Review Article

The Calcium Sensor CBL-CIPK Is Involved in Plant's Response to Abiotic Stresses

S. M. Nuruzzaman Manik,[1,2] Sujuan Shi,[1,3] Jingjing Mao,[1,2] Lianhong Dong,[1,2] Yulong Su,[1,2] Qian Wang,[1] and Haobao Liu[1]

[1]*Key Laboratory of Tobacco Biology and Processing, Tobacco Research Institute of CAAS, Ministry of Agriculture, Qingdao 266101, China*
[2]*Chinese Academy of Agricultural Sciences, Beijing 100081, China*
[3]*Qingdao Agricultural University, Qingdao 266109, China*

Correspondence should be addressed to Qian Wang; wangqian01@caas.cn and Haobao Liu; liuhaobao@caas.cn

Abiotic stress halts the physiological and developmental process of plant. During stress condition, CBL-CIPK complex is identified as a primary element of calcium sensor to perceive environmental signals. Recent studies established that this complex regulates downstream targets like ion channels and transporters in adverse stages conditions. Crosstalks between the CBL-CIPK complex and different abiotic stresses can extend our research area, which can improve and increase the production of genetically modified crops in response to abiotic stresses. How this complex links with environmental signals and creates adjustable circumstances under unfavorable conditions is now one of the burning issues. Diverse studies are already underway to delineate this signalling mechanism underlying different interactions. Therefore, up to date experimental results should be concisely published, thus paving the way for further research. The present review will concisely recapitulate the recent and ongoing research progress of positive ions (Mg^{2+}, Na^+, and K^+), negative ions (NO_3^-, PO_4^-), and hormonal signalling, which are evolving from accumulating results of analyses of CBL and CIPK loss- or gain-of-function experiments in different species along with some progress and perspectives of our works. In a word, this review will give one step forward direction for more functional studies in this area.

1. Introduction

Unlike animals, plants are not mobile organism and cannot go away from adverse environmental conditions. Owing to these reasons, they create special system to adjust themselves in external stress conditions through instant transmit signals. Due to the temporary fluctuations in cytosolic calcium concentration, plant cells receive the signals from external stimuli, so they can accept the signals using their own machineries and decode the signals to secondary messenger [1–4]. Calcium is broadly well known as a ubiquitous secondary messenger because of its diverse functions in plants. Ca^{2+} is encoded in various stimuli of abiotic and biotic stresses. Abiotic stresses caused by high magnesium, high sodium, low potassium, low phosphorus, ABA, and others affect the rate of germination, photosynthesis, seedling growth, leaf expansion, total biomass accumulation, and overall growth effects of plants [5, 6].

In recent decades, Calcineurin B-like (CBL) protein-CBL-interacting protein kinase (CIPK) complex is widely accepted as Ca^{2+} signalling mechanism, which is involved in response to different external stresses signals [5, 7]. In adverse stresses conditions, plants evolve a stress signal that is specifying Ca^{2+} signature [8–10]. The specific Ca^{2+} signatures are received by closely controlled activities of plasma membrane and other organelles channels and transporters [1, 10–12]. In addition, this signature binds to EF hands domains of the CBL proteins. Consequently, the CBL proteins bind the NAF/FISL domain of C-terminal of the CIPK, thus stimulating the kinase [13]. On the other hand, N-terminal of the CBL protein

directs the CBL-CIPK system to an exact cellular target region ensuing in the stimulated CIPK phosphorylating the proper target proteins [11, 14–17].

Bioinformatics and comparative genomic analyses in plants have provided details about the sequence specificity, conservation, function and complexity, and ancestry's history of CBL and CIPK proteins families from lower plants to higher plants. Bioinformatics research reports showed that *Arabidopsis thaliana* has 10 CBLs and 26 CIPKs [13] while in other plants *Populus trichocarpa* has 10 CBLs and 27 CIPKs [18], *Oryza sativa* has 10 CBLs and 31 CIPKs [19], *Zea mays* has 8 CBLs and 43 CIPKs [19], *Vitis vinifera* has 8 CBLs and 21 CIPKs [20], *Sorghum bicolor* has 6 CBLs and 32 CIPKs [20], and *Nicotiana sylvestris* has 12 CBLs and 37 CIPKs (unpublished). Recently some reviewers have focused on functions, structural features, gene expression, and regulation of the CBL-CIPK complex with different pathways [20–24]. Although some reviewers have described the mechanisms, functions, and interaction between the CBL and CIPK, their functional mechanism and regulation with calcium are yet unclear. There is still a huge need to synthesize and understand ongoing findings from current CBL and CIPK studies, so that signalling systems research can be fully harnessed [5, 25–27]. This review will briefly present underlying mechanism of the CBL-CIPK in response to different environmental stresses with emphasis on important pathways. Indeed, it will recap the recent discoveries of these signalling components along with ongoing research progress.

2. CBL-CIPK Signalling System Responses to Environmental Stresses

Mutants studies of *Arabidopsis* have demonstrated that the CBL-CIPK complexes are involved in mediating Ca^{2+} signals elicited by different stresses, such as low magnesium, low potassium, high salt, nitrate, low phosphorus, ABA, high pH, cold, and osmotic stress [4, 14, 15, 28–32]. Crosstalk between the CBL-CIPK network and other pathways can limit the distances of improving the tolerant crops in adverse conditions. Different pathways like Mg^{2+}, Na^+, K^+, NO_3^-, PO_4^-, and ABA are now burning issues for abiotic stresses. Overexpressing the CBL/CIPK complex in plants might develop their tolerance to concurrently occurring different abiotic stresses and enhance the yield [33]. This complex can posttranslationally phosphorylate its downstream target proteins like transcriptional factors and nutrient pathway to respond to different external environmental stimuli, and thus plant can adapt to unfavorable condition.

To date, research on the CBL-CIPK system has shown that influx/efflux mechanisms of different ions are involved to create an adjustable condition under unfavorable stages in cell. Next session will briefly discuss the mechanism of different pathways.

2.1. Magnesium Signalling. Maintaining Ca^{2+}/Mg^{2+} homeostasis is not only critical for sufficient supply of mineral nutrients [34] but also important for serpentine-tolerant plants [35].

Recently, a new function has been identified for the CBL-CIPK signalling network in vacuole-mediated detoxification of high external Mg^{2+} [36]. Analysis of double mutant functions of CBL2 and CBL3 (*cbl2-cbl3*) revealed that they are regulating vacuole-mediated Mg^{2+} ion homeostasis in cell [36]. The *cbl2-cbl3* double mutant was hypersensitive to high concentrations of external Mg^{2+} condition, and also ionic profiles analysis showed that a reduced amount of Mg^{2+} accumulation was found in the *cbl2-cbl3* double mutant plants. Tang et al. found that CIPK3/9/23/26 physically interacted with the CBL2/3 on the tonoplast, and the multiple *cipks 3/9/23/26* mutant could fully show hypersensitivity of Mg^{2+}, and a similar ionic profile was found as like as the *cbl2-3* mutant [36, 37]. These results strongly suggested that the CIPK3/9/23/26 work together with the CBL2/3 at the tonoplast to alleviate the toxic effects of external high Mg^{2+} concentrations via vacuolar sequestration, but it is not clear which pairs of CBL-CIPK play a vital role in this pathway (Figure 2) [36].

