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Role of Calcium in Regulating Potassium-Sodium Homeostasis and Potassium as Nutrient Signal during Abiotic Stress Conditions

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ABSTRACT

Calcium is a ubiquitous cation, which serves as a second messenger for numerous signals and confers specific cellular responses in eukaryotes. Recent studies have established a concept termed 'Ca^{2+} signature' that specifies Ca^{2+} changes triggered by each signal. However, it is very fascinating how this pervasive cation can translate an infinite number of stimuli into unique stimulus-dependent responses. Ca^{2+} is a fundamental component of nutrition signaling under stress condition. It interacts with various calcium sensors, which are directly involved in various molecular, biochemical and cellular changes occurring during the plant's adaptation to nutritional stress. Recently, in calcium signaling in plants, the CBL-CIPK protein network has been implicated in phytohormone (ABA), abiotic stress and potassium nutrition signaling. This review will mainly focus on the functional relationship of calcium-mediated salt stress tolerance, potassium nutrition, and potassium-sodium homeostasis by involvement of the CBL-CIPK complex.

Keywords: calcium signaling, CBL, CIPK, K^+/Na^+ homeostasis, Na^+/H^+-antiporter, signal transduction
Abbreviations: ABA, abscisic acid; **AKT1**, *Arabidopsis* K^+ transporter 1; **CBL**, calcineurin B-like protein; **CDPK**, Ca^{2+}-dependent protein kinase; **CIPK**, CBL-interacting protein kinase; **GORK**, gated outwardly-rectifying K^+ channel; **HKT**, high-affinity K^+ transporter; **MAPK**, mitogen-activated protein kinase; **NHX**, Na^+/H^+ exchanger; **ROS**, reactive oxygen species; **SOS**, salt overly sensitive

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INTRODUCTION

Plants often grow in soils that contain very low availability of the nutrients. To adapt this nutrient-deprived environment they must sense changes in environment and internal mineral nutrient concentrations. In the biological system, entire living organisms accumulate food and utilize it for growth and development and this phenomenon is referred as "nutrition". Nutrients are categorized as "macronutrients" and "micro-nutrients" absorbed by roots and are essential for optimal plant growth. Macronutrients are required in large amount while micronutrients are required in small quantity. Plants require almost fourteen different mineral elements and absorption of these nutrients is affected by several factors. Deficiency of any one of these elements results in abnormalities in growth and development of plant.

Potassium (K) is one of the major macronutrient required by plants at relatively higher concentration. It has already been well established that K plays an important role in metabolism, growth and stress adaptation of plant. Whereas, plant require sodium (Na), a micronutrient at relatively low concentration and is extremely harmful to plant at higher concentration. Both in K deficient and saline soil, the

Received: 27 January, 2009. Accepted: 16 March, 2010.

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growth of plant is adversely affected. Under normal condition, plant always tries to maintain a higher homeostatic ratio of K^+/Na^+ in the cytosol of plant cell while in the saline soil, excess entry of Na^+ which leads to a high Na^+/K^+ ratio in the cytosol which leads to lethality. Because of similar ionic radius and hydration energy, Na^+ might compete with K^+ for its entry into the root cell and hence disrupt the K^+-uptake and nutrition. There are K^+ outward channels, which also help in maintaining K^+ homeostasis by their K^+ efflux activity (Luan *et al*. 2009). Under salt stress, it is crucial for the plant to differentiate between the beneficial K to toxic sodium ions in the root cell and the molecular mechanism underlying this process requires detail investigation. There could be several aspects to understand this process where root cells distinguish essential K and toxic sodium ions. Some of the important mechanisms could be the selective uptake, distribution of ions and ions homeostasis. Several different genes have been identified, which encode proteins for different transporters/channels, involved in signaling of Na^+ and K^+ ions homeostasis. Moreover, a complex regulation at both expression and post-translational level of these proteins tightly control homeostasis of K and sodium ions under saline stress condition. Under different nutrient deprivation condition, there is a cross talk and overlap of signaling pathways, which also leads to interaction of these essential plant nutrients such as K, nitrogen (N) and calcium (Ca^{2+}).

Along with a nutrient-deprived soil microenvironment, various other external stimuli such as light, pathogen attack, abiotic stresses such as salt, drought and freezing lead to generation of second messangers such as Ca^{2+}, ROS (reactive oxygen species), inositol 1, 4, 5 triphosphate (IP_3), molecules which are involved in several signaling pathways (Tuteja 2009a, 2009b). IP_3 and ROS are also responsible for transient release of Ca^{2+} stored in organelles. Being a versatile and ubiquitous cation, Ca^{2+} is involved in numerous general housekeeping functions in the cell. At the same time, it is extremely important to realize that Ca^{2+} is involved in myriad of signaling processes to impart specific responses (Marschner 1995). Ca^{2+}-triggered events are critical for both normal cellular activity and for adapted responses (Sanders *et al.* 2002). Ca^{2+} is also involved in the regulation of K^+/Na^+ homeostasis, K nutrition, ionic selectivity under saline condition and exogenous Ca^{2+} can improve the salt tolerance (LaHaye and Epstein 1969; Liu and Zhu 1997a).

The spatial and temporal transient increase in Ca^{2+} level is known as "Ca^{2+} signature" which leads to changes in range of responses at molecular, biochemical, cellular and morphological level, including changes in cytosolic and organellar ions concentrations in the cells (Sanders *et al.* 1999, 2002). In response to nutrient deprivation and abiotic stress conditions, various Ca^{2+} sensors proteins like CBLs and other sensors (Luan *et al.* 2002) bind to free cytosolic Ca^{2+} and transduce signal downstream through various kinases like CIPKs, MAPKs and CDPKs (Rudd and Franklin-Tong 2001; Pandey 2008; Tuteja and Sopory 2008; Mahajan *et al.* 2008). These downstream signals are responsible for ion homeostasis including uptake of ions, extrusion, and sequestration of ions, long distance transport and metabolic changes by a precise control of transporters, pumps or channels located in the plasma membrane or in organellar membranes (Xiong and Zhu 2002). Under nutrient deficient condition both short-term and longer-term responses will be important in understanding the progression of signaling events (Schachtman and Shin 2007).

In this review, we mainly discuss the events that lead to plant responses to sodium and K ion homeostasis under saline condition and the regulation of K nutrition and signaling under nutrient deficient conditions. First, we will discuss the regulation of salt stress by SOS/CBL-CIPK protein complex. Second, we will be emphasizing Ca^{2+}-mediated regulation of K nutrient signaling, especially K^+/Na^+ homeostasis and signaling under stress condition.

