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To cite this article: Rongbin Hu, Yinfeng Zhu, Guoxin Shen & Hong Zhang (2017) Overexpression of the PP2A-C5 gene confers increased salt tolerance in Arabidopsis thaliana, Plant Signaling & Behavior, 12:2, e1276687, DOI: 10.1080/15592324.2016.1276687

To link to this article: http://dx.doi.org/10.1080/15592324.2016.1276687

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Rongbin Hu, Yinfeng Zhu, Guoxin Shen, and Hong Zhang
Accepted author version posted online: 03 Jan 2017.
Published online: 03 Jan 2017.
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Protein phosphatase 2A (PP2A) plays numerous roles in plants such as in cell cycle progression, root cortical cell elongation, tissue development, and plant responses to biotic and abiotic stresses.\(^2\)PP2A was shown to play important roles in biotic and abiotic stress signaling pathways in plants. PP2A is made of 3 subunits: a scaffolding subunit A, a regulatory subunit B, and a catalytic subunit C. It is believed that the B subunit recognizes specific substrates and the C subunit directly acts on the selected substrates, whereas the A subunit brings a B subunit and a C subunit together to form a specific PP2A holoenzyme. Because there are multiple isoforms for each PP2A subunit, there could be hundreds of novel PP2A holoenzymes in plants. For an example, there are 3 A subunits, 17 B subunits, and 5 C subunits in Arabidopsis, which could form 255 different PP2A holoenzymes. Understanding the roles of these PP2A holoenzymes in various signaling pathways is a challenging task. In a recent study, we discovered that PP2A-C5, the catalytic subunit 5 of PP2A, plays an important role in salt tolerance in Arabidopsis. We found that a knockout mutant of PP2A-C5 (i.e., ppa2a-c5–1) was very sensitive to salt treatments, whereas PP2A-C5-overexpressing plants were more tolerant to salt stresses. Genetic analyses between ppa2a-c5–1 and Salt-Overly-Sensitive (SOS) mutants indicated that PP2A-C5 does not function in the same pathway as SOS genes. Using yeast 2-hybrid analysis, we found that PP2A-C5 interacts with several vacuolar membrane-bound chloride channel proteins. We hypothesize that these vacuolar chloride channel proteins might be PP2A-C5’s substrates in vivo, and the action of PP2A-C5 on these channel proteins could increase or activate their activities, thereby result in accumulation of the chloride and sodium contents in vacuoles, leading to increased salt tolerance in plants.

**SHORT COMMUNICATION**

**Overexpression of the PP2A-C5 gene confers increased salt tolerance in Arabidopsis thaliana**

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**ABSTRACT**

Protein phosphatase 2A (PP2A) was shown to play important roles in biotic and abiotic stress signaling pathways in plants. PP2A is made of 3 subunits: a scaffolding subunit A, a regulatory subunit B, and a catalytic subunit C. It is believed that the B subunit recognizes specific substrates and the C subunit directly acts on the selected substrates, whereas the A subunit brings a B subunit and a C subunit together to form a specific PP2A holoenzyme. Because there are multiple isoforms for each PP2A subunit, there could be hundreds of novel PP2A holoenzymes in plants. For an example, there are 3 A subunits, 17 B subunits, and 5 C subunits in Arabidopsis, which could form 255 different PP2A holoenzymes. Understanding the roles of these PP2A holoenzymes in various signaling pathways is a challenging task. In a recent study, we discovered that PP2A-C5, the catalytic subunit 5 of PP2A, plays an important role in salt tolerance in Arabidopsis. We found that a knockout mutant of PP2A-C5 (i.e., ppa2a-c5–1) was very sensitive to salt treatments, whereas PP2A-C5-overexpressing plants were more tolerant to salt stresses. Genetic analyses between ppa2a-c5–1 and Salt-Overly-Sensitive (SOS) mutants indicated that PP2A-C5 does not function in the same pathway as SOS genes. Using yeast 2-hybrid analysis, we found that PP2A-C5 interacts with several vacuolar membrane-bound chloride channel proteins. We hypothesize that these vacuolar chloride channel proteins might be PP2A-C5’s substrates in vivo, and the action of PP2A-C5 on these channel proteins could increase or activate their activities, thereby result in accumulation of the chloride and sodium contents in vacuoles, leading to increased salt tolerance in plants.