Transporter family AtMHX was the first identified plant Mg^{2+}/H^+ antiporter localized on the tonoplast, which apparently contributes to vacuolar Mg^{2+} uptake [38], and also MGT2 and MGT3 are known as Mg^{2+} transporters localized on the tonoplast [39], but mutant results did not show significant phenotypic changes under high Mg^{2+} conditions [36]. Thus there is further identification of the transporters which are activated under Mg^{2+} toxicity conditions, which are a key step to understand the underlying mechanism of this ion detoxification in plants.

2.2. Sodium Signalling. The salt overly sensitive (SOS) pathway is the first identified CBL-CIPK pathway for maintaining ion homeostasis in plant cells [40]. Genetic and biochemical tactics with SOS mutants presented a molecular mechanism in which the CBL-CIPK complex mediates the salt stress-induced Ca^{2+} signal and shows tolerance to salt [41]. Under salt stress situation, this pathway can enhance salt tolerance in plant by multiple ways; for example, it can allow transporter to send back Na^+ into soil, sequester sodium ion into vacuole, or transport it to the older leaves [24]. The SOS pathway is mainly based on SOS3 (AtCBL4), SOS2 (AtCIPK24), and the plasma membrane Na^+/K^+ antiporter; SOS1, a combined component pathway, plays a vital role in effluxing Na^+ from the cell through SOS1; thus it can enhance the salt tolerance of plants [40]. In salt stress condition, plants can form SOS3-SOS2 complex in their roots and permit the SOS2 to phosphorylate and activate the SOS1 [40]. If plants are unable to activate SOS1 (such as *sos3* mutants), which can store extra Na^+ through a reduced efflux capacity, thus they inhibit growth under salty conditions [14].

Different CBLs can interact with the CIPK24 and therefore form a complexity system in response to salt stress. External salt stresses trigger the AtCBL4/SOS3-AtCIPK24/SOS2 complex to stimulate Na^+/H^+ exchange activity of the SOS1 (Figure 1) [42], which can exclude cell from extra Na^+ [40]. *AtCBL10*, one of the CBL family members, was later included in the salt tolerance pathway. It is thought that tonoplast Na^+/H^+ NHX antiporters are activated by
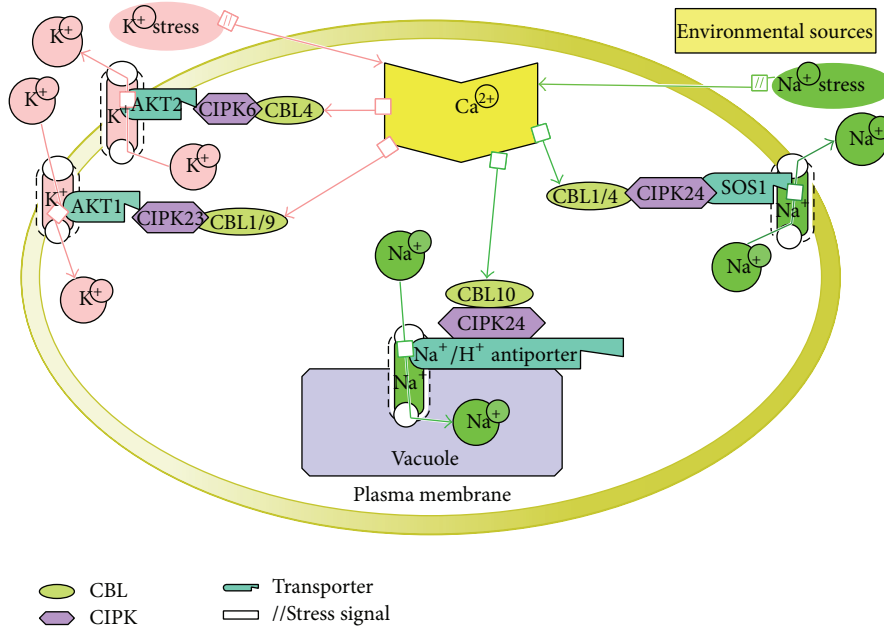
Figure 1: This model represents the identified CBLs-CIPKs interactions process and how they respond to abiotic stresses from environmental sources and maintain homeostasis in cell. All signals are centrally controlled by Ca^{2+}. Different colors indicate different pathways. AKT1: *Arabidopsis* K^+ transporter 1, AKT2: *Arabidopsis* K^+ transporter 2, and SOS1: salt overly sensitive 1. Mechanism in short: environmental stresses trigger Ca^{2+}; Ca^{2+} transmits signal to sensor molecule Calcineurin B-like (CBL) protein-CBL-interacting protein kinase (CIPK) to activate the transporters to create ion homeostasis in cell.
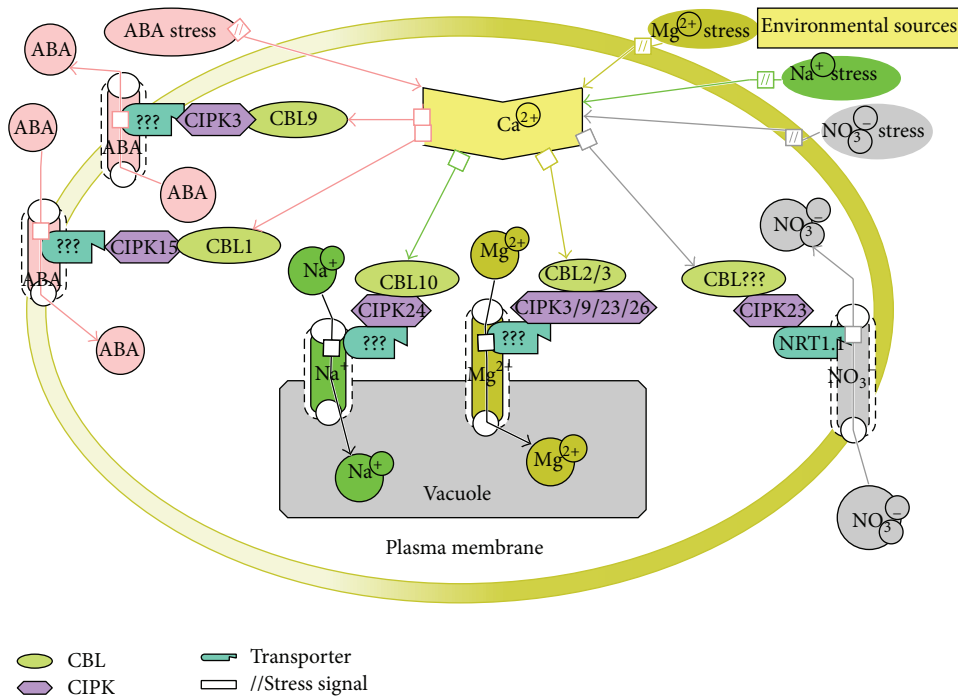

Figure 2: This model represents partially identified CBLs-CIPKs signalling system. Here, question marks (???) indicate that components have not yet been identified. Different colors indicate different pathways. NRT1.1: nitrate transporter 1.1.

the AtCIPK24/SOS2 through a mechanism related to the AtCBL10 to sequester intracellular extra Na^+ in the vacuole (Figure 1) [43]. Moreover, both CBL4/SOS3 and CBL10 are involved in mediating salt tolerance, but they perform their functions in different ways because of their distinct subcellular localizations and expression pattern.