Calcium as second messenger

The Ca^{2+} emerged as a versatile, ubiquitous second messenger, imparts physiological contribution in normal plant growth and development and in responses to stress through Ca^{2+}-signaling (Tuteja and Sopory 2008; Tuteja 2009a). In plants, number of external stimuli such as biotic, abiotic or hormonal response lead to changes in cytoplasmic Ca^{2+} concentration, which in turn regulate a wide variety of responses (Tuteja 2007, 2009b). The extracellular stress signal is first perceived by the membrane receptors, thereby activating complex intracellular signaling cascade including the generation of second messenger such as Ca^{2+}. Like other eukaryotes, Ca^{2+} concentration approximately varies from 100-200 nM in plants at the resting state (Gilroy *et al*.1993) while 1-10 mM constant supply of Ca^{2+} is required for normal plant growth and development (Epstein 1972). In response to the external stimuli, the spatially and temporally unique Ca^{2+} oscillations are generated through the coordinated activities of Ca^{2+} permeable channels/pumps and active transporters located on either at plasma membrane or at different organelles. These transporters/channels allow the downhill flow of Ca^{2+} at relatively higher concentration from one compartment to another compartment in which Ca^{2+} is at lower level, thereby ultimately leads to initiate stress signaling pathways (Knight 2000; Sanders *et al.* 2002). One general question arises that how Ca^{2+} operates different signaling pathways in response to various extracellular stimuli with high degree of precision and specificity. The answer of this question is the spatial and temporal expression of Ca^{2+} wave and oscillation in the cells called "Ca^{2+} signatures" for specific response (Knight *et al*. 1995).

CALCIUM AND SALT STRESS SIGNALING

More than 30% agricultural land in the world is affected by salt stress. To cope up with salinity, future crops need to be designed by genetic engineering, which can withstand and grow in the saline soil without compromising the yield and quality of the crop. Understanding the maintenance of intracellular ionic homeostasis is of key importance to develop crops which can sustain higher degree of stress without loosing yield and productivity. Under normal physiological conditions, the plant cell needs to keep the concentration of toxic ions below a threshold level and accumulate essential ions. Salt tolerance is a complex trait and involves ionic and osmotic re-equilibrium to maintain a state of homeostasis in the cell. It is a coordinated interplay of a various sets of genes, which are modulated as a consequence of salt-stress perception, whose products finally lead to a state of homeostasis and salt tolerance. In saline soil, Na^+ can generates low water potential zone in the soil, making difficult for uptake of water as well as nutrients by roots from the soil, which is the cause of physiological drought for plants (Mahajan and Tuteja 2005; Mahajan *et al.* 2008). Salt tolerant plant can be engineered by enhancing the osmotic adjustment and salt compartmentalization processes of plant cell. Ca^{2+} is involved in plant salt tolerance and leads to a coordinated action of various sets of genes which are activated in response to stress signals (Zhu 2002; Luan 2008). Cytosolic Ca^{2+} perturbations are precisely decoded by Ca^{2+}-sensing proteins or binding protein to relay the signaling cascade. Genetic, biochemical, molecular and cell biological approaches in recent years have resulted in significant progress in identifying several Ca^{2+}-sensing proteins in plants and in understanding the function of Ca^{2+}-regulated downstream components in various signaling network in plants. The various Ca^{2+} sensors or Ca^{2+}-binding proteins bind Ca^{2+} either to sequestrate or to perform some other complex tasks. The intracellular increase in the Ca^{2+} is perceived by various Ca^{2+}-binding proteins, such as recently discovered Ca^{2+} sensor, calcineurin B-like proteins (CBLs), and Ca^{2+}-dependent protein kinases (CDPKs) (Luan *et al*. 2002; Sanders *et al*. 2002; Batistic and Kudla 2004; Ludwig *et al*. 2004). CIPKs, CDPKs, MAPK and other kinases

initiates a phosphorylation cascades in response to Ca^{2+} and Ca^{2+} sensors, are the most common regulatory components in signal transduction which further transduce the signal downstream by modulating transcription factors, transporter, channels/pumps and other components for stress tolerance (Tena *et al.* 2001; Luan *et al.* 2002; Batistic and Kudla 2004; Luan 2008). CBL-CIPK network is extensively studied system in response to salt tolerance in plants. These CBL-CIPKs complexes evolutionary evolve from algal system to angiosperm (Batistic and Kudla 2009). Rice, *Arabidopsis*, and *Populus tricocarpa* genome encodes at least 10 CBLs in each and 30, 25, and 26 CIPKs respectively (Luan *et al.* 2002; Kolukisaoglu *et al.* 2004; Yu *et al.* 2007). In lower plants, *Physcomitrella patensa*, a bryophyte, genome encodes 4 CBLs and 7 CIPKs while green algae like *Ostreococcus tauri* and the related species *O. lucimarinus* encode only a single CBL protein and single CIPK gene while other green algae species such as *Chlamydomonas reinhardtii* and *Volvox carteri* do not have a single CBL or CIPK (Batistic and Kudla 2009; Luan 2009). This plant specific novel CBL-CIPK signaling pathway evolutionary less evolved in lower plants and gradually become complex in higher plants. Because each CBL protein may interact with more than one CIPK protein and *vice versa* leads to complex and functional diversification of the CBL–CIPK pathways that are relevant for stress signaling (Luan *et al.* 2002; Kolukisaoglu *et al.* 2004; Pandey *et al.* 2008; Luan 2009).

SOS pathway

Sodium is a micronutrient in plant cell. Salinity due to excessive availability of Na^+ in the soil either by natural cause or irrigation is a major problem for agricultural production in the world (Epstein *et al.* 1980). To understand molecular mechanism of salt tolerance in the higher plants various signaling pathways have been discovered in recent years. Two major pathways for salt tolerance have been discovered as SOS pathway and SOS genes associated CBL10-SOS2/CIPK24 pathway in *Arabidopsis*. The higher plants tolerate salinity either by SOS pathway by pumping out Na^+ ions out of cell by the plasma membrane Na^+/H^+-antiporter, SOS1 in roots (Shi *et al.* 2000), and/or by the CBL10–SOS2/CIPK24 complex in shoots by Na^+ ions sequestration from cytoplasm to vacuole (Kim *et al.* 2007; Quan *et al.* 2007).

The SOS pathway is studied in detailed in *Arabidopsis* while some homologous component or genes are isolated from algae, bryophytes (Luan 2009) and other angiosperms (Zhou *et al.* 2006; Martínez-Atienza *et al.* 2007; Wu *et al.* 2007) indicates the universality of SOS pathways from lower plants to higher plants. The SOS pathways work in Ca^{2+}-dependent manner, consist of Ca^{2+} sensor SOS3 or CBL4 belong to CBL family which physically interact with SOS2/CIPK24, a CBL-interacting protein kinase and transduce signal to activate SOS1, a plasma membrane Na^+/H^+-antiporter to efflux the Na^+ out from cell. The different *sos* mutants were isolated to understand the basic mechanism of SOS pathway. Salt hypersensitivity is a basic cellular traits exhibited by *sos* mutants at all developmental stages. These *sos* mutants are hypersensitive specifically to Na^+ and Li^+ ions and did not show any sensitivity to most of the divalent cations and anions, moreover *sos1* accumulates more proline under salt stress in comparison to wild type (Liu and Zhu 1997b). All three *sos* mutants i.e. *sos1*, *sos2* and *sos3* shows similar phenotype and double mutants did not show a cumulative effect on salt sensitivity suggesting that these genes exist in the same pathways, leading to salt tolerance (Liu and Zhu 1997b; Zhu 2000). These three components of SOS pathway and associated genes in other system are discussed below in detail.

SOS1 and ion transporter in Na^+ homeostasis

The ion transporters, channels/pumps are playing critical role in maintenance of ion homeostasis and plant adaptation. To maintain the optimal concentration of Na^+ in the cytoplasm, plant cell machinery use different type of transporters, channels/pumps either on plasma membrane or organellar membrane. One of these are the Na^+/H^+-transporters which catalyze the exchange of Na^+ for H^+ across the plasma membrane as well as vacuolar membrane to efflux Na^+ ions to apoplast or to compartmentalize it into the vacuole, respectively in order to maintain internal pH in the cell and ion homeostasis (Horie and Schroeder 2004).

