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Review

Relationship between calcium decoding elements and plant abiotic-stress resistance

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Serving as an important second messenger, calcium ion has unique properties and universal ability to transmit diverse signals that trigger primary physiological actions in cells in response to hormones, pathogens, light, gravity, and stress factors. Being a second messenger of paramount significance, calcium is required at almost all stages of plant growth and development, playing a fundamental role in regulating polar growth of cells and tissues and participating in plant adaptation to various stress factors. Many researches showed that calcium signals decoding elements are involved in ABA-induced stomatal closure and plant adaptation to drought, cold, salt and other abiotic stresses. Calcium channel proteins like AtTPC1 and TaTPC1 can regulate stomatal closure. Recently some new studies show that Ca^{2+} is dissolved in water in the apoplast and transported primarily from root to shoot through the transpiration stream. The oscillating amplitudes of $[Ca^{2+}]_o$ and $[Ca^{2+}]_i$ are controlled by soil Ca^{2+} concentrations and transpiration rates. Because leaf water use efficiency (WUE) is determined by stomatal closure and transpiration rate, so there may be a close relationship between Ca^{2+} transporters and stomatal closure as well as WUE, which needs to be studied. The selection of varieties with better drought resistance and high WUE plays an increasing role in bio-watersaving in arid and semi-arid areas on the globe. The current paper reviews the relationship between calcium signals decoding elements and plant drought resistance as well as other abiotic stresses for further study.

Key words: Calcium signals decoding elements; Stomatal closure; Water use efficiency (WUE); Abiotic stress-resistance

1. Introduction

Calcium signals decoding elements mainly include calcium-permeable ion channels, Ca^{2+}/ H^+ antiporters and calcium ATPases, which have been investigated with electrophysiological, biochemical and molecular approaches [1]. A large body of electrophysiological studies elucidated that plants have Ca^{2+} channels with different types of gating mechanisms: ligand, voltage and stretch-activated [2]. However, only a limited number of genes encoding Ca^{2+} channels have been isolated and functionally expressed. In plants, calcium ion has emerged as a secondary messenger mediating the actions of many hormone and environmental factors, including biotic and abiotic stresses. It is reported that Ca^{2+} is involved in regulating such diverse and fundamental processes as cytoplasmic streaming, thigmotropism, gravitropism, cell division, cell elongation, cell differentiation, cell polarity, photomorphogensis, and plant defense and stress responses [3-6]. It is believed that calcium influx and cytoplasmic calcium ($[Ca^{2+}]_{cyt}$) increases are important for guard cell ABA transduction, while ABA can regulate stomatal aperture in guard cells after inducing $[Ca^{2+}]_{cyt}$ to increase. It was also found recently in *Arabidopsis thaliana* that the oscillating amplitudes of $[Ca^{2+}]_o$ (extracellular Ca^{2+} concentration) and $[Ca^{2+}]_i$ (cytosolic Ca^{2+} concentration) are controlled by soil Ca^{2+} concentrations and transpiration rates. The phase and period of oscillations are likely determined by stomatal conductance [7], showing a close relationship between Ca^{2+} and transpiration stream as well as transpiration rate. Furthermore, leaf water use

efficiency (WUE) (namely, transpiration efficiency = photosynthesis rate/ transpiration rate) is determined by transpiration rate and closely related with stomatal closure. Thus, the relationship between Ca^{2+} and leaf WUE should be intensively studied in future.

Among all the stresses to plant growth and development, drought is a major stress to agricultural production [8-14]. Plants synthesize ABA in response to drought, triggering a signaling cascade in guard cells that results in stomatal closure, thus reducing water loss, which may influence WUE in plants. In addition, drought is also related to salt stress, cold stress, high temperature stress, acid stress, alkaline stress, pathological reactions, senescence, growth, development, cell circle, UV-B damage, wounding, embryogenesis, flowering, signal transduction and so on [8, 9, 13-23]. Many advances in relation to this hot topic, including molecular mechanism of anti-drought and corresponding molecular breeding have emerged [24, 25] and calmodulin and calmodulin-related proteins (CMLs) were shown to participate in the complex responses of plant cells to environmental stress. This work helps provide a base of knowledge in stress physiology and valuable information for the technology for engineering improved stress-tolerance in important crop species [26].