Tissue specific and subcellular localization experiments showed that the CBL4/SOS3 works primarily in the roots and is localized at the plasma membrane, respectively [40]. Thus the CIPK24/SOS2 functions at the same place where it phosphorylates Na^+/H^+ antiporter SOS1, thereby enhancing Na^+ efflux rate [40]. Compared with the CBL10, it is expressed predominantly in the shoots and leaves and localized at the vacuolar membrane (tonoplast) [44]. It is postulated that the CIPK24/SOS2 employed by the CBL10 on the tonoplast may phosphorylate and activate as a yet unknown Na^+ channel or transporter, which is the tonoplast bound and performs a role in transporting cytoplasmic Na^+ into the vacuolar space (Figure 2). That assumption is supported by knockout Arabidopsis mutant cbl10, which showed the salt-sensitive phenotype specifically in the leaves or shoots and accumulated less Na^+ than the wild type under high salt conditions [44].

Additionally, other studies have shown that a calcium sensor, CBL1, can also interact with the CIPK24 to mediate the regulation of Na^+ in the plant cell (Figure 1) [13]. Thus cbl1 mutant plants showed less tolerance to salt stress [45]. Subcellular localization assay demonstrated that the CBL1 is localized in the plasma membrane and interacted with the CIPK24/SOS2 as the CBL4/SOS3, and expression pattern analysis showed that it is expressed in the shoots and roots [45]. So, it can be said that Na^+ extrusion mediated by the CIPK24/SOS2-SOS1 system may also occur in the shoots.

Not only do CBLs show the salt sensitivity but also CIPKs are sensitive to salinity conditions. Arabidopsis cipk6 was described to be more sensitive to salt stress compared to the wild type and it is thought that CIPK6 might be involved in salt tolerance [46]. Interaction between the CIPK6 and the CBL4/SOS3 was proved by yeast two-hybrid system, which indicated the participation of the CIPK6 in this pathway [16]. Possibly, the CBL4/SOS3 also targets the CIPK6 in vivo as well as the CIPK24/SOS2. Further research can shade more light on this complex mechanism involved in response to salt stress.

Apart from the experiments on Arabidopsis, recently, researchers have done some experiments on other species and tried to understand the salt pathway clearer. For instance, apple MdCIPK6L-OE conferred tolerance to salt [47] and its ectopic expression could functionally complement Arabidopsis sos2 mutant, even though it was not homologous to the Arabidopsis CIPK24/SOS2 [47]. Besides MdCIPK6L-OE, MdSOS2 was cloned from apple, which showed the highest similarity to the AtCIPK24/SOS2, and also it positively responds to salt stress and functionally complements the Arabidopsis sos2 mutant [48]. The structural and functional analysis of BjSOS3 was established in the SOS pathway in Brassica juncea [49]. In rice OsCBL4 was the most homologous to the AtCBL4/SOS3 and it was able to functionally complement sos3-1 mutant in Arabidopsis, indicating

that it has the same function as the AtCBL4/SOS3 [50]. ZmCBL4 is the most similar to the OsCBL4 and it can also complement the sos3-1 mutant in Arabidopsis [51]. In Nicotiana sylvestris CBL10 also showed salt sensitivity in Arabidopsis, which demonstrated more tolerance phenotype than wild type Colombia plants under salt stress condition (unpublished). Among the identified CBLs and CIPKs in response to salt stress, only a few have been implicated as negative regulators of salt pathway. For example, AtCBL1 and poplar (Populus euphratica) PeCBL1 were found to negatively influence Na^+ efflux from the cell under saline conditions while the mechanisms behind this are still unclear [52].

2.3. Potassium Signalling. Potassium (K^+) is one of the most important mineral nutrients, which participates in various plant physiological processes and governs yield of crop production. Plants recognize external K^+ fluctuations and create preliminary K^+ signal in root cells [53]. Root cell then transfers signals into cytoplasm, which signals are sensed by calcium sensors [53]. Since 1992, AKT1 is called a low affinity inwardly rectifying K^+ channel, which is involved in the cellular uptake of K^+ signal via calcium sensors [15, 31, 32, 55]. The calcium sensor CBL-CIPK acts as a regulator of the AKT1 to maintain the homeostasis of potassium in cell [31, 56].

If the amount of external K^+ became low, one of the CIPKs, CIPK23, is targeted to the plasma membrane, which is concurrently stimulated by CBL1 and CBL9 to phosphorylate the AKT1; thus movement of K^+ will be inwardly into the cells (Figure 1) [15, 31, 32, 56]. Experiments on mutants cipk23, cbl1/cbl9, and akt1 showed similar reduced growth and chlorotic leaves under low K^+ conditions [15, 32, 36, 57]. It is hypothesized that the cbl1/9 are functionally overlapped, because they individually did not show any significant differences. But their tissue specific localization assay demonstrated that they are expressed in root cells and aerial tissues, such as guard cells and vascular cells as like as localization of the AKT1 [15, 32]. Although the AKT1 expressed low level in hydathodes and stomatal guard cells, the AtCIPK23 may be regulated by the AtCBL1 or AtCBL9 in aerial tissue to redistribution of K^+, turgidity of guard cell, and repolarization of cell membrane [55, 58–60]. Instead of mutant experiments, AKT1 overexpressed (OE) Arabidopsis plants did not show any significant performance in growth when they were grown in low K^+ conditions, while At/PeCBL1, AtCBL9, and AtCIPK23 OE Arabidopsis plants gave comparative tolerance compared to control plants under the same condition [61, 62]. Recently overexpressed AtCIPK23 in potato [63], coexpression of AtCBL9-AtCIPK23-AKT1 in sugarcane [63, 64], OsCBL1-OsCIPK23-OsAKT1 in rice [64], VvCBL1-CIPK4-VvKT1.1 and VvCBL2-CIPK3-VvKT1.2 in grapevine (Vitis vinifera) [65] showed improved tolerance under the low potassium conditions. Moreover, the activity of AKT1 can be negatively regulated by a PP2C-type phosphatase AKT1-interacting PP2C1 (AIP1) [56]. Therefore, the CBL1/CBL9-CIPK23 complex can phosphorylate and activate the AKT1, but dephosphorylation by the AIP1 may regulate the deactivation of the AKT1 [56].

Another study showed that CBL4 interacts with CIPK6, so CBL4-CIPK6 complex is controlling the plasma

membrane targeting of the *Arabidopsis* K$^+$ channel AKT2 by facilitating translocation to the plasma membrane (Figure 1) [16]. In addition, alone the regulatory C-terminal domain of CIPK is sufficient to mediate the CBL4- and Ca^{2+}-dependent channel translocations from the ER membrane to the plasma membrane [66]. This interaction system of the CBL4 is accomplished through a unique targeting pathway that is dependent on the dual site (myristoylation and palmitoylation) [16]. Thus this is a unique system designated as a critical mechanism of ion-channel regulation, in which a calcium sensor controls K$^+$ channel activity by promoting the translocation of the channel to the plasma membrane [66] that is together in kinase interaction-dependent and phosphorylation-independent manner [16]. These studies suggest that the *Arabidopsis* K$^+$ channel AKT2 proficiently translocates to the plasma membrane through the CBL4- and Ca^{2+}-dependent targeting pathway that entails the scaffolding task and the kinase activity of the CIPK6. This is consistent with the hypothesis that there are multiple pathways for K$^+$ channel operating. Besides, CIPK9 responds to various abiotic stresses, such as salinity, osmotic stress, chilling, and cellular injury, and also it plays a critical role in plant tolerance to low K$^+$ [67]. The knockout T-DNA mutant lines of *cipk9* displayed a hypersensitive response to low K$^+$ conditions. However, further analysis specified that K$^+$ uptake and content were not affected in the mutant plants [67]. It has been inferred that the *Arabidopsis* CIPK9 might have a different mode of action than the CIPK23 and CIPK6. It is possible that unknown CBLs interact with the CIPK9 to regulate K$^+$ homeostasis by activating a vacuolar potassium channel [68]. It can also be hypothesized that the unknown CBLs may interact with different CIPKs to sense Ca^{2+} signals in low K$^+$ stress conditions [68]. Indeed, there is still needed further research to qualify this assumption.

