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Advances of calcium signals involved in plant anti-drought

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Abstract

Considerable progresses have taken place, both in the methodology available to study changes in intracellular cytosolic calcium and in our understanding of calcium signaling cascades, but how calcium signals function in plant drought resistance is questionable. In plant cells, calcium plays roles as a second messenger coupling a wide range of extracellular stimuli with intracellular responses. Different extracellular stimuli trigger specific calcium signatures: dynamics, amplitude and duration of calcium transients specify the nature, implication and intensity of stimuli. Calcium-binding proteins (sensors) play a critical role in decoding calcium signatures and transducing signals by activating specific targets and corresponding metabolic pathways. Calmodulin is a calcium sensor known to regulate the activity of many mammalian proteins, whose targets in plants are now being identified. Higher plants possess a rapidly growing list of calmodulin targets with a variety of cellular functions. Nevertheless, many targets appear to be unique to higher plants and remain characterized, calling for a concerted effort to elucidate their functions. To date, three major classes of plant calcium signals, including calcium permeable ion channels, Ca^{2+}/H^{+} antiporters and Ca^{2+}-ATPases, have been responsible for drought-stress signal transduction. This review summarizes the current knowledge of calcium signals involved in plant anti-drought and plant water use efficiency (WUE) and presents suggestions for future focus of study. ***To cite this article: H.-B. Shao et al., C. R. Biologies 331 (2008).***

Keywords: Plant; Calcium signals; Calmodulin; Anti-drought; WUE; Guard cells; Breeding

1. Introduction

Calcium ion (Ca^{2+}) has emerged as an important messenger mediating the actions of many hormone and environmental factors, including biotic and abiotic stresses in higher plants. More evidence implicates that Ca^{2+} is involved in regulating such diverse and fundamental processes such as cytoplasmic streaming, thigmotropism, gravitropism, cell division, cell elongation, cell differentiation, cell polarity, photomorphogenesis, plant defense and stress responses [1–26]. It is believed that calcium influx and cytoplasmic calcium increases are important for guard cell abscisic acid (ABA) transduction [27–39]. It is addressed that Ca^{2+}-dependent and Ca^{2+}-independent signaling processes in plants are related to certain putative parallels between initial guard

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cell signaling and both the initiation of defense responses and phytochrome-induced signaling [40–46]. It is generally accepted that a rapid increase in cytosolic calcium concentration is mediated by calcium channels located on the plasma membrane and endomembranes such as vacuolar and endoplasmic reticulum membranes [47–52]. Electrophysiological studies elucidated that plants have Ca^{2+} channels with different types of gating mechanisms: ligand, voltage, and stretch-activated [53–61]. However, only a limited number of genes encoding Ca^{2+} channels have been isolated and functionally expressed. Drought is one of the biggest stresses to agricultural production and quality [62–66]. Plants synthesize mainly the stress hormone ABA in response to drought, triggering a signaling cascade in guard cells that results in stomatal closure, thus reducing water loss that may influence WUE in plants. It was reported that ABA triggers an increase in cytosolic calcium in guard cells, having been proposed to include Ca^{2+} influx across the plasma membrane [67–71]. ABA is known to evoke increases in cytosolic-free $[Ca^{2+}]$, which is dependent on flux through Ca^{2+} channels in the plasma membrane and release from intracellular Ca^{2+} stores [72–79]. It was also reported that ABA induces an increase in cytosolic $[Ca^{2+}]$ in guard cells, which precedes the reduction in stomatal aperture [80–82]. Therefore, it is believed that such $[Ca^{2+}]$ leads to the reduction in stomatal aperture [83,84]. Calcium signal-encoding elements mainly include calcium-permeable ion channels, Ca^{2+}/H^{+} antiporters and calcium ATPases. Calcium permeable channels have been investigated with electrophysiological, biochemical and molecular approaches [85–90]. It has been known that in guard cells, membrane hyperpolarization is directly associated with the elevation of cytosolic $[Ca^{2+}]$, which follows ABA application [91,92]. Specific patterns of Ca^{2+} elevation may be also involved in controlling both the stomatal closure response and the final steady state of stomatal aperture [93,94]. As a physiological trait of great importance regarding plant drought resistance and yield, much more attention is paid to WUE [95–101]. The molecular research regarding the enhancement of WUE plays important parts in the selection and cultivation of drought-resistant or drought-tolerant crop varieties. When breeding for drought tolerance, biomass productivity and water use efficiency are considered important agronomic characters. Guard cells represent the best characterized plant cell type with respect to ion transport and signal transduction. Stomatal closure can be triggered by raising the cytosolic Ca^{2+} concentration to approximately 1 μM or by drought stress due to ABA production [32,56]. It is clear that the possible relations between calcium signals and plant WUE are involved in the regulation of stomatal closure in guard cells [61,72,76,95,102].