With regard to the above description, this paper hopes to review advances in plant calcium signals decoding elements and the relationship between these elements and plant drought, salt and cold resistances, which will provide more valuable information for the research on plant abiotic stress resistance related with calcium signals decoding elements.

2. Advances in plant calcium signals decoding elements

Plant calcium signals decoding elements mainly include calcium permeable ion channels, Ca^{2+}/ H^+ antiporters and Ca^{2+}-ATPases [1]. Therefore, this paper firstly summarizes the main advances in three such kinds of elements.

2.1 Calcium-permeable ion channels

The previous definition of a Ca^{2+} -permeable channel, simply as a channel permeable to Ca^{2+}, merely assumed that its physiological function was to mediate Ca^{2+} influx from the apoplast into the cytoplasm. Although calcium-permeable channels have been investigated with electrophysiological, biochemical and molecular approaches, only a limited number of genes encoding Ca^{2+} -permeable channels have been isolated and functionally studied. It was 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. Removal of extracellular Ca^{2+} with the chelator EGTA or blockage of entry with a number of ion channel blockers suggested that low temperature-induced closure primarily involved in entry of Ca^{2+} across the plasma membrane, while intracellular mobilization appears to dominate if stomatal closure is initiated with ABA or mechanical stimulus. Amtmann et al [27] found that a wheat gene LCT1, encoding a low-affinity cation transporter, could complement a yeast mutant disrupted in the MIDI gene, which encodes a stretch-activated Ca^{2+} permeable non-selective cation channel. AtTPC1 (*Arabidopsis* two-pore voltage-gated channel 1), 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). $[Ca^{2+}]_{cyt}$ was enhanced by overexpressing of AtTPC1 or suppressed by antisense expression of it under sucrose stress. A unique gene in *Arabidopsis*, TPC1 (At4 g03560), whose general structure resembles that of the pore-forming subunits of mammalian and yeast Ca^{2+} channels that contain four Shaker-like domains, shows some sequence similarity. TPC1 expression enhances Ca^{2+} uptake in yeast Ca^{2+}-channel mutant. OsTPC1, the homolog of AtTPC1, was also identified and characterized [28]. In 2005, the TaTPC1 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. 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 control plants, indicating that the overexpression of TaTPC1 accelerated stomatal closing in the presence of Ca^{2+} [29]. It is also reported that plasma membrane calcium can transduce intracellular signaling and may exert effects on metabolism, gene expression and integrated physiological processes including cell division and cell elongation through regulating $[Ca^{2+}]_{cyt}$ [30]. Kinesin-like calmodulin binding protein (KCBP) is a microtubule motor protein involved in the regulation of cell division and trichome morphogenesis. A novel KCBP-interacting Ca^{2+} binding protein (KIC) with its single EF-hand motif, binds Ca^{2+} at a physiological concentration, suggesting that KIC modulates the activity of KCBP in response to changes in cytosolic Ca^{2+} and regulates trichome morphogenesis[31]. It is thought that the inward Ca^{2+} current, which generates the $[Ca^{2+}]_{cyt}$ gradient, is mediated by the clustering of

catalytically active (perhaps mechanosensitive) Ca^{2+} channels at the apex of the root hair [32-34]. It is noteworthy that these channels are inhibited by La^{3+} but not by nifedipine or verapamil. 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 indicates the presence of a voltage-dependent Ca^{2+}-permeable channel, whose activity may be regulated by extracellular CaM in pollen cells [35].