2.4. Nitrate Signalling. Nitrogen is a key limiting element for crop production and overall plant growth. NO$_3$$^-$ form of nitrogen, which is the principal nitrogen source of plants [69], research on NO$_3$$^-$ uptake system, provides a test case to define the nutrient transport system to unravel plant nutrient acquisition signalling pathways. However, the molecular mechanisms of NO$_3$$^-$ sensing and signalling have just started to be unraveled in *Arabidopsis thaliana*. The members of three nitrate transporter families, such as 53 of AtNRT1, 7 of AtNRT2, and 7 of AtCLC, have been identified in this plant [70–72]. Among the three families, four plasma membrane transporters members of AtNRT1 and AtNRT2 families are occupied in uptake of NO$_3$$^-$ by root cells [72, 73]. Members of AtNRT2.1 and AtNRT2.2 are engaged in high-affinity uptake that drive either a high affinity (nitrate concentration < 1 mM) or a low affinity (nitrate concentration > 1 mM) [74, 75], and AtNRT1.2 is worked in low-affinity uptake whereas AtNRT1.1 (CHL1) is performed as a dual-affinity transporter involved in both high- and low-affinity uptake of NO$_3$$^-$ [76, 77].

The CHL1 functions as a high-affinity nitrate transporter when threonine residue 101 (T101) is phosphorylated and as a low-affinity nitrate transporter when this residue is dephosphorylated [78, 79]. The first report of a potential role for the CHL1 in nitrate signalling originated from the studies of loss-of-function mutant (*chl1*) in *Arabidopsis*, which demonstrated that the CHL1 regulates the expression of AtNRT2.1 in response to nitrate stress [77]. In microarray system, it showed that AtCIPK23 was downregulated in the *chl1* mutant (Figure 2). However, the AtCIPK23 is not only the target of the AtNRT1.1-dependent signalling but also a regulator of the AtNRT1.1, which is responsible for its phosphorylation at the T101 residue [78]. The AtCIPK23 therefore governs both transport and signalling activities of AtNRT1.1, which infers that the incidence of retrocontrol loop for the AtNRT1.1-dependent gene acts in response to NO$_3$$^-$. Remarkably, the mechanisms leading to the AtCIPK23-mediated phosphorylation of the CHL1 are required to fully understand the possible role of the CHL1 in direct sensing of external nitrate.

In addition, *Arabidopsis* CBL9 is required to activate the AtCIPK23 to mediate the phosphorylation of CHL1 for high-affinity nitrate transportation but the activity of this signalling system remains obscure [80]. Transcriptomic study presented that *Arabidopsis* CIPK8 is involved as a low-affinity nitrate response under stress conditions [81]. Results of continuous experiments on *cipk8* mutant lines showed that the AtCIPK8 is involved in long-term nitrate-regulated root growth and it positively sets the primary nitrate response. In short, the *Arabidopsis* CIPK8 precise regulation of AtNRT1.1 is still unclear and needs further analysis [81].

2.5. Phosphorus Signalling. Phosphorus is known as a secondary macronutrient in plant [82]. Pi (inorganic phosphorus) form of phosphorus is readily absorbed by plants in phosphorous deficient condition [83]. Pi is involved in controlling major enzymatic reactions and switching the metabolic pathways [84]. A report by Chen et al. has published that the CBL-CIPK system is involved during the response to low Pi in *Brassica napus*. Under Pi deficient conditions, BnCBL1 and BnCIPK6 were upregulated and both proteins can interact with each other in yeast two-hybrid screens and split-YFP system [85]. Under low Pi treatment, overexpression of either BnCBL1 or BnCIPK6 showed better plant growth and accumulated more biomass in *Arabidopsis*, mostly found in the lateral roots development [85]. So, the BnCBL1 and BnCIPK6 might control the processes involved in the plant's response to Pi deficiencies, even though the mechanism and pathways are still unknown. It is not clear whether AtCIPK6 is involved in low Pi pathway, though the complementary experiment of the BnCIPK6 with *cipk6* mutant showed that it also responded to low Pi treatment. There is still need for further research in this area [57, 85].

3. Hormonal Signalling

Abscisic acid (ABA) is one of the most essential phytohormones in plants. It performs different roles in plants ranging from seed germination to growth and development as well as responses to abiotic stresses [86]. A specific Ca^{2+} signature responder is found in an early step of the ABA signalling pathways system [87–89], which implies that Ca^{2+} sensors

are involved in this signalling pathway. Moreover, studies on several overexpressed/mutant lines of CBL/CIPK inferred that the CBL-CIPK system is involved in the ABA signalling pathway (Figure 2).

Although the ABA signalling pathways are mainly regulated by two ways, such as ABA-dependent and ABA-independent ways, which are simultaneously controlled stress-responsive genes, ABA-dependent pathway shows a vital role in regulating osmotic stress-responsive genes [90]. The *Arabidopsis* mutant plants lacking CBL9 (*cbl9*) displayed hypersensitivity to ABA in the early developmental stages, such as seed germination and postgermination seedling growth [4]. Experimental results also showed that the *cbl9* accumulated much higher levels of ABA than the control plants under stress conditions [4]. Therefore, the AtCBL9 performs as a negative regulator in abscisic acid signalling [4]. Besides, the expression of AtCIPK3 is induced by cold, high salt, wounding, drought, and ABA. Seed germination analyses of *cipk3* mutants indicated that these lines were more inhibited by the ABA than wild type plants, and results indicated that the AtCIPK3 functions as a negative regulator in ABA signalling during seed germination [91]. It was also demonstrated that the AtCBL9 can form a specific complex with the AtCIPK3 to act together in regulating the ABA responses [92] and suggesting that the AtCBL9-CIPK3 complex negatively regulates the ABA signalling during seed germination (Figure 2) [92].

Furthermore, CBL1 is the most similar isoform of the CBL9 in *Arabidopsis*. Evaluation of the CBL1 function based on loss-of-function mutant showed that (*cbl1*) lines are hypersensitive to abiotic stresses [28, 45]. The *cbl1* did not show significant changes in response to the ABA, but CBL1 and CBL9 mutant lines both displayed less tolerance to drought and salt stress [28, 45]. These results indicated that the CBL1 is not involved in the ABA signalling system dissimilar to the CBL9. Meanwhile, it is remarkable to note that CIPK1 can interact with the CBL1 and CBL9, which mediates ABA responses as well as osmotic stress, drought, and salt responses. Above those factors infer that CBL1-CIPK1 complex is involved in the ABA-dependent way; however CBL9-CIPK1 complex is occupied in the ABA-independent way in *Arabidopsis* [29]. One more research informed that knockdown *cbl1* and *cipk15* generated an ABA-hypersensitive phenotype [93]. Thus CBL1-CIPK15 complex works as a negative regulator in the ABA signalling pathway (Figure 2) [93]. A recent study found that CIPK6 loss-of-function lines (*cipk6*) accumulated high level of ABA in seedlings after treatment, compared to the primary level of expression. This finding implies that the CIPK6 is also involved in responses to ABA [46].