2. Typical plant calcium signals

Plant calcium-signal-encoding elements mainly include calcium permeable ion channels, Ca^{2+}/H^{+} antiporters and Ca^{2+}-ATPases.

2.1. Calcium permeable ion channels in plants

The previous definition of a Ca^{2+}-permeable channel, simply as a channel permeable to Ca^{2+}, tacitly assumed that its physiological function was to mediate the Ca^{2+} influx from the apoplast into the cytoplasm [6,8,12,16,26,29,33]. Reports found that the importance of the cellular location of ion channels in determining stimulus specificity is emphasized by a study of Ca^{2+}-mediated stomatal closure in tobacco [71,82,98–100]. Removal of extracellular Ca^{2+} with the chelator EGTA or blockage of the entry with a number of ion channel blockers suggested that low-temperature-induced closure involves primarily entry of Ca^{2+} across the plasma membrane, while intracellular mobilization appears to dominate if stomatal closure is initiated with ABA or mechanical stimulation. Another evidence showed that a wheat gene *LCT1*, encoding a low-affinity cation transporter, can complement yeast mutant with a disruption in the *MIDI* gene, which encodes a stretch-activated Ca^{2+}-permeable non-selective cation channel. *AtTPC1* (*Arabidopsis* two-pore voltage-gated channel1), encoding a two-pore voltage-gated channel with high affinity for Ca^{2+} permeation, was found to rescue the Ca^{2+} uptake activity of a yeast mutant cch1 (which encodes a homologous L-type Ca^{2+} channel) [42]. Cytosolic $[Ca^{2+}]$ was enhanced by overexpressing of *AtTPC1* or suppressed by antisense expression of it under sucrose stress [72,89]. The molecular basis of plasma membrane Ca^{2+}-permeable channel activity is only just becoming apparent, and there is a number of intriguing candidate genes. A unique gene in Arabidopsis, *TPC1* (At4 g03560), encodes a channel with two Shaker-like domains (i.e., 2×6 transmembrane spans, each of which contains a putative 'pore' region) connected by a hydrophilic domain that includes two EF hands. The general structure resembles that of the pore-forming subunits of mammalian and yeast Ca^{2+} channels that contain four Shaker-like domains, and there is some sequence similarity. TPC1 expression enhances Ca^{2+} uptake in yeast Ca^{2+}-channel mutant [92,97]. *OsTPC1*,