**ARTICLE HISTORY**

Received 9 December 2016
Accepted 18 December 2016

**KEYWORDS**

Chloride channel protein; plant phosphatase; salt stress; signaling

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PP2A-C5 interacts with AtCLCc in vivo, we performed bimolecular fluorescence complementation (BiFC) experiments using N. benthamiana leaves. In this system, we first fused PP2A-C5 to nYFP (the N-terminal part of the yellow fluorescence protein) to form the nYFP-C5 fusion construct, and fused AtCLCc to cYFP (i.e., the C-terminal part of YFP) to form the CLCc-cYFP fusion construct. Then we introduced Agrobacterial cells into tobacco leaf cells through the infiltration technique and the Agrobacterial cells contained our gene constructs in 3 combinations: nYFP-C5 with cYFP constructs, nYFP and CLCc-cYFP constructs, and nYFP-C5 and CLCc-cYFP constructs. Only in the third combination we observed fluorescence signals in the infiltrated leaf tissues (Fig. 1C), indicating that it was the interaction between PP2A-C5 and AtCLCc that brought nYFP and cYFP together to produce green fluorescence in the tobacco leaf cells.

The physical interaction between PP2A-C5 and AtCLCc and similar salt tolerant phenotype of PP2A-C5-overexpressing plants and AtCLCc-overexpressing plants suggested a functional correlation. We then investigated the genetic relationship between PP2A-C5 and AtCLCc by overexpressing AtCLCc in the pp2a-c5-1 mutant background and we could not rescue the salt sensitive phenotype of the pp2a-c5-1 mutant, indicating that PP2A-C5 and AtCLCc function in the same pathway and AtCLCc functions downstream of PP2A-C5.1 Our data suggest that increasing PP2A-C5 expression might lead to higher activities of chloride channel proteins. This assumption appears consistent with the biochemical analysis of chloride (Cl⁻) concentrations in these plants. We observed the highest Cl⁻ concentration in PP2A-C5-overexpressing plants and AtCLCc-overexpressing plants, and the lowest concentration in the pp2a-c5-1 mutant.1 The Cl⁻ concentration in the pp2a-c5-1 mutant that overexpresses AtCLCc is similar to that of the pp2a-c5-1 mutant.1 To maintain the charge neutrality inside vacuoles of AtCLCc-overexpressing plants, we expected that AtCLCc-overexpressing plants should have higher levels of cations. Indeed our analyses of Na⁺ contents indicate similar results as Cl⁻ contents: PP2A-C5-overexpressing plants and AtCLCc-overexpressing plants contain the highest amount of Na⁺, whereas the pp2a-c5-1 mutant contains the least (Fig. 2).

Based on our study, we propose a working model to show how PP2A might participate in the salt signaling pathway in plant cells (Fig. 3). We believe that AtCLCc and AtCLCa are substrates of PP2A-C5 in plant cells and these vacuolar membrane bound chloride channel proteins exist in 2 forms: dephosphorylated form (active or high activity form) and phosphorylated form (inactive or low activity form). When PP2A-C5 is overexpressed in transgenic Arabidopsis plants,
Figure 3. A working model on how PP2A-C5 might be involved in salt signaling pathway in Arabidopsis. The specific PP2A holoenzyme containing the C5 subunit up-regulates activities of AtCLCc and/or AtCLCa on vacuolar membrane by removing phosphates from its substrate proteins, leading to more anions (i.e., $\text{Cl}^-$ and $\text{NO}_3^-$) to move into vacuole, thereby resulting in increased salt tolerance or better growth and development under treatment of NaCl, KCl, and KNO3. AtCLCc, H^+ / Cl^- antiporter; AtCLCa, H^+ / Na^+ antiporter; V-ATPase, vacuolar ATPase; V-PPase, vacuolar pyrophosphatase.

Disclosure of potential conflict of interest

No potential conflicts of interest were disclosed.