Very few reports have been published of interaction between GA and CBL-CIPK. Research showed that rice CBL gene OsCBL2 was upregulated by gibberellin acid in the aleurone layer in rice [94]. It also showed that this CBL is positively regulating the GA pathway. Using microarray analyses and RNA blots, they have found that the upregulation of the OsCBL2 expression occurs within specific time period after GA treatment [94]. Taken together, these data indicate that CBL-CIPK system plays an important role in the hormonal signalling pathway.

4. Conclusions and Perspectives

Studies on CBLs and CIPKs over the past few years have greatly advanced our knowledge of the function of single proteins in distinct physiological processes. Major advances in our understanding of this signalling system have been made possible by the identification of an increasing number of targets regulated by the CBL-CIPK complexes.

The unraveling of the crosstalk among different pathways will provide more information about the physiological responses of plants, including transpiration, germination of seeds, seedlings growth, and uptake of mineral nutrient under different stress conditions. The progress of the research on the CBL and CIPK families in different plant species other than *Arabidopsis thaliana* is still at an infant stage; in most cases it is limited to interaction studies and expression analyses of these families. Recently, some experiments have been done on the CBL-CIPK complex on poplar, rice, pea, and maize [27]; which experiments indicate an overall participation studies of the research on CBL-CIPK in responses to different abiotic stresses. A few members of the CBLs and CIPKs from above species have been functionally identified, and expression profile has been done in response to stresses, such as salt, drought, cold, and plant hormones [50, 51, 95–98].

Future research should put emphasis on identifying further signalling components over a period generation of mutants by gene knockout approaches and subsequent dissecting of gene functions. Fascinating new insights and prospects are emerging as a result of the increasing number of available genome sequences, which will assist the investigation of the ancestries and functional diversification of these calcium sensors and their interacting protein kinases into the extant complex interaction network. The mechanisms conferring this complex interaction specify the regulatory capabilities to rely on the intermolecular interactions between CBLs and CIPKs [99]. The CBL-CIPK signalling model emphasizes the importance of future research that focuses on the molecular mechanisms underlying the regulation of transporters that allow us to better understand plant's response to abiotic stress and also establish a proficient method of identifying molecular targets for genetically engineered resistant crops with enhanced tolerance to various environmental stresses. Therefore, the most important challenge for future research is not only functional thesis but also the elucidating of the details of synergistic functions in this interaction network and revealing of the molecular mechanisms of the complexes regulating target proteins.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

References

[1] O. Batistič and J. Kudla, "Analysis of calcium signaling pathways in plants," *Biochimica et Biophysica Acta—General Subjects*, vol. 1820, no. 8, pp. 1283–1293, 2012.

[2] V. Albrecht, O. Ritz, S. Linder, K. Harter, and J. Kudla, "The NAF domain defines a novel protein—protein interaction module conserved in Ca^{2+}-regulated kinases," *The EMBO Journal*, vol. 20, no. 5, pp. 1051–1063, 2001.

[3] J. Shi, K.-N. Kim, O. Ritz et al., "Novel protein kinases associated with calcineurin B-like calcium sensors in Arabidopsis," *The Plant Cell*, vol. 11, no. 12, pp. 2393–2405, 1999.

[4] G. K. Pandey, H. C. Yong, K.-N. Kim et al., "The calcium sensor calcineurin B-like 9 modulates abscisic acid sensitivity and biosynthesis in *Arabidopsis*," *The Plant Cell*, vol. 16, no. 7, pp. 1912–1924, 2004.

[5] J. Zhou, J. Wang, Y. Bi et al., "Overexpression of PtSOS2 enhances salt tolerance in transgenic poplars," *Plant Molecular Biology Reporter*, vol. 32, no. 1, pp. 185–197, 2014.

[6] R. Munns, D. P. Schachtman, and A. G. Condon, "The significance of a two-phase growth response to salinity in wheat and barley," *Australian Journal of Plant Physiology*, vol. 22, no. 4, pp. 561–569, 1995.

[7] Q. Yu, L. An, and W. Li, "The CBL–CIPK network mediates different signaling pathways in plants," *Plant Cell Reports*, vol. 33, no. 2, pp. 203–214, 2014.

[8] J. J. Rudd and V. E. Franklin-Tong, "Unravelling response-specificity in Ca^{2+} signalling pathways in plant cells," *New Phytologist*, vol. 151, no. 1, pp. 7–33, 2001.

[9] D. Sanders, J. Pelloux, C. Brownlee, and J. F. Harper, "Calcium at the crossroads of signaling," *The Plant Cell*, vol. 14, supplement 1, pp. S401–S417, 2002.

[10] A. N. Dodd, J. Kudla, and D. Sanders, "The language of calcium signaling," *Annual Review of Plant Biology*, vol. 61, pp. 593–620, 2010.

[11] O. Batistič, R. Waadt, L. Steinhorst, K. Held, and J. Kudla, "CBL-mediated targeting of CIPKs facilitates the decoding of calcium signals emanating from distinct cellular stores," *The Plant Journal*, vol. 61, no. 2, pp. 211–222, 2010.

[12] D. Sanders, C. Brownlee, and J. F. Harper, "Communicating with calcium," *The Plant Cell*, vol. 11, no. 4, pp. 691–706, 1999.

[13] Ü. Kolukisaoglu, S. Weinl, D. Blazevic, O. Batistic, and J. Kudla, "Calcium sensors and their interacting protein kinases: genomics of the *Arabidopsis* and rice CBL-CIPK signaling networks," *Plant Physiology*, vol. 134, no. 1, pp. 43–58, 2004.

[14] R. Quan, H. Lin, I. Mendoza et al., "SCABP8/CBL10, a putative calcium sensor, interacts with the protein kinase SOS2 to protect *Arabidopsis* shoots from salt stress," *The Plant Cell*, vol. 19, no. 4, pp. 1415–1431, 2007.

[15] Y. H. Cheong, G. K. Pandey, J. J. Grant et al., "Two calcineurin B-like calcium sensors, interacting with protein kinase CIPK23, regulate leaf transpiration and root potassium uptake in *Arabidopsis*," *The Plant Journal*, vol. 52, no. 2, pp. 223–239, 2007.

[16] K. Held, F. Pascaud, C. Eckert et al., "Calcium-dependent modulation and plasma membrane targeting of the AKT2 potassium channel by the CBL4/CIPK6 calcium sensor/protein kinase complex," *Cell Research*, vol. 21, no. 7, pp. 1116–1130, 2011.

[17] M. M. Drerup, K. Schlücking, K. Hashimoto et al., "The calcineurin B-like calcium sensors CBL1 and CBL9 together with their interacting protein kinase CIPK26 regulate the *Arabidopsis* NADPH oxidase RBOHF," *Molecular Plant*, vol. 6, no. 2, pp. 559–569, 2013.