the homolog of *AtTPC1*, was also identified and characterized [67,75]. *TaTPC1* gene, a gene encoding a Ca^{2+} permeable channel, was cloned from wheat and located on the plasma membrane through the application of a TATPC1-GFP fusion protein [35,77]. Expression of *TaTPC1* in the yeast mutant lacking CCH1 (homologous to the 1-subunit of a voltage-gated Ca^{2+} channel) can recover its growth through functional complementation, and *TaTPC1*-overexpression in transgenic plants could accelerate the stomatal closing in the presence of Ca^{2+} when compared with the control plants, indicating that the overexpression of *TaTPC1* accelerated the stomatal closing in the presence of Ca^{2+} [99, 102]. It was also found that hyperpolarization-activated Ca^{2+}-permeable channels play a critical role in the response to ABA-induced stomatal closure through the production of reactive oxygen species, notably hydrogen peroxide [87,89,103–105]. In *Arabidopsis* guard cells, hydrogen peroxide stimulates hyperpolarization-activated Ca^{2+}-permeable channels, thereby increasing cytosolic $[Ca^{2+}]$ [105]. Ca^{2+} channels involved in supplying the shoot with calcium are expected to be located primarily in the plasma membrane of root endodermal cells [106,107]. Plasma membrane Ca^{2+} channels from plant roots have been characterized both from calcium flux measurements in isolated vesicles and electrically, either after incorporating vesicles into planar lipid bilayers (PLB) or by patch-clamping root-cell protoplasts. All studies indicate the presence of depolarization-activated Ca^{2+} channels with contrasting pharmacologies. Two distinct Ca^{2+} channel activities have been observed when plasma membrane vesicles derived from rye or wheat roots were incorporated into PLB [108–110]. The inward Ca^{2+} flux through the maxi cation channel is inhibited by ruthenium red, but diltiazem, verapamil and quinine at micromolar concentrations and TEA^+ at millimolar concentrations inhibited the outward K^+ flux through this channel only. The second Ca^{2+} channel observed in PLBs has a lower unitary conductance and is termed voltage-dependent cation channel two (VDCC2). It is reported that plasma membrane calcium channels intracellular signaling and may exert effects on metabolism, gene expression and integrated physiological processes, including cell division and cell elongation through regulating cytosolic $[Ca^{2+}]$ [111]. It is thought that the inward Ca^{2+} current, which generates the cytosolic $[Ca^{2+}]$ gradient, is mediated by the clustering of catalytically active (perhaps mechanosensitive) Ca^{2+} channels at the apex of the root hair. This arrangement would be analogous to the apical clustering of mechanosensitive Ca^{2+} channels involved in osmoregulation and extension of hyphae of the oomycete *Saprolegnia ferax* or rhizoids of *Fucus serratus* [112–115]. It is noteworthy that these channels are inhibited by La^{3+}, but not by nifedipine or verapamil. A model of calcium-permeable channels involving plant temperature sensing was established based on the fact that calcium influx into the cytoplasm is mediated by calcium-permeable channels, which are assumed to be solely dependent on the cooling rate (dT/dt), whereas calcium efflux is mediated by calcium pumps, which have been shown to be dependent on the absolute temperature [116]. Such model suggests that the primary temperature sensor in plants might be a Ca^{2+}-permeable channel [117,118]. A hyperpolarization-activated Ca^{2+}-permeable channel, which can be suppressed by EGTA, trivalent cations, verapamil, nifedipine or diltiazem, was identified on the plasma membrane of *Lilium davidii* D pollen protoplasts with whole-cell patch-clamp recording. This primary evidence showed the presence of a voltage-dependent Ca^{2+}-permeable channel, whose activity may be regulated by extracellular CaM, in pollen cells [119,120].