To understand the basic mechanism of salt tolerance, Wu *et al.* (1996) first isolated the salt-hypersensitive mutant *sos1* mutants by using a root-bending assay on NaCl-containing agar plates in *Arabidopsis*. Phenotypically these mutants were normal as wild type while they exhibited more growth inhibition and salt-hypersensitivity to NaCl (Wu *et al.* 1996). The *sos1* mutants show more hypersensitivity to Na^+ and Li^+ ion than *sos2* and *sos3* mutants (Zhu *et al.* 1998). SOS1 locus contributed more in salt tolerance in comparison to two other SOS loci. These analyses revealed the fact that the regulation of SOS1 was governed by SOS3-SOS2 pathway (Shi *et al.* 2000). SOS1 encodes Na^+/H^+-antiporters, largest known Na^+/H^+ antiporter, shows sequence homology with Na^+/H^+-antiporters of microbial and animals origins. Moreover, SOS1 shows homology with other organellar Na^+/H^+-antiporters such as AtNHX1 or NHE6 confirming that SOS1 also efflux excess Na^+ ions into the apoplastic region (Shi *et al.* 2000). The hydrophilic cytosolic c-terminal region of SOS1 was unique for SOS1 and shows no similarity to other antiporters in the GenBank database. The hydrophilic c-terminal region of SOS1 provide vital platform to interact with various cytosolic regulators for the Na^+/H^+-antiport activity of SOS1. The mutation in c-terminal region of SOS1 may disturb the physical interaction with cytosolic regulators. It is interesting to note that SOS1 transcripts were up regulated in root than shoot by salt stress. In *sos3* mutants, *SOS1* transcripts were not up regulated in response to NaCl both in root and shoot. However, in *sos2* mutants, SOS1 was up regulated in response to NaCl in roots but not in shoots.

Similarly also in rice, SOS1 Na^+/H^+-antiporter homologous gene OsSOS1 was isolated. The yeast expressing OsSOS1 exchange Na^+ in plasma membrane vesicles and decreased Na^+ level in cell and confers salt tolerance to the yeast mutant, *axt3k*, mutated for different Na^+/H^+-antiporters (Δena1–4 Δnha1 Δnhx1) and also to *Arabidopsis sos1* mutant (Martínez-Atienza *et al.* 2007; Gao *et al.* 2008). Salt inducible, plasma membrane localized *Populus euphratica SOS1*, *PeSOS1* was isolated and showed 64% sequence identity with *Arabidopsis* SOS1 (Wu *et al.* 2007). These results strongly suggest that components of SOS pathway are effectively functional and conserved in higher plants.

The vacuolar Na^+/H^+-antiporter, OsNHX1 can also sequester Na^+ into vacuoles. Over-expression of OsNHX1 improves salt tolerance in transgenic rice plants without adversely affecting plant growth and Na^+ and K^+ balance (Fukuda *et al.* 2004). Recently, salt induced plasma membrane localized OsNHA1, a Na^+/H^+-antiporter, was cloned from rice, with significant similarity with *Arabidopsis* plasma membrane localized Na^+/H^+-antiporter AtNHA1 (Zhou *et al.* 2006). For optimal Na^+ concentration in the cell, vacuolar sequestration also play important role in Na^+ ion homeostasis. Many antiporters such as NHX1, NHX5, AVP1 and AVP2 are known which sequester excess Na^+ in vacuole in higher plants (Mullan *et al.* 2007).

In addition to Na^+/H^+-antiporter, other transporters such as high affinity K^+ transporter (HKT) plays an important role in ionic homeostasis. The *Arabidopsis* genome encodes single HKT gene having role to balance optimal Na^+ concentration by Na^+ entry into the cell while rice cv. 'Nipponbare SKC1' encodes seven HKT-type Na^+-selective transporters (Garciadeblas *et al.* 2003). Rice SKC1 can unload Na^+ ion from xylem sap and recirculation it to root hence maintaining K^+-homeostasis and ultimately enhancing salt tolerance (Ren *et al.* 2005). Some of the other ion transporters such as CAX1, a vacuolar H^+/Ca^{2+}-antiporter1 and

AHA1, a plasma membrane H^+-ATPase were also modulated by CBL-CIPK complex in maintenance of ion homeostasis (Cheng *et al.* 2004; Batelli *et al.* 2007).

SOS2/CIPK24 in salt tolerance

Phosphorylation by protein kinases such as CIPKs, CDPKs, MAPK and other kinases is one of the most significant regulatory mechanisms in plant signaling cascade (Guo *et al.* 2001; Luan *et al.* 2002; Batistic and Kudla 2004; Luan 2009). The yeast two-hybrid assay was used as an effective strategy for identifying targets of Ca^{2+} sensors CBLs. CBLs interact with protein kinase unlike its homolog CNB (Calcineurin B subunit), which interact with a protein phosphatase CNA (Calcineurin A subunit) in yeast and mammals. Using a yeast two-hybrid assay a novel family of protein kinase was identified named as Calcineurin B-Like (CBL)-interacting protein kinases (CIPKs) which physically interact with CBLs in *Arabidopsis* (Shi *et al.* 2000). The N-terminal kinase domain shows sequence similarity to kinase domain of sucrose non-fermenting (SNF) protein kinases in yeast and AMP-dependent kinases (AMPKs) in animals while C-terminal regulatory domains are unique to the plant CIPKs (Liu *et al.* 2000).

The SOS2/CIPK24 gene was isolated through positional cloning by Liu and Zhu (1997a) and was shown to be required for sodium and K ion homeostasis and salt tolerance. SOS2 encodes a serine/threonine protein kinase with an N-terminal catalytic domain and a C-terminal regulatory domain. CBL function as Ca^{2+}sensors and target downstream effectors protein kinases to plasma membrane or different organelle in different stress condition. Under stress condition, the Ca^{2+} bound CBL activate CIPKs by release of auto-inhibitory action of regulatory domain to kinase domain and provide space for substrate to access the catalytic site. In Ca^{2+}-dependent manner the Ca^{2+} sensor SOS3/CBL4 physically interact to 21 amino acid residues NAF/FISL motif in the SOS2 regulatory domain of SOS2/CIPK24, releases the catalytic domain that subsequently activates the substrate phosphorylation activity of SOS2/CIPK24 in salt stress condition (Halfter *et al.* 2000; Liu *et al.* 2000). Analyses of minimal domain search for interaction of SOS3/CBL4 with SOS2/CIPK24 have led to identification of NAF/FISL motif necessary and sufficient for interaction. SOS2 and SOS3 interaction is also supported by *sos2sos3* double mutant analysis, which indicated that they exist in the same pathway (Halfter *et al.* 2000). In addition to regulate SOS1, SOS2 has been shown to modulate *Arabidopsis* H^+/Ca^{2+}-antiporter CAX1 and activates Ca^{2+} transport and salt tolerance (Cheng *et al.* 2004).