2.2 Ca^{2+}/ H^+ antiporters

The Ca^{2+}/ H^+ antiporter plays a key role together with Ca^{2+}-ATPase in the accumulation of Ca^{2+} in vacuoles which constitute the primary pool of Ca^{2+} among several organelles of plants. The Ca^{2+}/ H^+ antiporters are 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. 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 [36]. 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 [1, 37]. The first plant Ca^{2+}/ H^+ antiporter was cloned by its ability to suppress the Ca^{2+}-hypersensitive phenotype of a *Saccharomyces cerevisiae* mutant. These genes have been termed as "cation exchangers" (CAX) [38]. CAX1 from *Arabidopsis thaliana* is a high-capacity and low-affinity Ca^{2+} transporter, which has been shown to be localized in the plant vacuole [39] and its activity appears to be regulated by a N-terminal autoinhibitory domain [40, 41]. *Arabidopsis* has up to 12 putative Ca^{2+}/H^+ cation antiporters (CAX1-11 and MHX) [42]. The *Arabidopsis* Ca^{2+}/ H^+ antiporter, CAX1, is a high-capacity and low-affinity Ca^{2+} transporter and several CAX1-like transporters are found in *Arabidopsis*. 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 [43]. Through using site-directed mutagenesis, 31 mutations in the repeats of the *Oryza sativa* CAX were generated, which translocate 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. 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+}; although 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 some results, the 451-amino acid protein OsCAX1a was predicted to have 11 TMs, like other CAX proteins [44, 45].

2.3 Ca^{2+}-ATPases

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 on the basis of protein sequence identity [46, 47]. 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 [48]. Isoform ECA1 appears to be located in the ER, as determined by membrane fractionation and immunodetection [49]. 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 [50]. In wheat root, a Ca^{2+}-ATPase in plasma membranes was characterized [51], and two endomembrane Ca^{2+} transporters were located in different cellular compartments: the secondary H^+/Ca^{2+} antiport located in the vacuolar membranes, and the primary Ca^{2+}-ATPases located in the endoplasmic reticulum.

3. Calcium signals decoding elements and plant drought resistance

Drought serves as a major stress factor in inhibiting plant growth and development and productivity. As shown in many studies in recent years, exogenous Ca^{2+} can enhance plant drought resistance, inhibit the synthesis of activating oxides, protect the structure of cellular plasma membranes, and maintain normal photosynthesis as well as regulate the metabolism of plant hormone and other important chemicals. In addition, as a second messenger, cellular Ca^{2+} also transmits drought signals, thus regulating the physiological responses induced by drought stress [52-54]. The relationship

between the nucleus of wheat seedling and Ca^{2+} under drought stress was examined with the cytochemical method of improved calcium antimonate precipitation. The results showed that there was a small concentration of Ca^{2+} in the nucleus, the nucleus was shaped like a ball and the chromatin was scattered. The longer the duration of drought stress the higher the free Ca^{2+} concentration in nucleus and the more serious the ultrastructure of the nucleus; meanwhile, the membrane of the nucleus wrinkled , the chromatin seriously agglutinated, and the shape of the nucleus was abnormal until its final disintegration [55]. It was also suggested that Ca^{2+}/CaM messenger system was involved in controlling stress resistance of rice seedling, blocking messenger transduction, drought resistance, salt resistance and decreasing chilling resistance [56]. It was also indicated that Ca^{2+} treatment increased protection against membrane lipid peroxidation and stability of membranes and therefore resulted in the increase of drought resistance of rice seedlings [57]. It was also found in wheat that Ca^{2+} appeared to reduce the devastating effects of stress by elevating the content of proline and glycine betaine, thus improving the water status and growth of seedlings and minimizing the injury to membranes [58]. All the above-mentioned results showed that Ca^{2+} plays important roles in plant responses to drought resistance.

WUE is given much attention as a physiological trait related to plant drought resistance. Especially, the molecular research regarding the enhancement of WUE playing a crucial part in the selection and cultivation of drought-resistant or drought-tolerant crop varieties. When breeding for drought tolerance, biomass productivity and WUE are considered as fundamental agronomic characteristics [59].

As a kind of signaling substance, Ca^{2+} is closely related with various abiotic stress resistance for plants. As a member of signaling networks, the cellular Ca^{2+} signal pathways are mainly regulated by special proteins called Ca^{2+} signals decoding elements, which are also responsible for Ca^{2+} transportation. It has also been well established that regulation of stomatal aperture is Ca^{2+}-dependent, with stimuli such as ABA and extracellular Ca^{2+} evoking a rise in $[Ca^{2+}]_{cyt}$ that precedes the loss of turgor by the surrounding guard cells. It was also reported that ABA induces an increase in $[Ca^{2+}]_{cyt}$ in guard cells, which precedes the reduction in stomatal aperture [60]. Therefore it is believed $[Ca^{2+}]_{cyt}$ leads to the reduction in stomatal aperture [61]. In addition, according to some relevant research, Ca^{2+} is involved into ABA-induced signal transduction in guard cells, thus directly regulating stomatal conductance (**Fig. 1**) [62, 63] and (**Fig. 2**) [64].