2.2. Ca^{2+}/H^+ antiporters in plants

The Ca^{2+}/H^+ antiporter plays a key role, together with Ca^{2+}-ATPase, in the accumulation of Ca^{2+} in vacuoles that constitute the primary pool of Ca^{2+} among several organelles of plants. The Ca^{2+}/H^+ antiporter is driven by a pH gradient generated by vacuolar proton pumps. Molecular cloning of the antiporters from *Saccharomyces cerevisiae*, *Arabidopsis thaliana* and mung bean revealed that the antiporter is a highly hydrophobic protein with an acidic motif in the centre [35–39,88]. The Ca^{2+}-transport activity and intracellular localization of the translation product of cDNA for mung bean Ca^{2+}/H^+ antiporter (VCAX1) were examined. When the cDNA was expressed in *Saccharomyces cerevisiae* that lacked its own genes for vacuolar Ca^{2+}-ATPase and the antiporter, VCAX1 complemented the active Ca^{2+} transporters, and the microsomal membranes from the transformant showed high activity of the Ca^{2+}/H^+ antiporter [121,122]. Ca^{2+}/H^+ antiporters may play an important role in specifying the duration and amplitude of specific cytosolic Ca^{2+} fluctuations through regulating Ca^{2+} efflux. The plant Ca^{2+}/H^+ antiporters were cloned by their ability to suppress the Ca^{2+}-hypersensitive phenotype of a *Saccharomyces cerevisiae* mutant. These genes have been termed as cation exchangers (CAX). CAX1 from *Arabidopsis thaliana* is a high-capacity and low-affinity Ca^{2+} transporter, which has been shown to be localized to the plant vacuole; its activity appears to be regulated by

an N-terminal autoinhibitory domain. *Arabidopsis* has up to 12 putative Ca^{2+}/H^{+} cation antiporters (CAX1–11 and MHX), in which CAX1 is a high-capacity and low-affinity Ca^{2+} transporter [123–125]. When heterologously expressed in yeast, CAX1 is unable to suppress the Ca^{2+} hypersensitivity of yeast vacuolar Ca^{2+} transporter mutants due to an N-terminal autoinhibition mechanism that prevents Ca^{2+} transport. Several results suggest that CAX1 is regulated by several signaling molecules that converge on the N-terminus of CAX1 to regulate H^{+}/Ca^{2+} antiporter [62,68,72]. Through using site-directed mutagenesis, 31 mutations in the repeats of the *Oryza sativa* CAX were generated, which translocates Ca^{2+} and Mn^{2+}. Mutant exchangers were expressed in a *Saccharomyces cerevisiae* strain that is sensitive to Ca^{2+} and Mn^{2+} because of the absence of vacuolar Ca^{2+}-ATPase and the Ca^{2+}/H^{+} exchanger. Such Ca^{2+}/H^{+} exchangers have 11 predicted transmembrane domains (TMs) and an acidic residue-rich region between TM6 and TM7. In CAX1, the 9-amino acid calcium domain exists in the hydrophilic loop between TM1 and TM2. This domain is thought to be involved in the selection of Ca^{2+}; however, the sequence has not been found in other CAXs. The C domain located in TM4 may be involved in the selection of Mn^{2+} by *Arabidopsis* CAX2, which is the only plant CAX known to be capable of Mn^{2+} transport. Based on results from the TMpred program 2, the 451-amino acid protein OsCAX1a was predicted to have 11 TMs, like other CAX proteins [67,92,98].

2.3. Ca^{2+}-ATPases in plants

Calcium pumps (Ca^{2+}-ATPases) belong to the superfamily of P-type ATPases that directly use ATP to drive ion translocation. Two distinct Ca^{2+} pump families have been proposed based on protein sequence identities [45–47,98,100]. Members of the type IIA and IIB families, respectively, include the ER-type calcium ATPases (ECAs) and the autoinhibited Ca^{2+}-ATPases (ACAs). In *Arabidopsis*, there are four ECA- and ten ACA-type calcium pumps. Isoform ECA1 appears to be located in the ER, as determined by membrane fractionation and immunodetection [78,105,109]. However, the potential for other isoforms targeting to non-ER locations must be considered. In tomato, there is evidence from membrane fractionation and immunodetection, suggesting that related ER-type calcium pumps (LCA1-related) are present in the vacuolar and plasma membranes [110,111]. It is concluded that activity and stability of Ca^{2+}-ATPase under 2 °C low temperature are the key factors in the development of cold resistance of winter wheat. It is also suggested that the cold-resistant agent CR-4 plays an important role in stabilizing plasma membrane calcium pump (Ca^{2+}-ATPase) under low temperature stress through the electron microscopical observations using the cytochemical method of cerium phosphate precipitation, which indicated that the Ca^{2+}-ATPase activity was mainly localized at the plasma membrane in wheat seedling cells growing at normal temperatures. Therefore, it can be inferred that Ca^{2+}-ATPase is involved in plant responses to drought, salt and water deficits [125–130].

