

USING 5' RACE TO AMPLIFY AND SEQUENCE VELVETLEAF EPSPS. Aaron L. Waltz, Alex R. Martin, and Fred W. Roeth, and Don J. Lee, Graduate Research Assistant, Professor, Professor, and Professor, Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68583-0915.

Glyphosate is a widely used pesticide in US crop production. With a large percentage of soybean acres (approximately 70%), and a growing number of corn acres being treated with glyphosate, an estimated 100,000,000 field acres could be treated annually with glyphosate. Tolerance/resistance to glyphosate has been engineered and selected for in many crop and common research species, and at least eighteen glyphosate-resistant biotypes of six weed species now exist. One biotype, glyphosate-resistant goosegrass, has one single amino acid substitution in common with commercially available, glyphosate-resistant corn hybrids. The goal of this research is to amplify and compare regions of the EPSPS DNA sequence within weed species, initially concentrating on velvetleaf, a primary weed of production agriculture in Nebraska and the Great Plains. PCR primers were developed with database techniques by aligning several EPSPS sequences available through GenBank. Highly conserved amino acid and base pair regions within this enzyme make this a possibility. Initial attempts to amplify products from DNA were unsuccessful. It appears that at least one of the primers has multiple targets within the Arabidopsis genome. A technique known as 5' RACE (Rapid Amplification of cDNA Ends) is being utilized to target and amplify the 5' end of EPSPS within velvetleaf. This process involves isolation of RNA, reverse transcription to make cDNA, tailing of the cDNA with base pairs, and then PCR amplification using one RACE-specific primer and one gene-specific primer. The EPSPS gene sequence from important weed species may yield information about the EPSPS enzyme across species. EPSPS gene primers that produce gene-specific products across multiple species would be beneficial in weed population surveys. Sequence information can also be used in blotting procedures to investigate EPSPS gene families and copy number, and to examine EPSPS mRNA expression. Understanding and utilizing information about EPSPS in weed populations can be an important basis for future weed management programs.