Njabulo Ngwenyama, Biology

Year in School: Junior
Hometown: Columbia, MO
Faculty Mentor: Dr. Shuqun Zhang, Biochemistry
Funding Source: MU Monsanto Undergraduate Research Fellowship

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

Mitogen-activated protein kinase (MAPK) cascades are major pathways involved in the transduction of extracellular signals into intracellular responses. A MAPK cascade consists of three kinases. MAPKK kinase (MAPKKK or MEKK) is at the top of this three-tier cascade. Upon its activation by a receptor/sensor, MAPKKK phosphorylates MAPK kinase (MAPKK or MEK), which in turn phosphorylates MAPK and activates it. The activated MAPK can then phosphorylate other protein kinases or be translocated to the nucleus where it can phosphorylate transcription factors and activate gene expression. About 20 MAPKs were identified in the fully sequenced Arabidopsis genome. To study the function of MPK3 and MPK6, the two most closely related MAPKs in Arabidopsis, we isolated the corresponding T-DNA mutants. However, no abnormal phenotype was observed in the single mutants. In order to determine if MPK3 and MPK6 have overlapping functions, we crossed the two single mutants to generate double mutants. Among the 172 F2 plants that we genotyped, no double homozygous plant was identified, indicating that this genotype is lethal. An attempt was then made to rescue this phenotype by introducing an inducible: MPK6 transgene. However, this construct led to only partial rescue of the lethal double mutants. 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 to maintain normal cell/tissue specific expression of the protein. The transgene products were tagged with YFP and GFP. Genomic DNA, as opposed to complementary DNA, was used as the coding regions in order to ensure the presence of introns. Indication of a full rescue will be verified in the T2 generation. Failure to observe completely rescued lines may indicate protein tag interference and further untagged constructs will then be attempted.