[18] H. Zhang, W. Yin, and X. Xia, "Calcineurin B-like family in *Populus*: comparative genome analysis and expression pattern under cold, drought and salt stress treatment," *Plant Growth Regulation*, vol. 56, no. 2, pp. 129–140, 2008.

[19] X.-F. Chen, Z.-M. Gu, F. Liu, B.-J. Ma, and H.-S. Zhang, "Molecular analysis of rice *CIPKs* involved in both biotic and abiotic stress responses," *Rice Science*, vol. 18, no. 1, pp. 1–9, 2011.

[20] S. Weinl and J. Kudla, "The CBL–CIPK Ca^{2+}-decoding signaling network: function and perspectives," *New Phytologist*, vol. 184, no. 3, pp. 517–528, 2009.

[21] O. Batistič and J. Kudla, "Plant calcineurin B-like proteins and their interacting protein kinases," *Biochimica et Biophysica Acta (BBA)—Molecular Cell Research*, vol. 1793, no. 6, pp. 985–992, 2009.

[22] O. Batistic and J. Kudla, "Integration and channeling of calcium signalling through the CBL calcium sensor/CIPK protein kinase network," *Planta*, vol. 219, no. 6, pp. 915–924, 2004.

[23] R. Li, J. Zhang, J. Wei, H. Wang, Y. Wang, and R. Ma, "Functions and mechanisms of the CBL-CIPK signaling system in plant response to abiotic stress," *Progress in Natural Science*, vol. 19, no. 6, pp. 667–676, 2009.

[24] S. Luan, W. Lan, and S. Chul Lee, "Potassium nutrition, sodium toxicity, and calcium signaling: connections through the CBL–CIPK network," *Current Opinion in Plant Biology*, vol. 12, no. 3, pp. 339–346, 2009.

[25] M. Nagae, A. Nozawa, N. Koizumi et al., "The crystal structure of the novel calcium-binding protein AtCBL2 from *Arabidopsis thaliana*," *The Journal of Biological Chemistry*, vol. 278, no. 43, pp. 42240–42246, 2003.

[26] M. J. Sánchez-Barrena, M. Martínez-Ripoll, J.-K. Zhu, and A. Albert, "The structure of the *Arabidopsis thaliana* SOS3: molecular mechanism of sensing calcium for salt stress response," *Journal of Molecular Biology*, vol. 345, no. 5, pp. 1253–1264, 2005.

[27] M. Akaboshi, H. Hashimoto, H. Ishida et al., "The crystal structure of plant-specific calcium-binding protein AtCBL2 in complex with the regulatory domain of AtCIPK14," *Journal of Molecular Biology*, vol. 377, no. 1, pp. 246–257, 2008.

[28] Y. H. Cheong, K.-N. Kim, G. K. Pandey, R. Gupta, J. J. Grant, and S. Luan, "CBL1, a calcium sensor that differentially regulates salt, drought, and cold responses in *Arabidopsis*," *The Plant Cell*, vol. 15, no. 8, pp. 1833–1845, 2003.

[29] C. D'Angelo, S. Weinl, O. Batistic et al., "Alternative complex formation of the Ca^{2+}-regulated protein kinase CIPK1 controls abscisic acid-dependent and independent stress responses in *Arabidopsis*," *The Plant Journal*, vol. 48, no. 6, pp. 857–872, 2006.

[30] A. T. Fuglsang, Y. Guo, T. A. Cuin et al., "*Arabidopsis* protein kinase PKS5 inhibits the plasma membrane H^+-ATPase by preventing interaction with 14-3-3 protein," *The Plant Cell*, vol. 19, no. 5, pp. 1617–1634, 2007.

[31] L. Li, B.-G. Kim, Y. H. Cheong, G. K. Pandey, and S. Luan, "A Ca^{2+} signaling pathway regulates a K$^+$ channel for low-K response in Arabidopsis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 33, pp. 12625–12630, 2006.

[32] J. Xu, H.-D. Li, L.-Q. Chen et al., "A protein kinase, interacting with two calcineurin B-like proteins, regulates K$^+$ transporter AKT1 in *Arabidopsis*," *Cell*, vol. 125, no. 7, pp. 1347–1360, 2006.

[33] G. Thapa, M. Dey, L. Sahoo, and S. K. Panda, "An insight into the drought stress induced alterations in plants," *Biologia Plantarum*, vol. 55, no. 4, pp. 603–613, 2011.

[34] T. Yamanaka, Y. Nakagawa, K. Mori et al., "MCA1 and MCA2 that mediate Ca^{2+} uptake have distinct and overlapping roles in *Arabidopsis*," *Plant Physiology*, vol. 152, no. 3, pp. 1284–1296, 2010.

[35] H. D. Bradshaw Jr., "Mutations in CAX1 produce phenotypes characteristic of plants tolerant to serpentine soils," *New Phytologist*, vol. 167, no. 1, pp. 81–88, 2005.

[36] R. J. Tang, F. Zhao, V. J. Garcia et al., "Tonoplast CBL–CIPK calcium signaling network regulates magnesium homeostasis in *Arabidopsis*," *Proceedings of the National Academy of Sciences*, vol. 112, no. 10, pp. 3134–3139, 2015.

[37] C. Gao, Q. Zhao, and L. Jiang, "Vacuoles protect plants from high magnesium stress," *Proceedings of the National Academy of Sciences*, vol. 112, no. 10, pp. 2931–2932, 2015.

[38] O. Shaul, D. W. Hilgemann, J. de-Almeida-Engler, M. van Montagu, D. Inzé, and G. Galili, "Cloning and characterization of a novel Mg^{2+}/H$^+$ exchanger," *The EMBO Journal*, vol. 18, no. 14, pp. 3973–3980, 1999.

[39] S. J. Conn, V. Conn, S. D. Tyerman, B. N. Kaiser, R. A. Leigh, and M. Gilliham, "Magnesium transporters, MGT2/MRS2-1 and MGT3/MRS2-5, are important for magnesium partitioning within *Arabidopsis thaliana* mesophyll vacuoles," *New Phytologist*, vol. 190, no. 3, pp. 583–594, 2011.

[40] Q.-S. Qiu, Y. Guo, M. A. Dietrich, K. S. Schumaker, and J.-K. Zhu, "Regulation of SOS1, a plasma membrane Na$^+$/H$^+$ exchanger in *Arabidopsis thaliana*, by SOS2 and SOS3," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 12, pp. 8436–8441, 2002.

[41] Z.-M. Pel, Y. Murata, G. Benning et al., "Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells," *Nature*, vol. 406, no. 6797, pp. 731–734, 2000.

[42] J.-K. Zhu, "Salt and drought stress signal transduction in plants," *Annual Review of Plant Biology*, vol. 53, pp. 247–273, 2002.

[43] N.-H. Cheng, J. K. Pittman, J. K. Zhu, and K. D. Hirschi, "The protein kinase SOS2 activates the Arabidopsis H$^+$/Ca^{2+} antiporter CAX1 to integrate calcium transport and salt tolerance," *The Journal of Biological Chemistry*, vol. 279, no. 4, pp. 2922–2926, 2004.

[44] B.-G. Kim, R. Waadt, Y. H. Cheong et al., "The calcium sensor CBL10 mediates salt tolerance by regulating ion homeostasis in Arabidopsis," *The Plant Journal*, vol. 52, no. 3, pp. 473–484, 2007.

