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Nucleoside Diphosphate Kinase 2 Interacts with Two Oxidative Stress-activated Mitogen-activated Protein Kinases to Regulate Cellular Redox State and Enhances Multiple Stress Tolerance in Transgenic Plants

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Nucleoside diphosphate kinases (NDPKs) are multifunctional proteins that regulate a variety of eukaryotic cellular activities, including cell proliferation, development, and differentiation. However, much less is known about the functional significance of NDPKs in plants. We show here that NDPK is associated with H2O2-mediated MAPK signaling in plants. H2O2 stress strongly induces the expression of the NDPK2 gene in Arabidopsis thaliana (AtNDPK2). Proteins from transgenic plants overexpressing AtNDPK2 showed high levels of autophosphorylation and NDP kinase activity, and they have lower levels of reactive oxygen species (ROS) than wildtype plants. Mutants lacking AtNDPK2 had higher levels of ROS than wildtype. H2O2 treatment induced the phosphorylation of two endogenous proteins whose molecular weights suggested they are AtMPK3 and AtMPK6, two H2O2-activated A. thaliana mitogen-activated protein kinases. In the absence of H2O2 treatment, phosphorylation of these proteins was slightly elevated in plants overexpressing AtNDPK2 but markedly decreased in the AtNDPK2 deletion mutant. Yeast two-hybrid and in vitro protein pull-down assays revealed that AtNDPK2 specifically interacts with AtMPK3 and AtMPK6. Furthermore, AtNDPK2 also enhances the myelin basic protein (MBP) phosphorylation activity of AtMPK3 in vitro. Finally, constitutive overexpression of AtNDPK2 in Arabidopsis plants conferred an enhanced tolerance to multiple environmental stresses that elicit ROS accumulation in situ. Thus, AtNDPK2 appears to play a novel regulatory role in H2O2-mediated MAPK signaling in plants.