It is important to conceive that there must be a desensitizing mechanism, which brings back the stress-activated pathway to normalcy once the signal is being transduce further down in the signaling pathway. The activated SOS3/CBL4-SOS2/CIPK24 complex in stress condition is being down regulated by ABI2 (Abscisic acid-Insensitive 2) protein phosphatase, a protein phosphatase 2C (PP2C). ABI2 interact with the protein phosphatase interaction motif (PPI) conserve in SOS2/CIPK24 family by which ABI2 interact and dephosporylate active kinase (Ohta *et al.* 2003). It was also speculated that ABI2 might dephosphorylate the proteins that are phosphorylated by SOS2 (Ohta *et al.* 2003).

In other plant system SOS2/CIPK24 homologues were also isolated such as salt-inducible *BnSOS* from *Brassica napus*, which belongs to a typical Ser/Thr protein kinase family (Wang *et al.* 2004). In green pea (*Pisum sativum*) salt inducibe CIPK (PsCIPK) was also isolated (Mahajan *et al.* 2006; Tuteja and Mahajan 2007b). SOS pathway is not only limited to *Arabidopsis* but also widely present in higher plants. In rice, many CIPKs have been identified having a diverse role in different stress condition and over-expression of OsCIPK15 improves salt tolerance (Xiang *et al.* 2007).

SOS3/CBL4 in salt tolerance

A typical Ca^{2+} signature generated by salt stress can be sensed by several Ca^{2+} sensors such as CaM, CaM-like proteins, CDPKs and recently discovered novel calcineurin B-like proteins (CBLs) in *Arabidopsis* (Luan 2009). CBL proteins played a diverse and critical role in salt stress signaling either through SOS pathway or through SOS-like pathways. SOS3/CBL4 is an EF-hand type Ca^{2+} binding protein which shows some similarity with yeast and mammalian Ca^{2+}-sensor CNB (calcineurin B subunit) of Ca^{2+} and CaM dependent calcineurin (Luan *et al.* 2002; Pandey 2008; Luan 2009). Calcineurin consist of phosphatase catalytic subunit calcineurin A (CNA) and regulatory subunit calcineurin B (CNB). Calcineurin play a vital role in different cellular processes including stress management to developmental processes in yeast and mammals. An exhaustive search for presence of calcineurin was done in higher plants such as *Arabidopsis* and rice but failed to yield its presence by both experimental and genome analyses methodologies (Luan *et al.* 2002; Kolukisaoglu *et al.* 2004; Yu *et al.* 2007). Functionally CBL proteins in plants are Ca^{2+}-binding protein, which interact and target a specific type of protein kinase instead of a protein phosphatase in yeast and mammals. The number and arrangement of canonical to non-canonical EF-hands in each specific CBL varies. In response to specific stimulus, the specific concentration of Ca^{2+} in cell favor specific binding affinity of each CBL to Ca^{2+} that leads to differential activation of CBL–CIPK complexes (Batistic and Kudla, 2004, 2008; Pandey 2008).

Two groups independently isolated SOS3 or CBL4 and identified as a Ca^{2+} binding protein, which belongs to CBL protein family (Liu *et al.* 1998; Kudla *et al.* 1999). In response to salt stress, the transient increase in the Ca^{2+} concentration lead to activation of SOS3/CBL4, which then binds to SOS2/CIPK24, and activate the downstream processes (Ishitani *et al.* 2000; Sanchez-Barrena *et al.* 2005). Mutant analyses have uncovered that the *sos3* and *sos1* mutants are specifically hypersensitive to Na^+ and Li^+ (Liu and Zhu 1997a) and also defective in high K uptake because they are unable to grow on media less than 4 mM K. These mutants provide primary evidence that salt tolerance somehow linked with Na^+/K^+-homeostasis in glycophytes (Wu *et al.* 1996). Surprisingly, increased extracellular Ca^{2+} suppresses the growth defect of *sos3* plants on low K^+ or 50 mM NaCl and improved K^+/Na^+ selectivity of both *sos3* and wild-type plants but not *sos1* plants. The *sos3* seedlings accumulated more Na^+ and less K^+ than the wild type in NaCl. However, this Ca^{2+} effect in *sos3* is more than twice of wild type. This study indicates that *SOS3* locus is essential for K^+-nutrition, K^+/Na^+ selectivity, and salt tolerance in higher plants (Liu and Zhu 1997a). The Ca^{2+} effect is specific to *sos3* and was not observed for *sos1* (Liu and Zhu 1998). Phenotypically *sos1* and *sos3 are* similar, means two genes probably function in a common pathway that regulates K nutrition and salt tolerance. Genetic evidence suggests that *sos1* is epistatic to *sos3* (Liu and Zhu 1997a). The *sos1* accumulates less Na^+ than the wild type in response to NaCl stress and this reduced Na^+ accumulation in *sos1* is due to a lower Na^+ influx rate. Therefore, the *sos1* mutation appears to disrupt low-affinity Na^+-uptake in addition to its impairment of high-affinity K^+-uptake (Ding and Zhu 1997).

Besides SOS3/CBL4-SOS2 and CBL10-SOS2 pathway, some other CBLs and CIPKs are also involve in salt tolerance in plants. In osmotic stress mediated signaling pathway some of the other CBL-CIPK genes are also involved in salt stress mediated responses such as CBL1 (Cheong *et al.* 2003) and CBL9 (Pandey *et al.* 2004) and CBL-interacting protein kinase, CIPK1 (D'Angelo *et al.* 2006). These CBL and CIPK components were suggested to play role in osmotic adjustment response of salt stress. At the same time CBL1 gene seems to be involved in variety of abiotic stress signaling pathways by showing responses to different stress including wounding, cold, drought and high salt (Kudla *et*

al. 1999; Cheong *et al.* 2003). *CBL1* expression and its mutant analysis shows that it is involved in salt tolerance along with other abiotic stress responses, unlike SOS3/ CBL4 and CBL10/SOS2 pathway which are specifically involved Na^+ ion homeostasis under high salt conditions.

CBL10- SOS2/ CIPK24 in salt tolerance

Differential sub-cellular localization for several CBLs such as CBL1, CBL4/SOS3, CBL9, and CBL10 was reported to localize to either plasma membrane or organellar membrane fractions (Ishitani *et al.* 2000; Luan *et al.* 2002; Kim *et al.* 2007; Quan *et al.* 2007; Batistic and Kudla 2009). Some of these CBL proteins are co-translationally modified by fatty acid myristate and can undergo optional post-translational modification at cysteine residue adjacent to myristoylated glycine by palmitoyl fatty acid (Bijlmakers and Marsh 2003; Batistic and Kudla 2009). In case of CBL1 both myristoyltion and palmitoylation are essential and required for salt tolerance recovery of *cbl1* mutant by cDNA complementation (Batistic *et al.* 2008) is important for protein–protein interaction or localization and stable attachment to the membranous fractions (Batistic and Kudla 2009). The N-terminal myristoylation of CBL4/SOS3 is important and necessary to recruit CIPK24/SOS2 to the plasma membrane in yeast (Quintero *et al.* 2002) and for phosphorylation of Na^+/H^+-antiporter, SOS1, for salt tolerance (Ishitani *et al.* 2000; Shi *et al.* 2000; Qiu *et al*. 2002). Therefore, it is generalized that plant Ca^{2+} sensors acquired certain protein domains that restrict their localization, serving as a mechanism for establishing location-dependent signal transduction pathways that initiate specific cellular responses (Luan 2009).

