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Molecular dissection of OsSAD1 conferring salt-, ABA- and drought stresses in rice

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Abstract

The RING (Really Interesting New Gene) finger proteins are known to play crucial roles in various abiotic stresses in plants. In this study, we report on RING finger E3 ligase, *Oryza sativa* salt-, ABA- and drought stress-induced RING finger protein1 gene (*OsSAD1*). *In vitro* ubiquitination assay demonstrated that unlike OsSAD1, a single amino acid substitution ($OsSAD1^{C168A}$) of the RING domain showed no E3 ligase activity, supporting the notion that the activity of most E3s is specified by a RING domain. Result of Yeast-Two hybridization, *In vivo* protein degradation assay supports that OsSAD1 interacting with 3 substrate, OsSNAC2, OsGRAS44 and OsPIRIN1, and mediates proteolysis of 3 substrates via the 26S proteasome pathway. Subcellular localizations of OsSAD1 while approximately 62% of transient signals were detected in cytosol, 38% of signals were showed nucleus. However, transiently expression of OsSAD1 was detected in cytosol 30% while as 70% of nucleus under 200 mM salt treated rice protoplasts. Results of bimolecular fluorescence complementation (BiFC) showed that two nucleus-localized proteins (OsSNAC2 and OsGRAS44) interacted with OsSAD1 in the both cytosol and nucleus. Heterogeneous overexpression of OsSAD1 Heterogeneous overexpresssion of *OsSAD1* in *Arabidopsis* exhibited sensitive phenotypes with respect to Salt- , mannitol-responsive seed germination, seedling growth. In ABA conditions, *OsSAD1* overexpression plants showed highly tolerance phenotypes, such as root length and stomatal closure. Our findings suggest that the OsSAD1 may play a negative regulator in salt stress response by modulating levels of its target proteins.

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