MECHANISM OF RESISTANCE TO PPO-INHIBITING HERBICIDES IN WATERHEMP. Patrick J. Tranel, William L. Patzoldt, and Aaron G. Hager, Associate Professor, Postdoctoral Research Assistant, and Assistant Professor, Department of Crop Sciences, University of Illinois, Urbana, IL 61801.

Genes encoding protoporphyrinogen oxidase (PPO) were identified in waterhemp biotypes resistant (R) and sensitive (S) to PPO-inhibiting herbicides, and evaluated to determine if they accounted for the resistance phenotype. Using reverse transcriptase polymerase chain reactions (RT-PCRs), three cDNA clones with high sequence similarities to known PPO genes were isolated from waterhemp. One of the PPO genes, designated *PPX1*, was isolated from both the R and S biotypes and was most similar to genes encoding plastid-targeted PPOs. A second PPO gene, designated *PPX2S*, was isolated by RT-PCR only from the S biotype, and was most similar to genes encoding mitochondria-targeted PPOs. Southern blot analysis also indicated that *PPX2S* was present in the S but not the R biotype. The third PPO gene, *PPX2L*, which was isolated from both R and S biotypes, was nearly identical to *PPX2S*, with the exception that it contained a 90-basepair extension at the 5' end. Presumably, the 5' extension can result in dual targeting – via the presence of a second in-frame translation initiation codon – of the encoded PPO to plastids and mitochondria.

Several sequence polymorphisms between the R and S biotypes were identified in both *PPX1* and *PPX2L*. Molecular markers were developed and used to determine whether the allele found in the resistant plant for either of these genes co-segregated with the resistance phenotype in a backcross population. The *PPX2L* marker, but not the *PPX1* marker, was significantly correlated with resistance, indicating that the *PPX2L* allele was responsible for resistance. Sequencing of additional *PPX2L* alleles from other R and S plants allowed us to identify a single amino acid that was consistently different between R and S plants. Specifically, *PPX2L* from R compared with S plants encoded a protein lacking a glycine amino acid at position 210, which is predicted to be proximal to the herbicide-binding site of the enzyme.

Confirmation that the Gly_{210} deletion was the molecular basis for resistance to PPO-inhibiting herbicides was obtained using a transgenic approach. PPO enzymes that differed only in the presence/absence of Gly_{210} were expressed in a PPO mutant strain of *E. coli*. Both enzymes were able to complement the mutant *E. coli*; however, *E. coli* expressing the minus-Gly_{210} PPO grew in 10-fold or higher concentrations of lactofen compared with *E. coli* expressing the plus-Gly_{210} PPO. This is the first elucidation of the molecular basis for evolved resistance to PPO-inhibiting herbicides.

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