Investigation of the function of Mitogen activated protein Kinase Kinase Kinase 20 (MKKK20) in Arabidopsis seedling development

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ABSTRACT

MYC2 is a bHLH (basic helix-loop-helix) transcription factor (TF) that works as a negative regulator of blue light (BL)-mediated photomorphogenic growth, however it positively regulates flowering time and lateral root formation. MYC2 is also known to crosstalk with multiple hormonal signaling pathways. MKKK20 (Mitogen-activated protein kinase kinase kinase 20) is an upstream triple kinase in the MAP kinase signaling pathway, which plays important roles in osmotic stress responses, abscisic acid (ABA)-mediated primary root growth and stomatal response, and it is also involved in root microtubule functions. Here, we show that MKKK20 acts as a repressor of photomorphogenic growth at various wavelengths of light, although it promotes flowering time and root growth. The expression of light-inducible genes such as *RBCS-1A*, *CAB1*, and *CHS* are negatively regulated by MKKK20. The double mutant analysis shows that MYC2 works downstream of MKKK20 to regulate the hypocotyl growth in BL. The phenotypic analysis of the double mutant reveals that MYC2 works downstream to MKKK20 in the regulation of jasmonic acid (JA)-mediated root growth, and both additively regulate ABA-mediated inhibition of seed germination. The expression of *MKKK20* is positively regulated by MYC2 in both white light (WL) and BL. The DNA-protein interaction studies by ChIP and yeast-one hybrid assays demonstrate that MYC2 directly binds to the G-box (CACGTG) and E-box (CATATG) of the *MKKK20* promoter to modulate its expression. This study further shows that CAM7 and HY5, two well-known promoting factors of photomorphogenic growth, negatively regulate the transcript level of *MKKK20*. The protein-protein interaction studies by yeast-two hybrid and BiFC assays indicate that MKKK20 physically interacts with MYC2. This interaction is likely to suppress the MYC2-mediated promotion of expression of *MKKK20*. This study also shows that MKKK20 negatively regulates the expression of some key light signaling pathway genes such as *HY5*, *SPA1*, and *GBF1*. Altogether, this work highlights the functional role of MKKK20 in photomorphogenesis, and functional interconnection between MYC2 and MKKK20 in light, JA, and ABA signaling pathways.