3. Calcium signals and molecular genetics of plant WUE

Plant WUE is an important index for measuring plant drought resistance and yield. In recent years, numerous progresses have been made in the investigation of plant WUE, especially at the molecular level. The ABA-responsive barley gene *HVA*1, a member of group-3 late embryogenesis abundant (LEA) protein genes, was introduced into spring wheat (*Triticum aesti*6*um* L.) cv. Hi-Line using the biolistic bombardment method [36,83,96]. Two homozygous lines and one heterozygous transgenic line expressing the *HVA*1 gene had significantly (PB0.01) higher WUE values, i.e. 0.66–0.68 $g\,kg^{-1}$, as compared to 0.57 and 0.53 $g\,kg^{-1}$, respectively, for the non-expressing transgenic and non-transgenic controls under moderate water deficit conditions. The two homozygous transgenic plant lines also had significantly greater total dry weight, root fresh and dry weight, and shoot dry weight compared to the two controls under soil water deficit conditions. Results of this study indicate that growth characteristics were improved in transgenic wheat plants constitutively expressing the barley *HVA*1 gene in response to soil water deficit [76,79,92,95]. A T-DNA insertion mutant for the *Arabidopsis* ABA-transporter AtMRP5 (mrp5-1) was isolated. Guard cells from mrp5-1 mutant plants were found to be intensive to the sulfonylurea compound glibenclamide, which in the wild type induces stomatal opening in the dark. The knockout in AtMRP5 affects several signaling pathways controlling stomatal movements. Stomatal apertures of mrp5-1 and wild-type Ws-2 were identical in the dark. In contrast, opening of stomata of mrp5-1 plants were reduced in the light. In the light, stomatal closure of mrp5-1 was insensitive to external calcium and ABA, a phytohormone responsible for stomatal closure during drought stress [126–130]. In contrast to Ws-2, the phytohormone auxin could not stimulate stomatal opening in the mutant in darkness. All stomatal phenotypes were complemented in trans-

genic mrp5-1 plants. Both whole-plant and single-leaf gas exchange measurements demonstrated a reduced transpiration rate of mrp5-1 in the light. Excised leaves of mutant plants exhibits reduced water loss, and water uptake was strongly decreased at the whole-plant level. If plants were not watered, mrp5-1 plants survived much longer, due to reduced water use. Analysis of CO_2 uptake and transpiration showed that mrp5-1 plants have increased the WUE.ERECTA gene, encoding a putative leucine-rich repeat receptor-like kinase (LRR-RLK) and known for its effects on inflorescence development, which was isolated and discovered as a major contributor to a locus, called D on *Arabidopsis* chromosome 2 [87]. Its mechanisms include, but are not limited to, effects on stomatal density, epidermal cell expansion, mesophyll cell proliferation and cell–cell contact. What is more, the results also indicate that the ERECTA gene can change both the stomatal number and structure of a leaf, thus regulating plant transpiration rate and WUE (biomass/amount of water used), which demonstrates excellent prosperities in improving crop drought resistance and high WUE [89,123].

4. Calcium signals and aquaporins involved in plant drought resistance

Plant aquaporins play an important role in water uptake and movement, which open and close a gate that regulates water movement in and out of the cells. Some plant aquaporins also play an important role in response to water stress. Since their discovery, advancing knowledge of their structures and properties led to an understanding of the basic features of the water transport mechanism and increased illumination to plant water relations. Meanwhile, molecular and functional characterization of aquaporins has revealed the significance of their regulation in response to the adverse environments such as drought and salinity [34,53,75,125].