[45] V. Albrecht, S. Weinl, D. Blazevic et al., "The calcium sensor CBL1 integrates plant responses to abiotic stresses," *The Plant Journal*, vol. 36, no. 4, pp. 457–470, 2003.

[46] L. Chen, Q.-Q. Wang, L. Zhou, F. Ren, D.-D. Li, and X.-B. Li, "Arabidopsis CBL-interacting protein kinase (CIPK6) is involved in plant response to salt/osmotic stress and ABA," *Molecular Biology Reports*, vol. 40, no. 8, pp. 4759–4767, 2013.

[47] R.-K. Wang, L.-L. Li, Z.-H. Cao et al., "Molecular cloning and functional characterization of a novel apple MdCIPK6L gene

reveals its involvement in multiple abiotic stress tolerance in transgenic plants," *Plant Molecular Biology*, vol. 79, no. 1-2, pp. 123–135, 2012.

[48] D.-G. Hu, M. Li, H. Luo et al., "Molecular cloning and functional characterization of MdSOS2 reveals its involvement in salt tolerance in apple callus and Arabidopsis," *Plant Cell Reports*, vol. 31, no. 4, pp. 713–722, 2012.

[49] H. R. Kushwaha, G. Kumar, P. K. Verma, S. L. Singla-Pareek, and A. Pareek, "Analysis of a salinity induced BjSOS3 protein from *Brassica* indicate it to be structurally and functionally related to its ortholog from *Arabidopsis*," *Plant Physiology and Biochemistry*, vol. 49, no. 9, pp. 996–1004, 2011.

[50] J. Martínez-Atienza, X. Jiang, B. Garciadeblas et al., "Conservation of the salt overly sensitive pathway in rice," *Plant Physiology*, vol. 143, no. 2, pp. 1001–1012, 2007.

[51] M. Wang, D. Gu, T. Liu et al., "Overexpression of a putative maize calcineurin B-like protein in *Arabidopsis* confers salt tolerance," *Plant Molecular Biology*, vol. 65, no. 6, pp. 733–746, 2007.

[52] J.-L. Zhang, T. J. Flowers, and S.-M. Wang, "Mechanisms of sodium uptake by roots of higher plants," *Plant and Soil*, vol. 326, no. 1-2, pp. 45–60, 2010.

[53] Y. Wang and W.-H. Wu, "Potassium transport and signaling in higher plants," *Annual Review of Plant Biology*, vol. 64, pp. 451–476, 2013.

[54] E. Kiegle, C. A. Moore, J. Haseloff, M. A. Tester, and M. R. Knight, "Cell-type-specific calcium responses to drought, salt and cold in the Arabidopsis root," *Plant Journal*, vol. 23, no. 2, pp. 267–278, 2000.

[55] M. Nieves-Cordones, F. Caballero, V. Martínez, and F. Rubio, "Disruption of the *Arabidopsis thaliana* inward-rectifier K$^+$ channel AKT1 improves plant responses to water stress," *Plant and Cell Physiology*, vol. 53, no. 2, pp. 423–432, 2012.

[56] S. C. Lee, W.-Z. Lan, B.-G. Kim et al., "A protein phosphorylation/dephosphorylation network regulates a plant potassium channel," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 40, pp. 15959–15964, 2007.

[57] E. L. Thoday-Kennedy, A. K. Jacobs, and S. J. Roy, "The role of the CBL–CIPK calcium signalling network in regulating ion transport in response to abiotic stress," *Plant Growth Regulation*, vol. 76, no. 1, pp. 3–12, 2015.

[58] J. I. Schroeder, J. M. Ward, and W. Gassmann, "Perspectives on the physiology and structure of inward-rectifying K$^+$ channels in higher plants: biophysical implications for K$^+$ uptake," *Annual Review of Biophysics and Biomolecular Structure*, vol. 23, no. 1, pp. 441–471, 1994.

[59] A. Szyroki, N. Ivashikina, P. Dietrich et al., "KAT1 is not essential for stomatal opening," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 5, pp. 2917–2921, 2001.

[60] K. L. Dennison, W. R. Robertson, B. D. Lewis, R. E. Hirsch, M. R. Sussman, and E. P. Spalding, "Functions of AKT1 and AKT2 potassium channels determined by studies of single and double mutants of *Arabidopsis*," *Plant Physiology*, vol. 127, no. 3, pp. 1012–1019, 2001.

[61] G. Pilot, F. Gaymard, K. Mouline, I. Chérel, and H. Sentenac, "Regulated expression of Arabidopsis Shaker K$^+$ channel genes involved in K$^+$ uptake and distribution in the plant," *Plant Molecular Biology*, vol. 51, no. 5, pp. 773–787, 2003.

[62] H. Zhang, F. Lv, X. Han, X. Xia, and W. Yin, "The calcium sensor PeCBL1, interacting with PeCIPK24/25 and PeCIPK26,

regulates Na$^+$/K$^+$ homeostasis in *Populus euphratica*," *Plant Cell Reports*, vol. 32, no. 5, pp. 611–621, 2013.

[63] X. Wang, J. Li, X. Zou et al., "Ectopic expression of AtCIPK23 enhances tolerance against low-K$^+$ stress in transgenic potato," *American Journal of Potato Research*, vol. 88, no. 2, pp. 153–159, 2011.

[64] J. Li, L. Yu, G.-N. Qi et al., "The Os-AKT1 channel is critical for K$^+$ uptake in rice roots and is modulated by the rice CBL1-CIPK23 complex," *The Plant Cell*, vol. 26, no. 8, pp. 3387–3402, 2014.

[65] T. Cuéllar, F. Azeem, M. Andrianteranagna et al., "Potassium transport in developing fleshy fruits: the grapevine inward K$^+$ channel VvK1.2 is activated by CIPK-CBL complexes and induced in ripening berry flesh cells," *The Plant Journal*, vol. 73, no. 6, pp. 1006–1018, 2013.

[66] G. A. Tuskan, S. DiFazio, S. Jansson et al., "The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray)," *Science*, vol. 313, no. 5793, pp. 1596–1604, 2006.

[67] G. K. Pandey, Y. H. Cheong, B.-G. Kim, J. J. Grant, L. Li, and S. Luan, "CIPK9: a calcium sensor-interacting protein kinase required for low-potassium tolerance in *Arabidopsis*," *Cell Research*, vol. 17, no. 5, pp. 411–421, 2007.

[68] A. Amtmann and P. Armengaud, "The role of calcium sensor-interacting protein kinases in plant adaptation to potassium-deficiency: new answers to old questions," *Cell Research*, vol. 17, no. 6, pp. 483–485, 2007.

[69] N. M. Crawford, "Nitrate: nutrient and signal for plant growth," *The Plant Cell*, vol. 7, no. 7, pp. 859–868, 1995.

[70] A. D. Angeli, D. Monachello, G. Ephritikhine et al., "CLC-mediated anion transport in plant cells," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 364, no. 1514, pp. 195–201, 2009.

[71] B. G. Forde, "Nitrate transporters in plants: structure, function and regulation," *Biochimica et Biophysica Acta—Biomembranes*, vol. 1465, no. 1-2, pp. 219–235, 2000.

