Functional Role of OTU2 in Early Development and Abiotic Stress Responses in Arabidopsis thaliana

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Abiotic stresses reduce global crop yields by up to 70%, threatening global food security. While stress-responsive pathways are well studied, the role of deubiquitination in plant development and stress adaptation remains poorly understood. The deubiquitinase OTU2, a cysteine protease, localizes to heat-induced stress granules in Arabidopsis thaliana, but its broader physiological function is unknown. This study aims to determine the role of OTU2 in early seedling development and responses to diverse abiotic stresses. CRISPR-generated otu2 knockout mutants, 35S::GFP:OTU2 overexpression lines, and pOTU2::GUS transcriptional reporters were analyzed under heat, hypoxia, salinity, osmotic, and carbon-limitation conditions. Germination assays were performed with four replicates (n=20 seedlings), while root assays used 30 seedlings per genotype. GUS staining and confocal microscopy assessed expression patterns and subcellular localization. Loss-of-function otu2 mutants showed a 42.5% reduction in germination compared with wild type under control conditions and severely impaired root development under carbon limitation; in one mutant line only 6.7% of seedlings formed primary roots. Under salt stress, germination was delayed in both wild-type and OTU2 overexpression lines, while otu2 mutant exhibited a more pronounced delay. OTU2 was strongly expressed in meristematic tissues, including root and shoot apical meristems, supporting a core developmental role. Its localization remained stable under hypoxia, salt, and osmotic stress, suggesting stress-specific molecular behavior. This study reveals a novel role for OTU2 across multiple abiotic stresses and highlights deubiquitination as a potential regulator of developmental resilience in plants.

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