CIPKs do not have a peptide signal for localization because CBLs target the activated CIPKs to their substrate on the plasma membrane or organellar membrane. CBL–CIPK interactions and localization studies was done for a few CBL-CIPK complex by recently developed technique called bimolecular fluorescence complementation (BiFC) and with this technique several CIPKs were shown to localized either to plasma membrane or vacuolar membrane of cell by their interaction with CBLs. For example, CIPK1 is targeted to the plasma membrane by CBL1 or CBL9, and CIPK23 is also targeted to plasma membrane by CBL1 and CBL9. The development of multicolor BiFC analyses also provides exact idea about simultaneous and alternative CBL-CIPK complex formation and localization in same cell (Waadt *et al.* 2008).

Simultaneous complex formation of CBL1–CIPK1 and CBL9–CIPK1 localized at plasma membrane while CBL10–CIPK24 complex localized at vacuolar membrane within one cell, established by multicolor BiFC (Waadt *et al.* 2008). The CBL-CIPK signaling is decoded by Ca^{2+}-signature to localize the complex to spatial and temporal compartment within the cell and provide evidence for decoding of Ca^{2+} signature and signaling (Batistic and Kudla 2009). This differentially localized interaction of CBL-CIPKs may leads to complex signaling network by interacting with different or same interactor within a single cell under different conditions (Batistic and Kudla 2009). The differential expression of either CBLs or CIPKs in different tissue provides tolerance to same stress response by different mechanism. The CIPK24 form alternative complex either with SOS3/CBL4 or CBL10 provides evidence for spatial partition of Ca^{2+} signatures in tissue specific expression of these genes.

SOS2/CIPK24 is expressed in both roots and aerial tissue such as leaves of the plant which forms important component of salt signaling by the SOS pathway (Liu *et al.* 2000) while different CBLs that physically interact and regulate CIPK24 activity, are differentially expressed in different tissue of plant. Recently, another member of CBL family, CBL10 was found to be involved in salt-stress tolerance beside CBL4/SOS3 of SOS pathway (Kim *et al.* 2007; Quan *et al.* 2007). Unlike other CBLs, CBL10 expresses only in the green tissues and not in the roots while CBL4 is abundantly expressing in the root, (Liu *et al.* 2000; Kim *et al.* 2007; Quan *et al.* 2007). In salt stress, CBL4-CIPK24 complex localize at the plasma membrane, regulates the SOS1 Na^+/H^+-antiporter to extrude toxic sodium out of the cell to the apoplast of the root to soil (Qiu *et al.* 2002). The CIPK24 interacts with CBL10, which mainly expressed in leaves, and localized to vacuolar membrane for intracellular sodium sequestration into vacuole (Kim *et al.* 2007). This might be an interesting mechanism where dual binding of CIPKs with different CBLs and regulating ion homeostasis in different tissue of plants on the basis of presence of differentially expressed Ca^{2+} sensors (Kim *et al.* 2007). It has been found that *cbl10* mutant is hypersensitive to salt that leads to cell death in leaf tissue, in contrary to other salt sensitive mutant such as *sos3*, *cbl10* accumulate less salt than the wild type under either normal or high salt conditions, suggesting that CBL10 might be involve in a novel Ca^{2+} signaling pathway for salt tolerance other than SOS pathway (Kim *et al.* 2007). It was also hypothesized that CBL10 might be involved in salt sequestration into vacuoles, thereby controlling cellular salt homeostasis. CBL10 was shown to be forming complex with SOS2/ CIPK24, a salt tolerance factor of SOS pathway in Ca^{2+} dependent manner, and localized to the vacuolar membrane (Kim *et al.* 2007). Hence CBL10-CIPK24 may be acting as an alternate salt-tolerance pathway that regulates the sequestration of Na^+ in plant cell besides SOS pathway (Kim *et al.* 2007). These evidences depict that CBLs play important role in cellular localization of CIPKs along with their roles in activation and regulation of the enzymatic activity of CIPKs. Thus CIPK24/SOS2, function depends on its interaction with particular CBLs, which recruit CIPKs to either the plasma membrane or vacuolar membrane, providing a salt tolerance mechanism based on location-dependent signal transduction pathways (Luan 2009).

It is quite possible that one gene might be involved in regulating a particular process by recruiting its interacting partners to different sub-cellular location. Study by Quan *et al*. (2007) deciphered also the function of the SCaBP8/ CBL10 mainly in the shoot in response to salt toxicity similar to Kim *et al*. (2007). They also found salt sensitivity in the shoot tissue of *scabp8/cbl10* mutant in response to high salt. Similarly, SCaBP8/CBL10 interacts with SOS2/ CIPK24 in Ca^{2+} dependent manner both *in vitro* and *in vivo*. But in contrary to Kim *et al*. (2007), SCaBP8/CBL10-SOS2/CIPK24 complex was localized to the plasma membrane, in a Ca^{2+}-dependent manner, and activated SOS1 in yeast to efflux Na^+ from cell to apoplast. Therefore, it is quite possible that CBL10/SCaBP8 might has dual function, first in sequestration of Na^+ ion into the vacuole (Kim *et al.* 2007) and second, the efflux of Na^+ ions outside to apoplastic region by regulating SOS1 (Na^+/H^+-antiporter) at plasma membrane (Quan *et al.* 2007) in maintaining salt homeostasis under saline conditions.

Very recently, Guo and co-workers (Lin *et al.* 2009) found the role of phosphorylation of a CBL, CBL10/ SCaBP8, by CIPK24/SOS2, which stabilized the bimolecular interaction of CBL10/SCaBP8-CIPK24/SOS2 complex at plasma membrane and enhanced plasma membrane Na^+/H^+-exchange activity *in vivo*. Site directed mutagenesis of a serine (Ser) at position 237 to (alanine) Ala in the CBL10/ SCaBP8 protein (the SOS2 phosphorylation target site) results in abolition of phosphorylation by CIPK24/SOS2 and mutant protein could not fully rescue the salt-sensitive phenotype of the *scabp8* mutant. At the same time when Ser-237 was mutated to (aspartate) Asp (to mimic the charge of a phosphorylated Ser residue) the mutant protein rescued the *scabp8* salt sensitivity) (Lin *et al.* 2009). This phenomenon of phosphorylation of a Ca^{2+} sensor and its involvement in SOS mediated salt tolerance in *Arabidopsis* is an important finding and shed new light in complexity of function of a Ca^{2+} sensor (CBL10/SCaBP8) and its interacting kinase (CIPK24/SOS2) in salt tolerance pathway.

CALCIUM AND POTASSIUM NUTRIENT SIGNALING

Role of potassium in plants

K is a macronutrient required in large quantities by plants and is the most abundant inorganic cation in plants (Leigh and Jones 1984). K plays an important role in the growth and development of plants. It activates many enzymes, maintains cell turgor, enhances photosynthesis, reduces respiration, and helps in sugar and starch transport (Marschner 1995). K functions in the cell by directly interacting with proteins resulting in enzyme activation, stabilization of protein synthesis, and neutralization of negative charges on proteins (Kochian and Lucas 1988; Marschner 1995; Maathuis and Sanders 1996). In addition to plant metabolism, K improves crop quality because it extends the grain-filling period, kernel weight, strengthens straw, increases disease resistance, and enables plant to withstand stress.

