

ARTICLE ADDENDUM



## Putative role of glutamine in the activation of CBL/CIPK signalling pathways during salt stress in sorghum

Rafael de Souza Miranda<sup>a</sup>, Juan Carlos Alvarez-Pizarro<sup>b</sup>, José Hélio Costa<sup>a</sup>, Stelamaris de Oliveira Paula<sup>a</sup>, José Tarquinio Prisco<sup>a</sup>, and Enéas Gomes-Filho<sup>a</sup>

<sup>a</sup>Departamento de Bioquímica e Biologia Molecular and Instituto Nacional de Ciência e Tecnologia em Salinidade (INCTSal/CNPq), Universidade Federal do Ceará, Fortaleza, Ceará, Brazil; <sup>b</sup>Centro de Ciências Agrárias e da Biodiversidade, Universidade Federal do Cariri, Crato, Ceará, Brazil

### ABSTRACT

The salt overly sensitive (SOS) pathway is the only mechanism known for Na<sup>+</sup> extrusion in plant cells. SOS pathway activation involves Ca<sup>2+</sup>-sensing proteins, such as calcineurin B-like (CBL) proteins, and CBL-interacting protein kinases (CIPKs). In this signalling mechanism, a transit increase in cytosolic Ca<sup>2+</sup> concentration triggered by Na<sup>+</sup> accumulation is perceived by CBL (also known as SOS3). Afterward, SOS3 physically interacts with a CIPK (also known as SOS2), forming the SOS2/SOS3 complex, which can regulate the number downstream targets, controlling ionic homeostasis. For instance, the SOS2/SOS3 complex phosphorylates and activates the SOS1 plasmalemma protein, which is a Na<sup>+</sup>/H<sup>+</sup> antiporter that extrudes Na<sup>+</sup> out of the cell. The CBL-CIPK networking system displays specificity, complexity and diversity, constituting a critical response against salt stress and other abiotic stresses. In a study reported in the journal *Plant and Cell Physiology*, we showed that NH<sub>4</sub><sup>+</sup> induces the robust activation of transporters for Na<sup>+</sup> homeostasis in root cells, especially the SOS1 antiporter and plasma membrane H<sup>+</sup>-ATPase, differently than does NO<sub>3</sub><sup>-</sup>. Despite some studies having shown that external NH<sub>4</sub><sup>+</sup> ameliorates salt-induced effects on ionic homeostasis, there is no evidence that NH<sub>4</sub><sup>+</sup> *per se* or some product of its assimilation is responsible for these responses. Here, we speculate about the signalling role behind glutamine in CBL-CIPK modulation, which could effectively activate the SOS pathway in NH<sub>4</sub><sup>+</sup>-fed stressed plants.

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Calcineurin B-Like (CBL) proteins are a family of small plant-specific peptides that function as Ca<sup>2+</sup> sensors. Some CBL Ca<sup>2+</sup> sensors, also referred to as SOS3-Like Calcium Binding Proteins (SCaBPs), are known to regulate ionic homeostasis.<sup>1</sup> The CBL-interacting protein kinase (CIPK) family, also known as sucrose non-fermenting (SNF)-related kinases (SnRKs), includes a serine/threonine protein kinase (SOS2) belonging to the SNF1-related kinase 3 (SnRK3) family. CBL/CIPK (SOS3/SOS2) proteins not only act as essential components of the salt overly sensitive (SOS) pathway, activating the Na<sup>+</sup>/H<sup>+</sup> SOS1 antiporter,<sup>2</sup> but also may interact and positively regulate key components during salt tolerance responses. These components include vacuolar Na<sup>+</sup>/H<sup>+</sup> antiporter (NHX) and H<sup>+</sup>-ATPase (V-ATPase) proton pumps, high-affinity K<sup>+</sup> transporters (AtHKT1) and channels (AKT1) and H<sup>+</sup>-ATPase proton pumps of the plasma membrane.<sup>3,4,5,6</sup>

It is known that numerous CBL (11 genes in Arabidopsis) and CIPK (26 genes in Arabidopsis) protein isoforms are present in plants; therefore, different combinations of CBL-CIPK complexes could occur to mediate physiological and biochemical responses for both growth and developmental patterns and tolerance to environmental stresses.<sup>14,15</sup> Thus, the exact flow of information will depend on the assembly of alternative and

