

Characterization of FBR6-interacting proteins:

AtAnnAt1, AtDHAR1, and AtNFYC.

by

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ABSTRACT:

The mechanisms of Programmed Cell Death (PCD) are relevant to plant development and protection from pathogen attack as well as the development of various human diseases. However, the molecular mechanisms are poorly understood. Previous research has uncovered an *Arabidopsis thaliana* mutant *fbr6*, which is resistant to a PCD-inducing toxin, Fumonisin B1 (FB1). *FBR6* encodes a transcription factor predicted to be regulated by additional proteins in response to developmental cues, stress, and pathogen attack. A yeast two-hybrid screen with *FBR6* revealed three interacting proteins – AtAnnAt1, AtDHAR1, and AtNFYC.

AtAnnAt1 is annotated as a Ca²⁺-binding annexin protein, which may interact with *FBR6* to alter DNA binding affinity. DHAR1 is a dehydroascorbate reductase enzyme involved in the metabolism of ascorbate to maintain redox homeostasis. DHAR1 is hypothesized to regulate *FBR6* DNA binding through S-glutathionylation of a cysteine residue in the DNA binding domain. NFYC is one subunit of a trimeric CCAAT-binding protein and is predicted to influence the specificity of *FBR6* binding at promoters.

Knock-out mutants for each protein were identified by PCR-based genotyping and confirmed by reverse-transcription PCR and Northern blots. Tissue localization studies were performed by observing GUS expression driven by the individual promoters, and intracellular localization was analyzed with GFP::protein fusions. Western blot analysis was also performed to find stable transgenic lines to be used for overexpression and *in vivo* interaction studies, including protein pull-down assays. Phenotypic analyses were performed on both knockout and overexpression lines for FB1 resistance, root growth, and susceptibility to various stresses.

Evidence of AnnAt1 and DHAR1 tissue localization and phenotypes continues to support a possible role for each in influencing PCD in conjunction with *FBR6*. However, the analysis of NFYC is less convincing but could negatively regulate *FBR6* function in PCD.

Further work to understand these proteins' roles in PCD include generation of double mutants with *fbr6*, identification of additional knockout mutant plants, complementation with epitope-tagged proteins, verification of *in vivo* interactions, and further characterization of overexpression lines. The outcomes of this project provide a number of tools necessary to determine the significance of the proposed interaction with *FBR6* and continue to elucidate the mechanism of plant PCD regulation.

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