K^+ starvation leads to growth arrest, impaired N balance, and reduced levels of sugars and impaired long distant transport (Marschner 1995). Because of function performed by K ion in cellular physiology, the K^+ deficiency is of a great agricultural importance (Laegreid *et al.* 1999). In agricultural land affected by either saline or low-K^+ soil, crop productivity and yields are severally affected. To improve and enhance this trait, an in-depth molecular understanding of the K^+-nutrition signaling and its cellular and tissue homeostasis need a greater attention which will ultimately leads to raise transgenic plants which will be able to grow on low-K^+ and saline soil without loosing crop quality and quantity (Amtmann *et al.* 2004; Pandey *et al.* 2008).

K^+ acquisition mechanisms in plants

There are two mechanisms, one is high affinity and the other is low affinity for K^+ ions uptake from the roots (Epstein *et al.* 1963; Epstein 1966; Siddiqi and Glass 1983; Kochian and Lucas 1988; Schachtman 2000; Very and Sentenac 2003; Luan *et al.* 2009). Deficiency of K^+ in plants generally switches to high-affinity system (Hirsch *et al.* 1998; Dennison *et al.* 2001; Gierth *et al.* 2005). Plants have various K^+ transporters, whose transport activity increases under K^+ deficient condition (Maathuis *et al.* 2003; Ashley *et al.* 2006). In case of *Arabidopsis*, root cell rapidly sense the changes in external K and exhibit a shift to high-affinity K uptake after 6 hours of K^+ deprivation (Shin and Schachtman 2004). The low availability of K^+ in soil might increases the production of ROS (reactive oxygen species) like H_2O_2, which trigger the elevation of Ca^{2+} and elevated level of Ca^{2+} leads to expression of number of low-K^+ responsive genes (Shin and Schachtman 2004).

Calcium and low-K responses

Numbers of phosphorylation-related events have been found to be involved in signal transduction pathway in responses to nutrient deprivation (Schachtman and Shin 2007). In yeast a number of protein kinases have been identified which were regulated by nutrient status (Wilson *et al.* 2002). Involvement of a novel CBL-CIPK signaling pathway in response to K deprivation was identified (Li *et al.* 2006; Xu *et al.* 2006) where a kinase, CIPK23 phosphorylate voltage gated channel, AKT1 and activate K uptake from root.

In Ca^{2+} mediated signaling pathway in plants, CDPK (Ca^{2+} dependent protein kinases) are the major players in several signal transduction network (Harper *et al.* 1991, 2004; Klimecka and Muszynska 2007). In stomatal guard cell, CDPKs (Ca^{2+}-dependent protein kinases) also get activated when cytosolic Ca^{2+} level increases which leads to phosphorylation of KAT1 (another K transporter) protein (Li *et al.* 1998). This K^+ channel in guard cell plays a major role in stomatal movements in Ca^{2+}-dependent manner (Li *et al.* 1998) and is also important component of K nutrition and homeostasis mediated by Ca^{2+} in guard cells.

In addition to regulation of K transporter by post-translation modification such as phosphorylation, there are several other genes, which were regulated at transcription level like "Ca^{2+}-related" proteins, including a Ca^{2+}-dependent protein kinase, a Ca^{2+} ATPase, and a calmodulin binding protein, after K deprivation (Schachtman and Liu 1999). Under K^+-starvation in roots, Ca^{2+} pump *ACA1* and Ca^{2+} transporter *CAX3* transcripts are quickly up regulated. Moreover, Ca^{2+} also act as osmoticum in fully expended cells but due to low mobility it cannot replace K^+ for its better osmoticum properties in fast growing tissue. Therefore under K^+-deficient condition, increased Ca^{2+} transporter activity leads to preferential influx of Ca^{2+} to vacuole of older tissue thus freeing K^+ for transport to expanding tissues which suggest that active Ca^{2+} homeostasis is indeed a component of plant low-K^+ condition adaptations (Armengaud *et al.* 2004).

CBL-CIPK and potassium nutrition

Both CBLs and CIPKs belong to multigene family and are involved in abiotic stress (Cheong *et al.* 2003; Batistic and Kudla 2004; Pandey 2008; Batistic and Kudla 2009; Luan 2009), ABA sensitivity and biosynthesis (Pandey *et al.* 2004; Kim *et al.* 2003) and K (Li *et al.* 2006, Xu *et al.* 2006; Pandey *et al.* 2007; Luan *et al.* 2009).

By genetic analysis Wu and colleagues (Xu *et al.* 2006) isolated the *lks1* (low-K^+-sensitive) mutant that showed sensitivity to low K as phenotypic observation of leaf chlorosis in the *lks1* mutant. *LKS1* encoded a CBL (calcineurin B-like protein) interacting protein kinase CIPK23, a critical component for K^+-uptake in plants.

Simultaneously, Luan and colleagues (Li *et al.* 2006; Cheong *et al.* 2007) also reported the involvement of CIPK23 in low-K nutrition by systematic reverse genetic screening for reduced root growth and arrested seedling development phenotype under low-K condition. Involvement of a protein kinase in low-K nutrition signaling prompted Wu co-workers (Xu *et al.* 2006) and Luan colleagues (Li *et al.* 2006; Cheong *et al.* 2007) to look for finding the both upstream and downstream target of this protein kinase. By both yeast two-hybrid assay and reverse genetic based mutant phenotypic analyses, they could identify a voltage-gated K-channel, AKT1 (Hirsch *et al.* 1998) as downstream target and two CBLs, CBL1 and CBL9 as upstream components of CIPK23. By both *in vitro* and *in vivo* studies, it has been defined that the C-terminal region of AKT1 channel interact and get phosphorylated by constitutively active CIPK23 or by CBL1/CBL9-CIPK23 complex in a Ca^{2+} dependent manner. Moreover, both CBL1 and CBL9 recruit the protein kinase, CIPK23 to the plasma membrane to promote the phosphorylation of AKT1 channel. By genetic analysis of CBL1 and CBL9 loss of function in their respective single mutants, *cbl1* and *cbl9*, no significant phenotype was observed, however, the double mutant of both CBL1 and CBL9, *cbl1cbl9* showed a similar low-K sensitive phenotype as seen in both *lks* (*cipk23*) and *akt1* mutants (Li *et al.* 2006; Xu *et al.* 2006; Cheong *et al.* 2007). In addition, the over-expression of CBL1 and CBL9 also leads to enhanced tolerance in low-K media as compared to wild type growth. However, single or double gene expression of CBL1 and CBL9 could not complement *lks* (*cipk23*) mutant phenotype, suggesting that CBL1 and CBL9 acts synergistically upstream of CIPK23 in low-K nutrition signaling pathway. The mechanistic regulation of voltage gated high affinity K transporter, AKT1 was studied both *in vitro* and *in vivo* system (Li *et al.* 2006; Xu *et al.* 2006). The inward K^+ currents were activated in the presence of CIPK23 as well as CBL1 and CBL9 (wild-type) and inactivated in the absence of CIPK23 (*lks1* mutant) or in the *cbl1cbl9* double mutant (Xu *et al.* 2006). The activation of AKT1 channel is in a Ca^{2+}-dependent manner, connecting the Ca^{2+} signal to enhanced K^+-uptake through activation of a K^+ channel. Disruption of both *CBL1* and *CBL9* or *CIPK23* gene in *Arabidopsis* reduced the AKT1 activity in the mutant roots,

confirming that the Ca^{2+}-CBL-CIPK pathway functions to orchestrate transporting activities *in planta* according to external K^+ availability (Li *et al.* 2006; Xu *et al.* 2006).