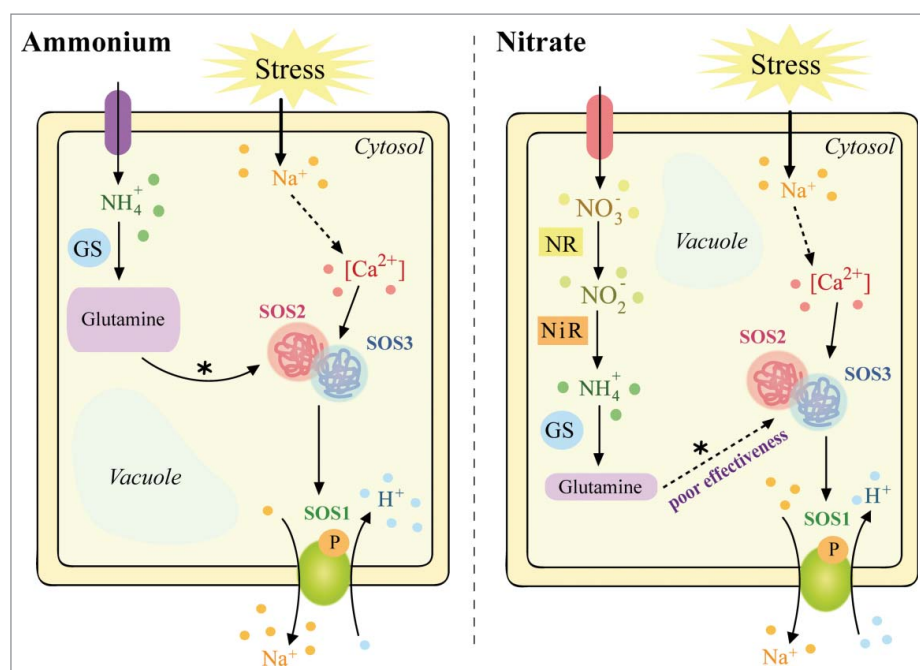
competing CBL-CIPK combinations.<sup>16</sup> Some CBL and CIPK proteins have been functionally characterized,<sup>15</sup> and the CBL4-CIPK24 (SOS3/SOS2) complex was revealed to specifically mediate salt stress signaling and tolerance.<sup>14</sup> Interestingly, like CBL4-CIPK24 in Arabidopsis, many other members (CBL1, CBL9, PsCBL, CIPK1, CIPK2, CIPK25, CIPK26 and CIPK31) are also involved in the network-like signaling system to mediate response to salt stress, including ABA-dependent signaling cascades.<sup>16,17,18,19,20,21,22</sup> However, although important progress has been achieved in understanding the downstream targets and physiological functions of some of these proteins, many queries remain to be explored.

To date, extensive studies have focused on identifying salt responsive regulators and focusing salt stress pathways at cellular, tissue and whole-plant level. Strictly, from salt exposure to SOS1 activation, stress signals always result in cytosolic Ca<sup>2+</sup> alterations, which are unique and precisely translated by Ca<sup>2+</sup> sensor proteins to relay the signaling network.<sup>1,23</sup> Surprisingly, although 14-3-3 protein has been found to interact with SOS2 and constitute an important feature for salt tolerance,<sup>24</sup> there is little or no evidence regarding synergistic chemical inducers in inducing CBL/CIPK proteins under salinity, especially in roots.<sup>16</sup>

**CONTACT** E. Gomes-Filho ✉ [egomesf@ufc.br](mailto:egomesf@ufc.br); R.S. Miranda ✉ [rafaelmyranda@hotmail.com](mailto:rafaelmyranda@hotmail.com) ✉ Departamento de Bioquímica e Biologia Molecular and Instituto Nacional de Ciência e Tecnologia em Salinidade (INCTSal/CNPq), Universidade Federal do Ceará, Fortaleza 60440-554, Ceará, Brazil.

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**Figure 1.** Putative model of CIPK modulation by glutamine. Under  $\text{NH}_4^+$  supply, an over-accumulation of glutamine occurs in the root cells of sorghum, especially in salt-stressed plants. The signal from glutamine\* is suggested to specifically modulate CIPK proteins at the allosteric, transcriptional or post-transcriptional level. Upon activation of CIPK proteins, the operation of the SOS pathway is augmented, activating  $\text{Na}^+$  extrusion by an SOS1 exchanger. Otherwise,  $\text{NO}_3^-$  nutrition may also modulate CIPK proteins, albeit at a lower intensity. Abbreviations:  $\text{Ca}^{2+}$ , calcium; GS, glutamine synthetase;  $\text{Na}^+$ , sodium;  $\text{NH}_4^+$ , ammonium; NiR, nitrite reductase;  $\text{NO}_2^-$ , nitrite;  $\text{NO}_3^-$ , nitrate; NR, nitrate reductase; SOS1, Salt Overly Sensitive 1, a plasma membrane-binding  $\text{Na}^+/\text{H}^+$  antiporter; SOS2, Salt Overly Sensitive 2, also referred to as CBL-interacting protein kinase (CIPK); SOS3, Salt Overly Sensitive 3, also referred to as calcineurin B-like protein (CBL).