[72] Y.-F. Tsay, J. I. Schroeder, K. A. Feldmann, and N. M. Crawford, "The herbicide sensitivity gene CHL1 of *Arabidopsis* encodes a nitrate-inducible nitrate transporter," *Cell*, vol. 72, no. 5, pp. 705–713, 1993.

[73] A. Gojon, P. Nacry, and J.-C. Davidian, "Root uptake regulation: a central process for NPS homeostasis in plants," *Current Opinion in Plant Biology*, vol. 12, no. 3, pp. 328–338, 2009.

[74] W. Li, Y. Wang, M. Okamoto, N. M. Crawford, M. Y. Siddiqi, and A. D. M. Glass, "Dissection of the *AtNRT*2. 1: *AtNRT*2. 2 inducible high-affinity nitrate transporter gene cluster," *Plant Physiology*, vol. 143, no. 1, pp. 425–433, 2007.

[75] D. Y. Little, H. Rao, S. Oliva, F. Daniel-Vedele, A. Krapp, and J. E. Malamy, "The putative high-affinity nitrate transporter NRT2.1 represses lateral root initiation in response to nutritional cues," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 38, pp. 13693–13698, 2005.

[76] K.-H. Liu, C.-Y. Huang, and Y.-F. Tsay, "CHL1 is a dual-affinity nitrate transporter of Arabidopsis involved in multiple phases of nitrate uptake," *Plant Cell*, vol. 11, no. 5, pp. 865–874, 1999.

[77] R. Wang, D. Liu, and N. M. Crawford, "The Arabidopsis CHL1 protein plays a major role in high-affinity nitrate uptake," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 25, pp. 15134–15139, 1998.

[78] C.-H. Ho, S.-H. Lin, H.-C. Hu, and Y.-F. Tsay, "CHL1 functions as a nitrate sensor in plants," *Cell*, vol. 138, no. 6, pp. 1184–1194, 2009.

[79] K.-H. Liu and Y.-F. Tsay, "Switching between the two action modes of the dual-affinity nitrate transporter CHL1 by phosphorylation," *The EMBO Journal*, vol. 22, no. 5, pp. 1005–1013, 2003.

[80] G. Vert and J. Chory, "A toggle switch in plant nitrate uptake," *Cell*, vol. 138, no. 6, pp. 1064–1066, 2009.

[81] H.-C. Hu, Y.-Y. Wang, and Y.-F. Tsay, "AtCIPK8, a CBL-interacting protein kinase, regulates the low-affinity phase of the primary nitrate response," *The Plant Journal*, vol. 57, no. 2, pp. 264–278, 2009.

[82] D. P. Schachtman, R. J. Reid, and S. M. Ayling, "Phosphorus uptake by plants: from soil to cell," *Plant Physiology*, vol. 116, no. 2, pp. 447–453, 1998.

[83] R. L. Bieleski, "Phosphate pools, phosphate transport, and phosphate availability," *Annual Review of Plant Physiology*, vol. 24, no. 1, pp. 225–252, 1973.

[84] M. E. Theodorou and W. C. Plaxton, "Metabolic adaptations of plant respiration to nutritional phosphate deprivation," *Plant Physiology*, vol. 101, no. 2, pp. 339–344, 1993.

[85] L. Chen, F. Ren, L. Zhou, Q.-Q. Wang, H. Zhong, and X.-B. Li, "The Brassica napus Calcineurin B-Like 1/CBL-interacting protein kinase 6 (CBL1/CIPK6) component is involved in the plant response to abiotic stress and ABA signalling," *Journal of Experimental Botany*, vol. 63, no. 17, pp. 6211–6222, 2012.

[86] K. Nakashima and K. Yamaguchi-Shinozaki, "ABA signaling in stress-response and seed development," *Plant Cell Reports*, vol. 32, no. 7, pp. 959–970, 2013.

[87] G. J. Allen, S. P. Chu, C. L. Harrington et al., "A defined range of guard cell calcium oscillation parameters encodes stomatal movements," *Nature*, vol. 411, no. 6841, pp. 1053–1057, 2001.

[88] G. J. Allen, S. P. Chu, K. Schumacher et al., "Alteration of stimulus-specific guard cell calcium oscillations and stomatal closing in *Arabidopsis* det3 mutant," *Science*, vol. 289, no. 5488, pp. 2338–2342, 2000.

[89] J. Leung and J. Giraudat, "Abscisic acid signal transduction," *Annual Review of Plant Biology*, vol. 49, no. 1, pp. 199–222, 1998.

[90] V. Chinnusamy, K. Schumaker, and J.-K. Zhu, "Molecular genetic perspectives on cross-talk and specificity in abiotic stress signalling in plants," *Journal of Experimental Botany*, vol. 55, no. 395, pp. 225–236, 2004.

[91] K.-N. Kim, Y. H. Cheong, J. J. Grant, G. K. Pandey, and S. Luan, "CIPK3, a calcium sensor-associated protein kinase that regulates abscisic acid and cold signal transduction in *Arabidopsis*," *Plant Cell*, vol. 15, no. 2, pp. 411–423, 2003.

[92] G. K. Pandey, J. J. Grant, Y. H. Cheong, B.-G. Kim, L. G. Li, and S. Luan, "Calcineurin-B-like protein CBL9 interacts with target kinase CIPK3 in the regulation of ABA response in seed germination," *Molecular Plant*, vol. 1, no. 2, pp. 238–248, 2008.

[93] Y. Guo, L. Xiong, C.-P. Song, D. Gong, U. Halfter, and J.-K. Zhu, "A calcium sensor and its interacting protein kinase are global regulators of abscisic acid signaling in *Arabidopsis*," *Developmental Cell*, vol. 3, no. 2, pp. 233–244, 2002.

[94] Y.-S. Hwang, P. C. Bethke, H. C. Yong, H.-S. Chang, T. Zhu, and R. L. Jones, "A gibberellin-regulated calcineurin B in rice localizes to the tonoplast and is implicated in vacuole function," *Plant Physiology*, vol. 138, no. 3, pp. 1347–1358, 2005.

[95] S. Mahajan, S. K. Sopory, and N. Tuteja, "Cloning and characterization of CBL-CIPK signalling components from a legume (*Pisum sativum*)," *The FEBS Journal*, vol. 273, no. 5, pp. 907–925, 2006.

[96] Y. Xiang, Y. Huang, and L. Xiong, "Characterization of stress-responsive CIPK genes in rice for stress tolerance improvement," *Plant Physiology*, vol. 144, no. 3, pp. 1416–1428, 2007.

[97] Y. Yu, X. Xia, W. Yin, and H. Zhang, "Comparative genomic analysis of CIPK gene family in Arabidopsis and Populus," *Plant Growth Regulation*, vol. 52, no. 2, pp. 101–110, 2007.

[98] Z. Gu, B. Ma, Y. Jiang, Z. Chen, X. Su, and H. Zhang, "Expression analysis of the calcineurin B-like gene family in rice (*Oryza sativa* L.) under environmental stresses," *Gene*, vol. 415, no. 1-2, pp. 1–12, 2008.

[99] M. J. Sánchez-Barrena, M. Martínez-Ripoll, and A. Albert, "Structural biology of a major signaling network that regulates plant abiotic stress: the CBL-CIPK mediated pathway," *International Journal of Molecular Sciences*, vol. 14, no. 3, pp. 5734–5749, 2013.