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Functional analysis of MAP kinases in Arabidopsis thaliana: Fully rescuing the mpk3/mpk6 mutant phenotypes

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Mitogen Activated Protein Kinase (MAPK) cascades are three-stage modules involved in signal transduction. MAPKs function at the lower tier of these cascades and they phosphorylate transcription factors and other protein kinases upon activation, ultimately leading to cellular responses. Twenty genes coding for MAPKs were identified in the fully sequenced Arabidopsis genome. MPK3 and MPK6 are the most closely related. Analysis of T-DNA insertional lines revealed no phenotype in the mpk3 and mpk6 single mutants; however, female sterility is observed in MPK3+/-/MPK6-/- plants and embryo lethality results from knocking out both genes. This indicates overlapping function of MPK3 and MPK6. To better understand the function of these two kinases, an attempt was made to rescue these phenotypes by introducing a Dexamethasone (DEX) inducible: MPK6 transgene. This construct led to only partial rescue of the lethal double mutants, and no signs of fertility were evident in MPK3+/-/MPK6-/- plants. In an attempt to attain complete rescue of these phenotypes, new MPK3 and MPK6 constructs were engineered with the following features: • Transgenes regulated by endogenous promoters were used in order to maintain normal cell/tissue specific expression of the protein, which may be essential for normal plant function. • The transgene products were tagged with Yellow Florescent Protein and Green Florescent Protein in order to ascertain their expression patterns. • Genomic DNA, as opposed to complementary DNA, was used as the coding regions in order to ensure the presence of introns, which may be significant for gene function. Currently, T1 generation transgenic plants have been isolated and transgenic lines with good expression of the transgene proteins, in vivo, will be identified by Western Blot analysis. Indication of a full rescue will be verified in the T2 generation. Failure to observe completely rescued lines may indicate protein tag interference, and untagged constructs will then be attempted.