Abstract

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Calcium-mediated mechanisms of plant response to salt stress during germination

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Abstract

Statement of the Problem: Salinity impairs plant seed germination and seedling establishment. The calcium ion (Ca2+) which functions as a ubiquitous second messenger in multiple plant responses to various internal and external stimuli, including exposure to salt (NaCl), is known to improve seed germination and seedling growth and development under salt stress conditions. However, the Ca2+-mediated mechanism that underlies the improved tolerance to salt stress is yet to be deciphered. Plant exposure to relatively high concentrations, among other harmful effects, destabilizes the plant's ion homeostasis required for normal metabolism, growth and development. We recently suggested a role of the Arabidopsis Na+/K+ transporter HKT1;1 as an important component of germinating seedling response to salt that is controlled by the Ca2+-regulated key regulator of gene expression in response to salt, the CAMTA6 transcription factor. The purpose of this study is to improve our knowledge regarding plant mechanisms of coping with salt stress in which Ca2+ signaling is involved, in order to outline potential approaches of improving crops performances under salt stress conditions that cause severe reductions in crop yields on a global scale.**Methodology and Theoretical Orientation**: Seed germination assay was performed in order to examine the germination rates of wild-type (WT) Arabidopsis thaliana (Col-0 ecotype), mutants and seeds of transgenic plants in response to control, NaCl, CaCl2 and combined NaCl + CaCl2 treatments. The effect of the treatments on the distribution of growth-related hormone auxin in germinating seedlings was examined in seedlings that express the auxin level indicative sensor DR5-RFP using fluorescent microscopy. To elucidate potential genes that are involved in the Ca2+ mediated response to salt, transcriptomic analysis was performed based on total RNA that was isolated from whole seedlings that were ejected from the seed coat at early stages of development. To further study the effect of Ca2+ on ion homeostasis under salt stress conditions, ion content analysis was performed by harvesting ejected and root and shoot of young seedlings followed by ICP-MS analysis. Gene expression profiling was performed using qRT-PCR for relative transcript quantification, fluorescent microscopy visualization of fluorescent-tagged protein and promoter activity was assessed by creating transgenic plants that express the GUS reporter gene, under the regulation of the studied promoters, followed by GUS staining and light microscopy imaging. **Findings:** Germination assay of seeds of hkt1 mutants compared with WT on agar solidified medium supplemented with NaCl (200 mM) or NaCl and CaCl2 (10 mM), revealed no Ca2+-mediated improved tolerance to salt among hkt1 mutants while WT seeds displayed germination rate of 30% that suggested a role of HKT1;1 in this improved tolerance. Control and CaCl2 treatments yielded ~100% germination rate. GUS staining of germinating seedlings that express the GUS protein under the regulation of HKT1;1 promoter (HKT1;1promoter-GUS) and were treated with NaCl or NaCl + CaCl2 showed elevated expression at the whole germinating seedling and enhanced radicle promoter activity in response to the combined treatment. These data were further reinforced by qRT-PCR analysis for relative transcript quantification. Ion content analysis revealed higher concentration of K+ in seedlings that were treated with the combined treatment than just with NaCl, suggesting that the Ca2+ mediated tolerance involves improved accumulation of K+. The transcriptomic analysis for identification of differentially expressed genes elucidated 147 down regulated and 664 upregulated genes in response to the combined treatment. Among the upregulated genes is the Type 2C protein phosphatase PP2C49 that is known to regulate the activity of HKT1;1. The elevation of PP2C49 in response to the combined treatment was verified using qRT-PCR analysis. Therefore, we tested the effect of sanguinarine, a polycyclic ammonium ion PP2C inhibitor, on seed germination and HKT1;1 promoter activity, under salt stress conditions. Interestingly, sanguinarine was found to improve the germination rate of WT seeds (~30%) in the presence of salt, while hkt1 seeds were no responsive to the chemical. Moreover, sanguinarine treatment of HKT1;1promoter-GUS expressing germinating seedlings was found to promote radicle focused expression of HKT1;1 that was more pronounced in response to sanguinarine and NaCl combined treatment. In addition, the distribution of auxin in DR5-RFP expressing germinating seedlings treated with NaCl or NaCl + CaCl2. Finally, differential auxin accumulation was detected in the radicle tips such that the auxin levels were found to be reduced in response to NaCl but significantly to a lesser extent in response to the combined treatment. **Conclusion & Significance:** The research offers new insights regarding the Ca2+-mediated improved tolerance to salt during germination. Ca2+ was found to promote a radicle focused and enhanced expression of HKT1;1 that improves germination rate in the presence of salt. Furthermore, the addition of Ca2+ in the presence of salt results in lower Na+/K+ ratio that is associated with improved tolerance to salt. Transcriptomic analysis was efficient in pointing out PP2C49 as involved in regulation of HKT1; 1's activity in response to Ca2+ under salt stress conditions. Collectively, the findings suggest that Ca2+ enhances the expression of HKT1;1 in the emerging radicle that results in improved accumulation of K+ which allows completing germination cycle in the presence of salt.

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Short Communication

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Biography

Doron Shkolnik is an assistant professor and faculty member of the Institute of Plant Sciences and Genetics in Agriculture at the Faculty of Agriculture, Food and Environment of the Hebrew university of Jerusalem. His research is focused on elucidating the molecular mechanisms that underlie seeds response to salt stress with greater focus on Ca2+ signaling and the possible crosstalk with abiotic stress and growth and development-related hormones, including abscisic acid and auxin. Furthermore, the research also assesses the involvement of various signals in seedling establishment and root tropic growth under salt stress conditions. The research utilizes common methods of molecular biology and cutting-edge fluorescent microscopy, including Light-Sheet fluorescence microscopy (LSFM).

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