Identification of CBL1, CBL9 and CIPK23 in a Ca^{2+}-dependent regulation of low-K uptake and signaling is a major breakthrough in understanding the long awaited mechanism of K uptake and nutrition. Discovery of this regulatory mechanism prompted Luan and co-workers to explore if there are more members of CBLs and CIPKs involved in K nutrition and signaling pathways. Therefore, they performed a systematic reverse genetic as well as expression profile of all the CBLs and CIPKs under low-K conditions and found another CIPK member, CIPK9 in K nutrition (Pandey *et al.* 2007). The transcript of *CIPK9* was highly inducible under low-K condition and the loss-of-function mutant of CIPK9 showed hypersensitive and impaired growth under K-deficient conditions (Pandey *et al.* 2007). Although, a detailed mechanistic regulation by CIPK9 was not described and need further investigation but it has been hypothesized that CIPK9 might be involved in regulating different aspects of K nutrition other than K uptake *via* AKT1 in the root of plant cell (Pandey *et al.* 2007; Amtmann and Armengaud 2007).

An important observation by Li *et al.* (2006) that there was not a complete abolition of AKT1 channel activity in both *cbl1cbl9* and *cipk23* mutant suggested that multiple CBL-CIPK complex might be involved in cooperative regulation of AKT1 activity (Lee *et al.* 2007). Therefore, Luan group (Lee *et al.* 2007) has identified two more CIPKs, CIPK6 and CIPK16 interacted with AKT1 in addition to CIPK23. Similarly, two more CBLs, CBL2 and 3 in addition to CBL1 and CBL9 that were already reported in earlier studies (Li *et al.* 2006; Xu *et al.* 2006) interacted with all three CIPKs such as CIPK6, CIPK16 and CIPK23 (Lee *et al.* 2007) in their detail biochemical analyses.

By electrophysiological analyses in *Xenopus oocytes*, a differential activation of AKT1 channel was observed between these four different CBLs and three CIPKs (Lee *et al.* 2007). Moreover binding domain determination of AKT1 with CIPKs led to identify an ankyrin repeat domain in the AKT1 protein appears to interact with the kinase domain of the CIPKs thereby determining which specific CIPKs can adhere on to the AKT1 channel protein. In addition to that they have found a PP2C-type phosphatase, AIP1, which interact and dephosphorylate AKT1 channel and negatively regulate AKT1 channel activity (Lee *et al.* 2007).

K^+/Na^+ homeostasis

There is selective uptake of K^+ under certain circumstances such as the preferential exclusion of Na^+ to maintain concentration of K^+ in cytoplasm is relatively high than Na^+ ions in cells. Because of the physico-chemical similarities between K^+ and Na^+ in the soils where plants are rooted, K and sodium ions might compete for entry into plant root cells. Not only this, Na^+ also competes for major binding site for various metabolic processes with K^+ (Marschner 1995; Shabala and Cuin 2007). Na^+-K^+ selectivity becomes more critical for plants growing in saline environment.

The concentration of both Na^+ and K^+ depends only on influx and efflux activity of these ions through transporters because neither Na^+ nor K^+ is altered in metabolism or incorporated into other biomolecules. Na^+ competes with K^+ for uptake sites at the plasma membrane of root cell, including both low-affinity (e.g. NSCC; non-selective cation channels) and high-affinity (e.g. HKT; high affinity K transporter) transporters in high concentrations of Na^+. This competition affects the plant growth in saline soils, and in this way salinity some time results in the K^+ deficiency.

Qi and Spalding (2004) studied the physiological interactions between these two ions through electrophysiological studies. The K^+-uptake ability of the *Arabidopsis* salt overly sensitive *sos* mutant under mild salt stress strongly inhibited root-cell K^+ permeability and growth rate in K^+-limiting conditions but not in wild-type plants. And even *sos1* growth phenotype is partially rescued upon increasing K^+ concentration. Therefore, this shows that the SOS1, Na^+/H^+-antiport activity is necessary for protecting the K^+ permeability under the salinity stress (Qi and Spalding 2004). Complementary studies were shown with AKT1 transporter in which salt sensitivity is one of the characteristics of *akt1* mutant in developing seedlings but there was no salt sensitivity observed in the *skor* mutant seedlings (Qi and Spalding 2004). These finding suggest that K transport is affected by sodium at the level of ion uptake by plant.

It is becoming clear that genetic variation for salt tolerance within a species is also linked to sodium-potassium selectivity of the plant (Munns 1993). Many plant researchers have now starting focusing on this for developing more salt tolerant plants. In addressing the question of what mechanism(s) make one plant type more tolerant than another, a high affinity K^+ transporter (HKT1) mRNA levels increase in wheat and barley roots under K^+ deprived condition (Wang 1998; Rubio *et al.* 1995). High-affinity K uptake transporter, HKT1, was shown to function as a high-affinity K^+-Na^+ co-transporter. High-affinity K^+-uptakes were activated by micromolar Na^+ concentrations; moreover high-affinity Na^+-uptake was activated by K^+. However, at physiologically detrimental concentrations of Na^+, K^+ accumulation mediated by HKT1 was blocked and low-affinity Na^+ uptake occurred, which correlated to Na^+ toxicity in plants. Point mutations in the sixth putative transmembrane domain of HKT1 increase Na^+ tolerance (Rubio *et al.* 1999). Na^+ uptake and Na^+ inhibition of K^+ accumulation indicate a possible role for HKT1 in physiological Na^+ toxicity in plants. K channel such as GORK, gated outwardly-rectifying K^+ channel are playing a crucial role in the stomatal movement and regulating turgor pressure (Hosy *et al.* 2003; Luan *et al.* 2009) and activity of this channel plays an important role in K^+ homeostasis in plants (Luan *et al.* 2009). The involvement of cytosolic K^+/Na^+ ratio as a key determinant of plant salinity tolerance have also been suggested by Chen *et al.* (2007) where they have shown in salt tolerant genotype of barley (*Hordeum vulgare*) multiple mechanisms do exist to withstand saline conditions. These includes a tight control of membrane voltage to retain a more negative membrane potential; inbuilt higher H^+ pump activity; higher performance of root cells to pump Na^+ from the cytosol to the external medium; and increased sensitivity to supplemental Ca^{2+}. However, no significant difference was observed in their unidirectional Na^+ influx or in the density and voltage dependence of depolarization-activated outward-rectifying K^+ channels (Chen *et al.* 2007).

In past years, crop improvement has been undertaken mostly by selective agronomic trait and breeding approaches. Several important agronomic traits in crop plants are shown to be control by quantitative trail loci (QTLs) including the salt stress tolerance. In order to understand and develop salt tolerant rice, Lin *et al.* (2004) have isolated a QTL, *SKC1*, which maintained K in salt tolerant variety. Molecular cloning of this salt tolerant QTL, SKC1 by positional cloning led to found a HKT-type transporter (Ren *et al.* 2005). Electrophysiological analyses have suggested that SKC1 is a Na^+ selective transporter and is involved in regulating K^+/Na^+ homeostasis under salt stress of root and shoot (Ren *et al.* 2005). Therefore, understanding the molecular basis of K^+/Na^+ homeostasis has become a potential area for improving salt tolerance in crops for plant biologists.

