MOLECULAR CHARACTERIZATION OF THE GENE ENCODING PROTOPORPHYRINOGEN OXIDASE FROM WATERHEMP. William L. Patzoldt, Aaron G. Hager, and Patrick J. Tranel, Graduate Research Assistant, Assistant Professor, and Associate Professor, Department of Crop Sciences, University of Illinois, Urbana, IL 61801.

Recently, a waterhemp biotype with resistance to protoporphyrinogen oxidase (PPO)-inhibiting herbicides was identified in Illinois. Since waterhemp is the first plant species to have evolved resistance to PPO inhibitors under field settings, little is known regarding the mechanism of resistance. As part of our investigation, we are comparing PPO inhibitor-resistant and -susceptible waterhemp biotypes at the molecular level to determine if the mechanism of resistance is mediated by alterations in the nucleotide sequence or over-expression of the gene(s) encoding the herbicide target-site.

Plants have two PPO isozymes, located in either plastids or mitochondria and encoded by *PPX-I* or *PPX-II*, respectively, both of which are inhibited by herbicides. Since *PPX-1* and *PPX-II* have not yet been sequenced in waterhemp, we began our research by obtaining the full-length sequence of *PPX-I*. Using known sequences of *PPX-I* from spinach, tobacco, and mouse-ear cress, primers were created to sequence a fragment of *PPX-I* from waterhemp. This fragment was used to create gene-specific primers to obtain the full-length sequence by 5' and 3' RACE (Rapid Amplification of cDNA Ends). Initial sequencing results from the fragment of *PPX-I* from waterhemp indicated that it was most similar in nucleotide sequence to the spinach gene. Interestingly, the PPO enzyme encoded by *PPX-II* from spinach has been identified in two isoforms due to the existence of dual initiation codons: one isoform is targeted to the mitochondria, while the other is targeted to the plastid. Given this scenario, a single alteration could potentially confer PPO inhibitor resistance to plastidic and mitochondrial PPO enzymes.

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