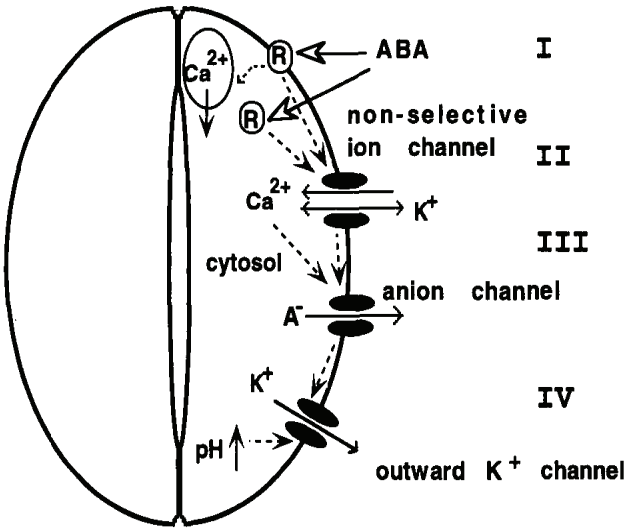

Fig. 1 The ABA-induced Ca2+ signal transduction in plant guard cells modified from [62, 63].

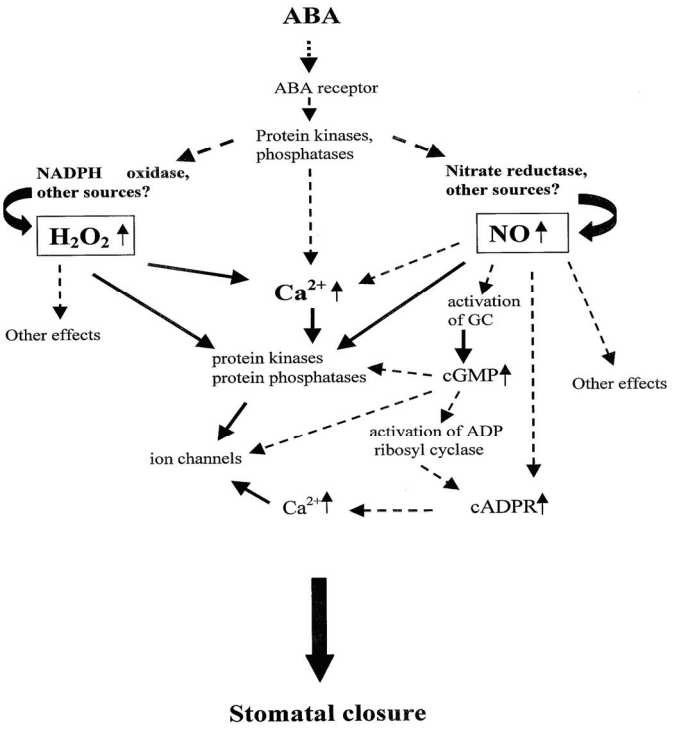

Fig. 2 The pattern and process of the ABA-induced signal transduction in plant cells modified from [64, 115].