CONCLUSIONS AND FUTURE PROSPECTS

Due to extensive agricultural practices the world is facing the problem of salinity and nutrition deficiency in the soil. Ca^{2+} has been widely accepted to plays an important role in several diverse physiological as well as developmental processes. Ca^{2+} acts as a major second messenger in several signaling pathways/networks. Identification of salt tolerance pathway in *Arabidopsis* has opened up several avenues for plant biologist to understand and improving salt stress problem. Similarly molecular analyses of nutrient signaling,

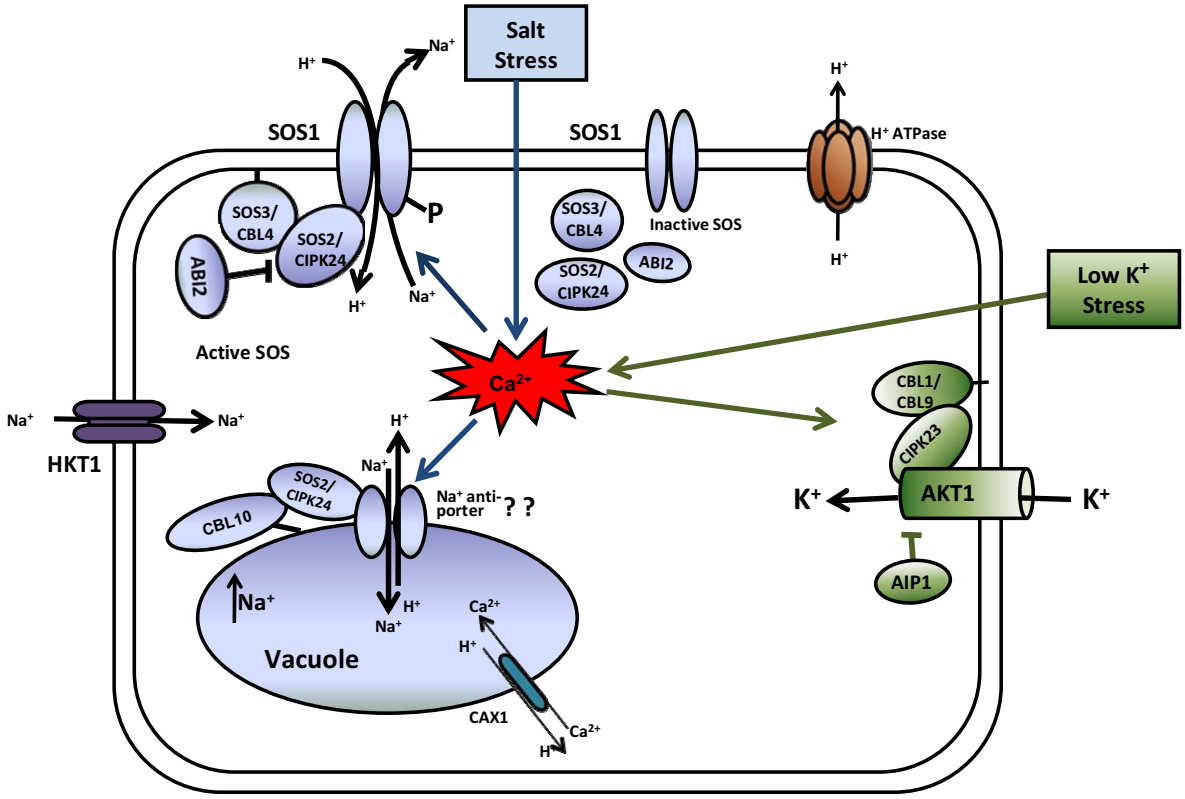

Fig. 1 Diagrammatic and hypothetic depiction of CBL-CIPK pathways including SOS pathways for Na^+-K^+ homeostasis and potassium nutrient signaling. Elevated calcium levels in response to different stress activate calcium sensors SOS3/CBL4 and CBL10 which in turn activate dual function protein kinase SOS2/CIPK24. The Na^+-K^+ balance is maintained by synergistic action of SOS pathways either by pumping out the Na^+ from cytosol to apoplast at plasma membrane in roots or sequester it into vacuole at tonoplast in shoot by alternate CBL10-SOS2/CIPK24 pathway for optimum physiological condition in the plant cell. Under low K^+ condition, CBL1 and CBL9 synergistically regulate CIPK23 which phosphorylate and activate voltage gated high affinity potassium channel, AKT1. PP2C phosphatases, ABI2 and AIP1 negatively regulates SOS1 and AKT1 transporter under saline and low-K condition, respectively. Most likely, components of SOS complexes are involved in regulating the other ions transporter like CAX1, HKT1 and H^+-ATPase for ion homeostasis under saline condition. Blue and green color represents the sodium (SOS pathway) and potassium signaling components, respectively. Arrow line shows the direction of signaling events and ions flow while question mark (?) represents probable unknown components of stress pathway.

especially K nutrition signaling is also being undertaken seriously. Recently, a novel Ca^{2+} mediated SOS/CBL-CIPK pathway has been implicated in salt tolerance as well as K nutrition signaling. The involvement of Ca^{2+} in regulating salt stress tolerance, K^+-Na^+-homeostasis and nutrient signaling is summerized in a hypothetical model in **Fig. 1**. Under stress condition the involvement of Ca^{2+}, Ca^{2+} sensors (CBLs), effector kinases regulated by these calcium sensors (CIPKs) and physiological targets of these kinases such as transporters (Na^+/H^+-antiporter and AKT1) is the breakthrough discovery in plant biology which has opened a new horizon to understand the salt stress and nutrient deficient signaling in plant. Following this several genes are being identified in various plant species, which encodes for calcium sensors, kinases and transporters/channels, and pumps involved in maintaining K^+/Na^+ homeostasis.

Future work will be required to direct the function of these various proteins in Ca^{2+}-mediated K^+/Na^+ homeostasis, signaling, and plant responses to abiotic stress. Our knowledge of sodium tolerance and the importance of K^+ transport systems and its cytosolic homeostasis under saline conditions has increased considerably in recent years. Indeed, it is becoming more and more accepted that the ability of a plant to maintain a high cytosolic K^+/Na^+ ratio is crucial in plant salt tolerance mechanisms. Not many genes responseble for K^+ as well as for Na^+ transport have been fully characterized physiologically. In addition, the majority of these studies on plants have been mostly carried out on *Arabidopsis*. The completion of rice genome and public availability of rice genome information will facilitate our understanding of potential transport mechanisms for K^+/Na^+ homeostasis in crop plants, which will enable plant biologist to design future crops able to tolerate and grow on saline and low-nutrient soil. Still a lot is needed to be discovered concerning the mechanisms of the maintenance of optimal K^+/Na^+ ratios under saline conditions. Loss of function mutants and RNA silencing by RNAi approach will greatly enable the task to understand many more candidate genes involve in K^+/Na^+ homeostasis and nutrition signaling. More detail efforts need to be invested for a better understanding of how and where the discrimination between K^+ to Na^+ selectivity occurs in plants. Ultimately, by engineering the crops, which can either tolerate or adapt to the saline and eroded soil deprived in nutrients, without adding chemical fertilizers for a sustainable agricultural practices will greatly benefit mankind to eradicate hunger.

ACKNOWLEDGEMENTS

The work in G.K.P' lab is supported by research and development grant from Delhi University. AKY and PK are getting JRF support from CSIR, India. The work in N.T.' lab is partially supported by grant from Department of Biotechnology (DBT), Government of India.

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