Aquaporins, or Major Intrinsic Proteins (MIPs), are channel-forming membrane proteins with the extraordinary ability to combine a high flux with a high specificity for water across biological membranes. They belong to a well-conserved and ancient family of proteins called the major intrinsic proteins (MIPS), with molecular weights in the range of 26–34 kDa, with members found in nearly all living organisms. The aquaporin family in plants is large, indicating complex and regulated water transport within the plant in order to adapt to different environmental conditions, which includes more than 150 membrane channel proteins. Regulation of aquaporin-mediated water flow, through indirect or direct means, appears to be a mechanism by which plants can control cellular and tissue water movement. All aquaporin isoforms probably work together in an orchestrated manner, where each individual aquaporin isoform displays a specific localization pattern, substrate specificity, and regulatory mechanism [6,46, 79,91,130].

Terrestrial plants have evolved to cope with rapid changes in the availability of water by regulating all aquaporins that lie within the plasma membrane [30, 43,56,68]. Regulation of aquaporin trafficking may also represent a way to modulate membrane water permeability, and the factors affecting and regulating aquaporin behaviors involve phosphorylation, heteromerization, pH, Ca^{2+}, pressure, solute gradients, drought, flooding and so on, which suggests that aquaporins are involved in a versatile and dynamic regulation of water movement [20,32,50,57]. The abundance and activity of aquaporins in the plasma membrane and tonoplast may be regulated, hence enabling the plant to tightly control water fluxes into and out of its cells, as well as within the cells [5,6,19,50].

Currently, powerful evidence indicates that cellular biochemistry and physiology of a living organism is seriously affected by ion homeostasis [32–41,56,98]. Mercury (Hg^{2+}) has been used extensively to provide evidence for the involvement of aquaporins in water transport process in animal and plant cells [67]. Due to mercury-induced conformational changes and identification of conserved surface loops in plasma membrane aquaporins from higher plants, mercury is thought to bind to sulphydryl groups of the aquaporin proteins, physically blocking the channels and reducing their hydraulic conductivity [9,111]. Partial recovery of the water flow rate following the application of mercuric chloride was also observed in tomato and aspen root systems, implying the presence of aquaporins as the regulators of plant water status [85,96]. However, the inhibition of water flow with mercurial reagents is not completely understood, and is not a general characteristic of aquaporins [28,38]. Some mercurial reagents, especially mercuric chloride, are highly membrane-permeate and are powerful metabolic inhibitors. That is why the effect of $HgC1_2$ on water permeation across the living cells should be interpreted with caution, since a possible outcome of $HgC1_2$ application could be the reduced phosphorylation of water channels [9–12]. Mercury can also induce conformational changes in the plasma membrane aquaporins of higher plants [87].

Calcium signaling is a common pathway in the response of plants to environmental stresses or hormones and cell-specific fluctuations in cytosolic Ca^{2+} occur in the epidermis, endodermis and pericycle of *Arabidop-*

sis roots in response to drought and salt [88,98,112]. Aquaporins in plant membranes can undergo Ca^{2+}-dependent phosphorylation, which can raise their water-channel activity [19,29,35]. On the other hand, calcium showed a clear effect on aquaporin activity, with two distinct ranges of sensitivity to free Ca^{2+} concentration [72,79]. Since the normal cytoplasmic free Ca^{2+} sits between these ranges, it allows for the possibility of changes in Ca^{2+} to finely up- or down-regulate water channel activity [72,79,89]. Ca^{2+} decreased the osmotic water permeability of PM vesicles from *Arabidopsis*, suggesting a potential relevance to intracellular Ca^{2+} signaling and further influencing plant WUE [14,92,93, 102–106]. At the whole plant's level, Ca^{2+} has also been shown to ameliorate the reduction of root hydraulic conductivity produced by salinity [81,97]. The effect of calcium is predominantly on the cytoplasmic side, and inhibition corresponds to an increase in the activation energy for water transport. However, a link between these observations and cell signaling and/or calcium-dependent water channel gating remains to be established [52,63,67,78,121–130].