What's more, Ca^{2+} /CaM were indicated to participate in the process of ABA-induced drought signal transferring under PEG stress, while ABA synthesis was related with cytoplasmic Ca^{2+} concentrations [65]. 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 [66]. 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 [66-69]. It has been known for some time that in guard cells, membrane hyperpolarization is directly associated with the elevation of $[Ca^{2+}]_{cyt}$ that follows ABA application [67]. Allen et al (2001) [70] showed that specific patterns of Ca^{2+} elevation may be involved in controlling both the stomatal closure response and the final steady state of stomatal aperture. A number of studies have shown that cytosolic-free concentration of calcium ($[Ca^{2+}]_{cyt}$) plays a central role in stomatal movement, and the change in $[Ca^{2+}]_{cyt}$ regulates stomatal opening or closing [60, 71-73]. Further studies have established a close relationship between $[Ca^{2+}]_{cyt}$ oscillation and stomatal status [70, 74, 75]. Relatively lower extracellular concentrations of calcium ($[Ca^{2+}]_{ext}$) have been shown to induce $[Ca^{2+}]_{cyt}$ increases or oscillations, resulting in stomatal closure or aperture fluctuations [70]. In addition, Ca^{2+} is dissolved in water in the apoplast (extracellular space) and transported primarily from the root to the shoot through the transpiration stream [76, 77-82]. The transpiration rate is governed by stomatal conductance, which displays diurnal oscillations [83]. It was also found recently in *Arabidopsis thaliana* that the oscillating amplitudes of $[Ca^{2+}]_o$ (extracellular Ca^{2+} concentration) and $[Ca^{2+}]_i$ (cytosolic Ca^{2+} concentration) are controlled by soil Ca^{2+} concentrations and transpiration rates. The phase and period of oscillations are likely determined by stomatal conductance [7]. These results show a close relationship between Ca^{2+} and transpiration steam as well as transpiration rate. Thus, relationship between Ca^{2+} and leaf WUE should be investigated because leaf WUE is determined by transpiration rate and closely related with stomatal closure.

From the results of Ca^{2+} channel protein research in *Arabidopsis* and other plants, we can conclude that the genes encoding these proteins may belong to a certain gene family, and play an important part in plant responses to various stress resistances of plants [11, 28, 29, 38, 84]. In 2005, Wang et al. of our institute cloned TaTPC1 gene, encoding a Ca^{2+} -permeable channel from wheat and located it on the plasma membrane through the application of a TATPC1-GFP fusion protein. 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 stomatal closing in the presence of Ca^{2+} [29]. What's more, we also implemented research work concerning wheat WUE evolution, gene location and molecular markers [85-88]. Currently, we conduct our research on the relationship between wheat Ca^{2+} channel proteins and leaf WUE as well as whole plant WUE by applying verapamil (a non-special inhibitor to Ca^{2+} channels) and $LaCl_3$ (a special inhibitor to Ca^{2+} channels), already showing there exist differences between the WUE of dry-land wheat varieties and wetland ones in the presence of both inhibitors (data not shown). Meanwhile, we are currently implementing the functional research of wheat Ca^{2+} channels and identifying their distribution in different organs, so that the gene cloning of such channels can be further conducted.

4. Calcium signals decoding elements and plant cold-resistance

It is commonly held that Ca^{2+} channels play tremendous roles in the growth of root hairs and the low temperature acclimation of chilling-resistant plants. It is concluded that activity and stability of Ca^{2+}-ATPase under 2℃ low temperature are the key factors in the development of cold resistance of winter wheat [89]. It is suggested that the cold-resistant agent CR-4 plays a momentous role in stabilizing plasma membrane Ca^{2+}-ATPase under low temperature stress, indicating that the Ca^{2+}-ATPase activity was mainly localized at the plasma membrane in wheat seedling cells growing at normal temperatures [90]. A model of calcium-permeable channels involved in 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 cooling rate (dT/dt) and calcium efflux is mediated by calcium pumps, which have been shown to be dependent on absolute temperature (T). Such a model suggests that the primary temperature sensor in plants might be a Ca^{2+}-permeable channel [91], indicating such channel proteins may participate in plant responses to cold stresses. It is also demonstrated in alfalfa as well as in *Arabidopsis* that Ca^{2+} influx acts as a signal transduction component in gene activation at low temperature [92]. It was found that the addition of Ca^{2+} chelators prevent cold acclimation and the expression of cold-regulated genes [93], while it was also found that addition of Ca^{2+} ionophore induces the expression of cold acclimation-specific genes at 25°C [94]. From studies on *Arabidopsis* mutants displaying reduced tonoplast Ca^{2+}/H^+ antiport (CAX1) activity, it appears that CAX1 participates in the development of the cold acclimation response [95]. It was reported that when exposed to cold conditions, the Ca^{2+}

concentration in cold-insensitive plants has been transiently increased, suggesting that Ca^{2+} acts as a second messenger during cold acclimation [94, 96, 97]. Therefore, with regard to the above-mentioned description, it can be equally inferred that Ca^{2+} transporters may be involved in plant responses to such other stresses as drought, salt and water deficit.

