

The maize E3 ubiquitin ligase ZmCER9 regulates the hypersensitive response by targeting activated resistance proteins for degradation

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Abstract

Activation of nucleotide-binding leucine rich repeat (NLR) resistance proteins (R-proteins) involves a structural transformation that triggers the effector triggered immunity (ETI) response, often featuring a rapid cell death hypersensitive response (HR). Plants require mechanisms to both induce and to suppress ETI appropriately. Rp1-D21 is an autoactive derivative of the maize Rp1-D NLR R-protein, which spontaneously triggers HR. ZmCER9 is homologous to a class of Doa10 E3 ligases that regulate the levels of proteins that enter the secretory pathway through the endoplasmic reticulum (ER)-associated degradation (ERAD) pathway. Here, we identify ZmCER9 as a suppressor of the Rp1-D21 HR phenotype. When transiently co-expressed with Rp1-D21, ZmCER9 caused a reduction in protein levels of Rp1-D21 and of other autoactive derivatives of Rp1-D, but not of the non-autoactive NLR Rp1-D and of other non-autoactive derivatives. Degradation of Rp1-D21 by ZmCER9 was proteasome-dependent. ZmCER9 displays self-ubiquitination activity, physically interacts with autoactive but not with non-autoactive derivatives of Rp1-D and appears to localize to the ER, consistent with it being an E3-ligase involved in ERAD. To determine whether ZmCER9 restrains Rp1-D21-triggered phenotypes in its native host, we used CRISPR/Cas9 to generate loss-of-function *Zmcer9* alleles in maize lines segregating for Rp1-D21. In crosses where Rp1-D21 was inherited maternally, *Zmcer9* mutant genotypes showed significant reductions in chlorophyll concentration index, plant height, and ear height relative to wild type. Arabidopsis lines homozygous for a mutation in the *ZmCER9* ortholog, *AtCER9*, displayed enhanced HR upon infection with the HR-inducing bacterial pathogen *Pseudomonas syringae* pv. *tomato*, while lines overexpressing *ZmCER9* exhibited suppressed HR. This appears to be a novel and, possibly, general mechanism that directs activated NLRs for proteasome-mediated degradation to regulate ETI.