5. Conclusions

The calcium signal metabolism performs a pivotal function in the whole multiple signaling networks that mediate a variety of cellular events, including proliferation, differentiation, and cell survival. The presence of it in mammalian cells and plant cells is no longer in any doubt, and this has been corroborated by the detection of the enzymes responsible for phosphoinositide metabolism, phosphoinositide kinases and phosphatases in animals and plants, which is directly linked with calcium signals. Plants have evolved multiple traits that provide resistance against a range of biotic and abiotic stress factors. The majority of studies on plant resistance have focussed on one particular trait and its effect on one particular stress factor. However, plants usually employ multiple lines of resistance against multiple stress factors simultaneously. For instance, in response to herbivore attack, plants both express traits that have a direct negative impact on the herbivore, and traits that enhance the efficacy of the herbivore's natural enemies. In order to better understand the functioning of plant resistance, we study how plants integrate the expression of multiple (inducible) resistance traits in response to various combinations of biotic and abiotic stress factors.

ABA is an endogenous anti-transpirant that induces stomatal closure, thereby leading to water conservation and change of WUE. There is more than 95% of the water that passes through plants exits via the stomatal pores, through which the vast majority of carbon dioxide required for photosynthesis enters. Stomata operate as a miniature homeostatic sensor and effector system that senses a number of stimuli to induce guard cell swelling or shrinking, resulting in stomatal opening or closing, and thus optimization of WUE, a measure of the efficiency with which plants facilitate CO_2 influx at the expense of water loss. Therefore, ABA-induced stomatal closure is closely related with WUE. From the above descriptions, we found out that the changes in cytosolic Ca^{2+} concentration, especially such changes in guard cells, can be regulated by ABA production, thus leading to the change of the stomatal aperture. Moreover, cytosolic Ca^{2+} concentration can be regulated by Ca^{2+} transporters such as calcium-permeable ion channels, Ca^{2+}/H^+ antiporters and Ca^{2+}-ATPases, which are also called calcium-signal-encoding elements. We can conclude that the latter, activated by ABA-induced signaling, are involved in regulating WUE through control of the influx and efflux of Ca^{2+} from guard cells. These elements may be involved in the midway process of ABA-induced stomatal closure. The above three kinds of calcium-signal-encoding elements have similar motifs in their molecular structure. We can think that calcium-permeable channels and Ca^{2+}/H^+ antiporters may also be involved in plant stress resistance as Ca^{2+}-ATPases do. In addition, we also found out that some genes, seemingly having nothing to do with WUE, can actually directly or indirectly regulate plant WUE, and that some of these genes can even regulate stomatal aperture and stomatal density, which are crucial to the change of WUE. Hence, we can equally conclude that calcium-signal-encoding elements are also involved in the change of plant anti-drought properties and plant WUE. In the presence of Ca^{2+}, the overexpression of *TaTPC1* (functioning in Ca^{2+} import in wheat cytosol) accelerated stomatal closure. Therefore, it is easy to reach the conclusion that calcium-signal-encoding elements (including calcium permeable ion channels, Ca^{2+}/H^+ antiporters and Ca^{2+}-ATPases) can regulate plant WUE through involving in the midway process of ABA-induced stomatal closure and the change of plant WUE; such process can be illustrated as:

ABA signaling transduction → activation of calcium-signal-encoding elements → change of cytosolic Ca^{2+} concentration $[Ca^{2+}]_{cyt}$ → Ca^{2+} signaling transduction → change of the stomatal aperture → change of plant WUE and plant anti-drought properties

The above is our hypothesis about the relations between calcium-signal-encoding elements and WUE in plants. Many more details concerning its molecular mechanism need to be further studied and clarified.

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