5. Calcium signals decoding elements and plant salt resistance

When Ca^{2+} is in deficit, plants are more susceptible to damage by low pH or high salt. Numerous results suggest that external and apoplastic Ca^{2+} directly alleviates symptoms produced by ion stresses or mineral toxicities, such as proton, Na^+, Al^{3+}, and Cl^- toxicities, and Ca^{2+} also helps to establish a favorable K^+: Na^+ ratio under salt stress [98].

It is suggested that intracellular calcium signaling through a calcineurin-like pathway mediates the beneficial effect of calcium on plant salt tolerance [99]. A salt stress induced Ca^{2+}-dependent signaling network was described and illustrated in detail to mediate Na^+ homeostasis and salt tolerance, indicating that Ca^{2+} transporters are closely related to plant salt tolerance. It was also suggested that CaM activation might be necessary in calcium promotion of the accumulation of proline in fig calli, and the addition of calcium to media alleviated the inhibition of fig callus growth under salt stress [100], demonstrating that CaM might act jointly with Ca^{2+} under the support of calcium signals decoding elements in plant responses to salt stress. It was found that in barley roots, the activation of tonoplast H+-ATPase and the regulation of Na^+ and K^+ uptake under NaCl stress may be related to Ca^{2+}-CaM system [52], showing that calcium signals decoding elements may participate in the process of plant signaling responses to salt stress through accordingly regulating cytosolic Ca^{2+} concentration. A number of studies by Jian-Kang Zhu and his co-workers also indicated that in *Arabidopsis*, Ca^{2+} signaling network is closely related with the activation of the salt overly sensitive (SOS) signal transduction pathway which regulates cellular Na^+ and K^+ homeostasis [101-103]. It is reasonable to assume that the salt induced $[Ca^{2+}]_{cyt}$ transients detected in plant cells [96](Knight, 1996), and perhaps, a new $[Ca^{2+}]_{cyt}$ steady-state in yeast are controlled by the ECA and ACA Ca^{2+}-ATPases and CAX1 and 2 transporters [104]. It was also evident that some physiological functions and molecular properties of the nonselective cation channels (NSCCs) are directly correlated with a multitude of stress responses, growth and development, uptake of nutrients and calcium signaling [105]. Therefore, it can be concluded that both types of Ca^{2+} transporters, namely, Ca^{2+}-ATPases and CAXs involve in plant responses to salt stresses through regulating $[Ca^{2+}]_{cyt}$, thus regulating cellular and intercellular Ca^{2+} signaling networks and improving resistances or tolerances.

6. Conclusions

It has been found by a growing number of studies that calcium plays a vital role in life processes by acting as a messenger to regulate plant growth and development, as well as by its response and adaptation to environmental stresses [11, 106]. From the above information, more evidence can be found for supporting the conclusion that calcium signals decoding elements, which could be activated by ABA-induced signaling transduction, can regulate some genes relating to leaf WUE through controlling cytosolic Ca^{2+} concentration to lead to stomatal closure. Namely, Ca^{2+} serves as a signaling messenger for ABA-induced stomatal closure. Based on this, it can be speculated that calcium signals decoding elements located on the plasma membrane also involve in ABA-induced stomatal closure, thus leading to the change of leaf WUE. ABA is an endogenous anti-transpirant that induces stomatal closure, thereby leading to water conservation and the change of WUE. It was reported 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 miniature homeostatic sensory and effector systems that sense 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 [64]. Therefore, ABA-induced stomatal closure is closely related with WUE. From the above description, we have found 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 stomatal aperture [60]. Besides, 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 decoding signals elements. Therefore, it can be suggested that calcium decoding signals elements, activated by ABA-induced signaling, involve in regulating WUE through controlling the influx and efflux of Ca^{2+} from guard cells. Namely, these elements may be involved in the midway process of ABA-induced stomatal closure. In addition, we also find some genes, which control other traits of plants, can actually indirectly or directly regulate plant WUE [107-109], and some of these genes can even regulate

stomatal aperture and stomatal density [29, 108, 109], which are crucial to the change of WUE. Hence, it can be concluded that calcium decoding signals elements also involve in the change of plant WUE. It was also reported that in the presence of Ca^{2+}, the overexpression of TaTPC1 (functioning in Ca^{2+} import in wheat cytosol) accelerated the stomatal closing. The phase and period of Ca^{2+} concentration oscillations are likely determined by stomatal conductance [7], which governs transpiration rate [83] by transporting Ca^{2+} primarily from the root to the shoot through the transpiration stream [76, 82]. Therefore, by a final analysis, it can be proposed that calcium decoding signals elements (including calcium-permeable ion channels, Ca^{2+}/ H^+ antiporters and Ca^{2+}-ATPases) can regulate plant WUE by involving the midway process of ABA-induced stomatal closure and the change of plant WUE, such sequent process can be illustrated as the following:

