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Entitled

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF TWO NOVEL RING-TYPE E3 LIGASES FROM SESUVIUM VERRUCOSUM

by

Fayas Thayale Purayil
Faculty Advisor
Dr. Shyam Kurup
Department of Integrative Agriculture
College of Agriculture and Veterinary Medicine

Date & Venue

7 November, 2023
11:00 AM
E1; Room 1028
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Abstract

Drought and salinity pose significant environmental challenges that impact the growth and productivity of plants. *Sesuvium verrucosum* is a succulent halophyte species of the *Aizoaceae* family. Halophytes have evolved specialized mechanisms to thrive in saline environments. Studying these adaptations can provide valuable insights into how plants cope with stress, thereby improving the resilience of crops and other plant species to combat salinity stress. In plants, the ubiquitin-proteasome system (UPS) is crucial in maintaining cellular homeostasis and regulating protein levels, particularly in stress responses. E3 ubiquitin ligases play a major role in identifying targets and transferring ubiquitin to those targets. Among them, RING-type E3 ligases are widely studied and essential in plants responses to environmental stresses. In the present study, identification and classification of various types of E3 ligases were performed via transcriptome profiling of *S. verrucosum* under salinity stress. We identified 433 E3 ligases expressed under salt stress in the roots. Single RING-type E3 ligases are the most abundant E3 ligases in *S. verrucosum*. Among the 13 differentially upregulated RING-type E3 ligases, the functional roles of two novel E3 ligases, *SvRNF170* and *SvRNF185*, were characterized. These E3 ligases were expressed in all tissues of *S. verrucosum* and were induced under salt stress. These two E3 ligases are evolutionarily conserved in plants as their orthologs are present in numerous plant families; however, no functional study of these ligases has been reported. In addition, these two novel RING-type E3 ligases showed similarities to their human counterparts, which are involved in endoplasmic reticulum-associated degradation (ERAD). Our ubiquitination experiments confirm that both SvRNF170 and SvRNF185 possess ligase activity, underscoring the importance of their RING domain. Subcellular localization studies revealed that both proteins are associated with the endoplasmic reticulum (ER) membrane. Moreover, when overexpressed in *Arabidopsis thaliana*, *SvRNF170* and *SvRNF185* enhanced tolerance to salt, osmotic, and ER stresses. The expression analysis of several ER stress-responsive genes in *SvRNF185* and *SvRNF170* overexpression lines reveals the possible involvement of these E3 ligases in the ERAD system. Mass spectrometry analysis of the co-immunoprecipitation of interacting proteins revealed the association of SvRNF185 proteins with numerous ER stress-responsive proteins. Taken together, these data indicate the potential of these E3 ligases in ERAD responses. This research marks the first functional exploration of these evolutionarily conserved E3 ligases and advances our understanding of the regulatory mechanisms during stressed conditions.

Keywords: *Sesuvium verrucosum*, salt stress; ubiquitination**,** root transcriptome, E3 ligase, abiotic stress, *SvRNF185*, *SvRNF170*, ERAD.