Some products of N assimilation might act as signalling molecules, activating specific members of the CIPK family in plants. It has been shown that *AtCIPK8* and *AtCIPK23* transcripts and N assimilation by-products accumulate to high levels under  $\text{NO}_3^-$  supply.<sup>7,8</sup> Despite a relationship between  $\text{NO}_3^-$  nutrition and CIPK signalling factors having been demonstrated regarding both morphogenetic processes and  $\text{NO}_3^-$  uptake in roots, the roles of these factors in salt tolerance responses are still elusive. On the other hand,  $\text{NH}_4^+$  supply also specifically activates other CIPK members, such as CIPK15, whose response is closely associated with the expression of N assimilation enzymes such glutamine synthetase (GS) and glutamate synthase (GOGAT).<sup>9</sup>

Recent studies have shown that glutamine functions as a signal molecule that induces several transcription factors responsible for plant defence against abiotic stress.<sup>10,11</sup> In rice, exogenous glutamine increases the expression of some members of the CIPK family, which are responsible for amplifying and transducing the signal that activates pathways involved in the regulation of plant growth and stress responses. We previously reported that  $\text{NH}_4^+$  supply severely restricts  $\text{Na}^+$  accumulation and transport to shoots through the efficient upregulation of SOS1 in sorghum roots, a response not observed for  $\text{NO}_3^-$  nutrition.<sup>12</sup> Our data suggested that enhanced SOS1 antiporter activity may mostly involve post-transcriptional and/or post-translational regulation, as *SOS1* transcript abundance was similar in salt-stressed plants treated with  $\text{NH}_4^+$  or  $\text{NO}_3^-$ . Thus, we hypothesised that glutamine exerts a role as a signal molecule to activate the SOS2/SOS3 system and, subsequently, the SOS1 antiporter (Fig. 1). This idea is supported by high glutamine accumulations in  $\text{NH}_4^+$ -grown

sorghum roots, as the accumulations were more prominent in response to salt stress.<sup>13</sup>

It is also plausible that activation of plasma membrane  $\text{Na}^+/\text{H}^+$  antiporters by the CBL-CIPK complex occurs in  $\text{NO}_3^-$ -fed stressed plants, but this response was less intense than that of  $\text{NH}_4^+$ -fed stressed plants.<sup>12</sup> Thus, this evidence supports the assumption that high levels of glutamine, as a consequence of enhanced GS activity, can stimulate the formation of CBL-CIPK-specific complexes acting on important mechanisms of ionic regulation during tolerance to salinity in  $\text{NH}_4^+$ -fed sorghum. Nevertheless, the roles of some complex combinations remain to be explored, including their interaction with specific signal molecules such as by-products of N metabolism under salt stress. For instance, specifically in sorghum, there is no evidence of a role for glutamine or  $\text{NH}_4^+$  in activating the CBL4-CIPK24 complex, which is mostly found to function in  $\text{Na}^+$  extrusion in root cells under salt stress conditions.<sup>14,15</sup> To clarify this mistake, much future effort should be addressed. Firstly, the crystal structures of CBL-CIPK complexes must be solved. Secondly, the CBL- and CIPK-interacting potential candidates should be studied with respect to antagonistic and synergistic effects. Finally, the physiological inputs and outputs of each CBL-CIPK combination should be investigated in order to complete understanding their biological roles.

The use of conventional plant physiology integrated with genetics and biochemical traits may constitute a reliable strategy for understanding the role of glutamine in CBL-CIPK signalling pathways. In this sense, the identification of glutamine as an element of the CBL-CIPK network emerges as a promising challenge for future studies to advance in knowledge and manipulation of signalling processes. Once enough

mechanisms are elucidated, specific signalling pathways and cross-talk could be properly applied to crop management and plant breeding programmes in order to improve the capacity of plants to tolerate salt stress.

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