ABA-induced signal transduction→The activation of calcium decoding signals elements → The change of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) →Ca^{2+} signaling transduction →The change of stomatal aperture → the change of transpiration stream →the change of transpiration rate→ the change of transpiration efficiency →The change of plant WUE.

It is our current hypothesis about the physiological relationship between calcium signals decoding elements and WUE in plants. More details concerning its molecular mechanism need to be further studied and clarified and complemented. Plant WUE is an important index for measuring plant drought-resistant and yield. Water scarcity is a major factor limiting agricultural production all over the world [110-115]. With the change of global environment, the impact of drought stress on crop yield becomes more serious. Thus, for sustainable agricultural development, irrigation practices must be implemented on the basis of applying the available water resource more efficiently. WUE can be observed at several levels [111-115]. In recent years, numerous progresses have been made in the investigation of plant WUE, especially at the molecular level.

As to the relationship between calcium signals decoding elements and plant abiotic stress resistance, according to numerous evidence described above, our speculation is that under cold and salt stresses, these elements act jointly to control cellular and intercellular Ca^{2+} concentrations, thus accordingly regulating Ca^{2+} signaling networks and further improving plant tolerance or resistance to cold and salt stresses. In view of the principles of "cross-talking", "cross stress" and "cross-resistance" [111, 112], plant resistance or tolerance to various stresses may be closely interrelated and mutually affected. In addition, some of the above research also tells us that Ca^{2+}-ATPases are involved in wheat cold resistance [89, 90]. According to this, it can also be speculated that Ca^{2+}-ATPases may be involved in other plant stress resistance based on the principle of "cross resistance". As we know, the three kinds of calcium decoding signals elements have similar motifs (EF-hands and transmembrane structures) in their molecular structure. Therefore, it can be conjectured that calcium permeable channels and Ca^{2+}/H^+ antiporters may also involve in plant stress resistance as Ca^{2+}-ATPases do. Because the acquirement of the tolerance or resistance to a certain stress can induce the emergence of other kind of tolerance or resistance, Ca^{2+} may confer many kinds of resistances or tolerances to plants. Therefore, calcium signals decoding elements serve as important regulators for plant responses under various stresses.

As an important element for the improvement of plant tolerance or resistance to various stresses, Ca^{2+} participates in many processes of plant responses to stresses and plays vital roles in maintaining both cellular and intercellular ionic balances under stresses. What's more, Ca^{2+} is also considered to be related with the improvement of plant acclimation and adaptation to various environments [93]. Under normal growth conditions, Ca^{2+} is also required for plant basic physiological activities, so, it is also assumed as a regulator of life itself [98]. Therefore, it can be equally assumed that as controllers for Ca^{2+} concentrations, calcium signals decoding elements are also important regulators for the machinery of life, and they play pivotal roles under both normal and stress conditions. With the advancement of Ca^{2+} signaling network research, such assumption will be expressly confirmed with the discoveries and clarifications of more molecular mechanisms for Ca^{2+}-dependent signaling networks.

Abbreviations

WUE: water use efficiency; AtTPC1: 1*Arabidopsis* two-pore voltage-gated channel; ABA: abscisic acid; $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_I$: cytosolic Ca^{2+} concentration; $[Ca^{2+}]_o$ and $[Ca^{2+}]_{ext}$: extracellular Ca^{2+} concentration; EGTA: Ethyleneglycol-bis-(2-aminoethyl)-tetraacetic acid; TaTPC1: *Triticum asetivum* two-pore channel 1; GFP: green fluorescent protein; CaM: calmodulin; CAX: cation exchanger; NSCCs: nonselective cation channels.

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Conflict of interest

The authors have declared that no conflict of interest exists.

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