}. The SOS2 C-terminal regulatory domain interacts with the kinase domain to cause auto-inhibition. Deletion of its auto-inhibitory domain or site-specific modifications (Thr to Asp mutation) to the catalytic domain resulted in constitutive expression of SOS2 kinase\textsuperscript{94}, opening the possibility of regulation of stress signalling that controls ion homeostasis. Likewise, modulating the expression or activation of SOS1 (Na\textsuperscript{+}/H\textsuperscript{+} antiporter on plasma membrane) would increase salt tolerance. The SOS1 gene has been shown to encode a putative plasma membrane Na\textsuperscript{+}/H\textsuperscript{+} antiporter in Arabidopsis\textsuperscript{92}. Mutations in SOS1 result in increasing the sensitivity to Na\textsuperscript{+} stress, while its over-expression lowers the Na\textsuperscript{+} content in shoots and improves salt tolerance. Calcineurin, a Ca\textsuperscript{2+}- and CaM-dependent protein phosphatase, as studied in yeast, is required for regulating homeostasis of Na\textsuperscript{+}, K\textsuperscript{+} and Ca\textsuperscript{2+}. Loss of function mutations in CnB (a regulatory subunit of calcineurin) makes the yeast cells more sensitive to Na\textsuperscript{+} inhibition\textsuperscript{125}. Calcineurin regulates the K\textsuperscript{+} transport system under salt stress in yeast by directly or indirectly regulating the phosphorylation of TRK1, a high-affinity K\textsuperscript{+} transporter in yeast cells\textsuperscript{125}. In higher plants, calcineurin-like activity has been reported and appears to regulate the inward K\textsuperscript{+} channel activity\textsuperscript{96} in guard cells, which may lead to modulation of stomatal control by manipulation of this putative phosphatase. Constitutive activation of yeast calcineurin in the host or in plants increased salt tolerance by predisposing the plants to survive stress\textsuperscript{123,126}. In a recent study, a cDNA encoding a novel Ca\textsuperscript{2+}-binding
protein from *Entamoeba histolytica* (EhCaBP) was transferred into tobacco, resulting in enhanced tolerance to salt. Cold-tolerance response has been increased by antisense inhibition of the gene for protein phosphatase 2CA (PP2CA). Transgenic plants altered for components of Ca\(^{2+}\) signalling have been observed to show enhanced production of activated oxygen species, tolerance to heavy metals, cold, salt and drought in rice. Engineering IP\(_3\) functioning has been a target to control Ca\(^{2+}\) expression. The genes encoding two of the phosphatases have been cloned and transgenic plants constructed having altered levels of inositol phosphatases and presumably of IP\(_3\) levels as well. This work is exciting in that IP\(_3\), as a common component of signalling, is an excellent target for manipulating signals from a variety of pathways, and it may be possible to modulate responses of plants to a variety of environmental stresses and several other responses like disease resistance, fertility and hormone perception. In an interesting and novel study being undertaken in the North Carolina State University (NCSU) laboratory of Robertson, the utility of a high-capacity Ca\(^{2+}\)-binding domain of calreticulin has been demonstrated, which might increase Ca\(^{2+}\) stores in plants. The Arabidopsis plants expressing a fusion protein of the C-domain of maize calreticulin and the green fluorescent protein (GFP) showed a delayed loss of chlorophyll after transfer to Ca\(^{2+}\)-depleted medium, 9–35% increase in total Ca\(^{2+}\) for induced C-domain plants compared to controls and, unexpectedly, a significantly increased salt and heavy-metal (aluminum) tolerance. Manipulation of mechanisms regulating heavy-metal transport was conducted by transferring an Arabidopsis gene, *CAX1* (for Ca\(^{2+}\) exchanger 1) into tobacco, which resulted in increasing stress sensitivity. Subsequently, *CAX2* (having low affinity for Ca\(^{2+}\) in yeast) was introduced into tobacco, which resulted in increased tolerance to high manganese levels, and plants were also able to accumulate more Ca\(^{2+}\), cadmium and manganese in the media in which they were grown; but they remained healthy. The *CAX2*-modified plants also had much higher cadmium and manganese transport in isolated root cell membrane vesicles. The present study has an important implication for developing tolerant plants effective in phyto remediation.

**Perspectives**

Calcium research has entered a ‘golden age’ and tremendous advances are being made in our understanding of the involvement of Ca\(^{2+}\) in cellular responses to environmental factors. The next few years will witness the identification of many novel stimulus-specific Ca\(^{2+}\)/CaM-dependent protein kinases and their genes, which will immensely increase our knowledge about functioning of Ca\(^{2+}\) in plants. The genes for various Ca\(^{2+}\) transporters are being identified and will hold the key to regulate cytosolic Ca\(^{2+}\) expression. The connections between membrane receptors and intracellular Ca\(^{2+}\) regulation still remain enigmatic and will be an important subject of research in future. ‘Listening’ to ‘cross-talk’ involving Ca\(^{2+}\) within cells, between cells, tissues and organs will be exciting, to know the coordination among them in response to various stimuli. The complex web of calcium signalling in plants has been equated with neural network of animals, and it may be a part of ‘intelligence’ and ‘learning’ behaviour of the plants to decide their response to at least 17 different environmental factors and their combinations. As the specificity of Ca\(^{2+}\) signalling for specific environmental signals gets unveiled, it will be possible to precisely engineer the Ca\(^{2+}\) expression in plants to make them more tolerant to a stressful environment.


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