Acer VanWallendael May 13 2014

Characterization of the glyphosate-sensitive element of the EPSPS gene by DNA sequence analysis

Abstract:

Changes to the EPSPS gene in plants and bacteria allow resistance to the common herbicide glyphosate. In order to analyze the variation in the EPSPS gene, DNA was extracted from five common perennial angiosperms. Degenerate primers recognizing conserved regions of the gene successfully amplified the active site of the EPSPS enzyme. None of the sequences of the amplified genes coded for amino acid changes in the binding site of EPSPS. However, surprising diversity was revealed both in the amino acid sequence directly adjacent to the binding site, and in the nucleotide sequence coding for the active site. This work corroborates findings that the active site is highly conserved, and offers a new method for surveying for escaped transgenes and evolved glyphosate resistance in weeds.

Introduction:

Glyphosate is a broad-spectrum herbicide (commonly known as RoundUp) that targets 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS or EPSP Synthase) (Figure 1). EPSPS is crucial to the shikimic acid synthesis pathway for synthesizing aromatic amino acids. It is found in most non-animal organisms.

Transgenic glyphosate-resistant crops are useful because they allow indiscriminate application of the herbicide. In plants, resistance to glyphosate is commonly engineered by inserting a gene with a point mutation G101A (Funke 2006). Resistance may evolve in weed populations either by hybridization with a transgenic crop or by evolution of the mutation P106S (Powles and Yu 2010). In addition, researchers have found that the T102I mutation confers resistance (Cao 2012). Glyphosate-resistant weeds are rare, but are of great interest to farmers whose crops they threaten and conservationists concerned about their spread. This study uses a new method to characterize the genetic basis of EPSP synthase and glyphosate resistance in less-studied plants.

Methods:

Plants were collected from Inwood Hill Park in New York City. DNA was extracted from plants using a Qiagen Fastprep kit. DNA for *Brassica rapa* was obtained from Dr. Steven Franks. Since none of the collected plants had sequence information available, primers were designed based on known sequence for *Brassica rapa* and four other angiosperms in the NCBI database. Degenerate primers were designed flanking the area coding for the binding site of EPSP synthase. PCR products were visualized on a 1% agarose gel with ethidium bromide. PCR products were purified using a Qiagen PCR purification kit, and sequenced by Sanger sequencing (Genewiz). Sequences were analyzed using Clustal Omega.

Results:

Amplicon size varied because the amplified fragment contained a variably sized intron. The intron is common to angiosperms, and begins just 18-30 nucleotides after the highly conserved binding site sequence. Amplicon sizes were consistent for a particular species over several PCR reactions at 58^{o} and 56^{o}C and at different concentrations of DNA.

Amino acid sequences were conserved across all plants in the binding site, but nearby regions showed diversity (Figure 3). In addition, variation in nucleotide sequence was high, but all observed polymorphisms were synonymous. For these sequences, a group of thirteen amino acids is highly conserved across divergent taxa.

Discussion:

Degenerate primers were able to amplify the EPSPS gene over a wide range of angiosperm clades. Variation in the amplicon size because of the variable intron means that this region is useful for differentiating plant species. Sequencing revealed that the active site of EPSPS is a highly conserved amino acid sequence, despite the nucleotide diversity in the region surrounding it.

This method offers a great opportunity to understand more about how EPSPS genes are conserved, and how glyphosate resistance may evolve. It also provides a new tool to differentiate plant species, and a method to survey for the genetically engineered mutation for glyphosate resistance and for evolved resistance to glyphosate.

Works Cited:

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