Identifying the Mechanism of Resistance to ALS-Inhibiting Herbicides in Spotted Spurge *(Euphorbia maculata)*

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Introduction

Spotted spurge (*Euphorbia maculata)* is a problematic weed commonly found in golf course putting greens and sports fields. Acetolactate synthase (ALS) inhibitors are widely used to combat its presence. The University of Georgia has discovered a spotted spurge biotype resistant to metsulfuron, a member of the sulfonylurea family of ALS inhibitors (Figure 1). ALS is critical in the biosynthesis pathway of three branch-chained amino acids: valine, leucine and isoleucine (Ray, 1984; Umbarger, 1978). Plants subjected to ALS inhibitors will be killed by starving of these three essential amino acids. Resistance to ALS inhibitors is usually caused by missense mutations in ALS encoding genes. Missense mutations can be identified through sequencing of the susceptible and resistant populations to determine if a substitution occurred.

Objective

The goal of this research is to deduce the mechanism of resistance to ALS-inhibiting herbicides in a metsulfuron resistant spotted spurge.

Materials and Methods

Rate Response Trial

Rate response trials were applied to the susceptible (S) and resistant (R) spotted spurge biotypes by Patrick McCullough at the University of Georgia (Figure 1) to confirm the suspected herbicidal resistance.

DNA Extraction

Three common methods were used in the DNA extraction, CTAB extraction, Omega BioTek e.z.n.a DNA Kit, and a Qiagen DNA Plant mini kit. The DNA was extracted from the fresh leaves of the spotted spurge. Extraction followed the protocol given in the kits. For CTAB DNA extraction, a modified procedure was used based on the procedure proposed by Porebski et al. (1997).

Polymerase Chain Reaction

Extracted DNA was used in a polymerase chain reaction (PCR) on an Eppendorf Mastercycler pro to amplify the ALS gene (approximately 2,000bp long) of spotted spurge in both the susceptible and resistant populations. Phusion DNA Polymerase is used for PCR following the reaction mixture and thermal cycling conditions as suggested by the protocol (Figure 2). PCR Primers (Figure 3) were designed through Primer3 and ordered from Eurofins genomics. Some of the primers utilized were designed by previous research (Duran Prado, 2004). Upon successful amplification, the PCR product was run through a 1% agarose gel electrophoresis. The desired fragment was extracted using a Qiagen Gel Extraction Kit. This fragment (DNA concentration must be > 25ng/uL) is then sent to the Auburn University Genomics and Sequencing Lab for capillary sequencing with the same forward and reverse primer pair used in PCR. The obtained nucleotide sequences and the translated protein sequences were aligned with the *Arabidopsis thaliana* ALS gene in CLC Genomic Workbench 6.0 (Figure 4). The alignment was used to identify whether missense mutations occurred in the resistance conferring codons. In figure 4, three common proteins that have been shown to confer resistance are indicated on the *A. thaliana* sequence.

Figure 1

Rate Response Trials

Spotted Spurge Treated with Metsulfuron-methyl (2 WAT)

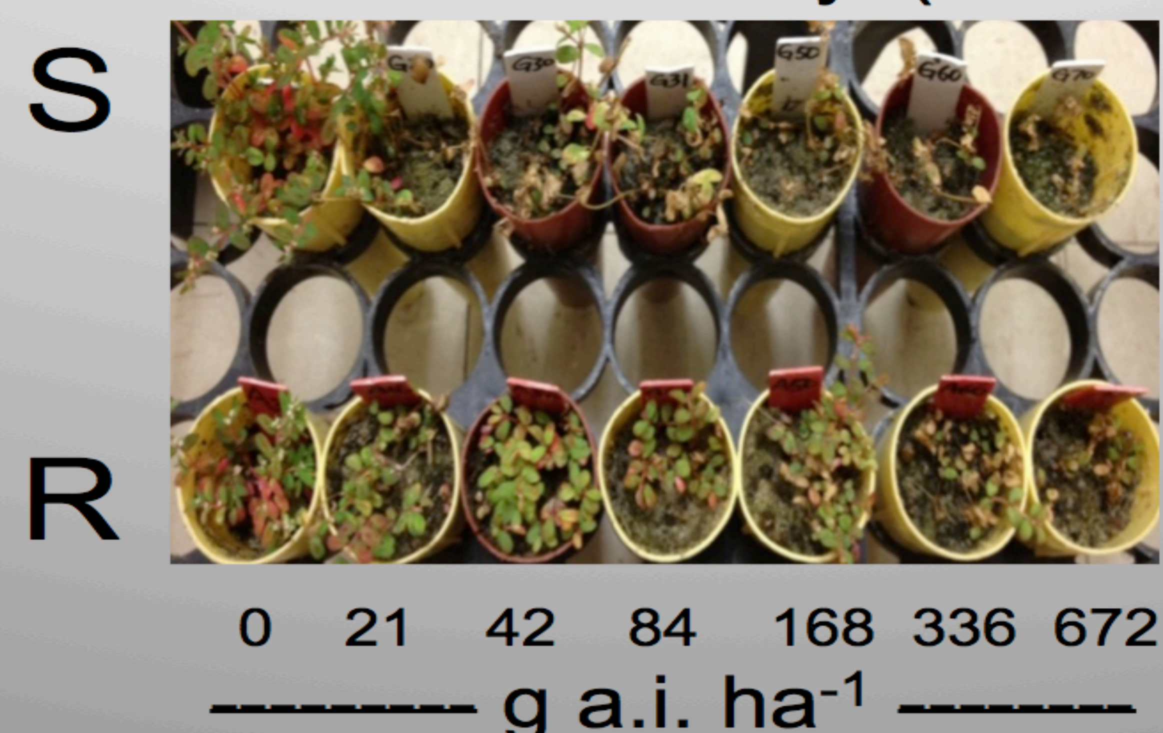


Figure 2

PCR Settings For ALS Amplification

1	2	3	4	5
98℃ 30 seconds	98℃ 10 seconds	60-68℃ 30 seconds	72℃ 45 seconds	72℃ 10 minutes
	←	35X	→	

Figure 3

PCR Primers (*denotes reverse primer)

GCCACCAATCTCGTCAGC		GGAGGCGCGTGCGTAT
GGAGGCGCGTGCGTATCCCT	A	AAATATCAATACTGTGTTCTCCCATC*
GTTGCCTAAAGACCCTAGCG		CGCGTGCGTATCCCT
GAACCACGAAAAGGCTCTGA		CCAGGACGACCTGAAGTAGC*
CCACGAAAAGGCTCTGACAT		TTGGGGATGAGTTGTCTTTACA
ACCCTAGCGAGTTGCAATTA		
Primers Designed by Previous Research		
AAGGGCGCCGATATYCTYGTBGARGC	B	ACAGTCCCATGCATNCCMARCAT*
TGTGGGGCAGCACARATGTGGG		ATCACCTTCTGTGATCAYRTCYTTRAA*

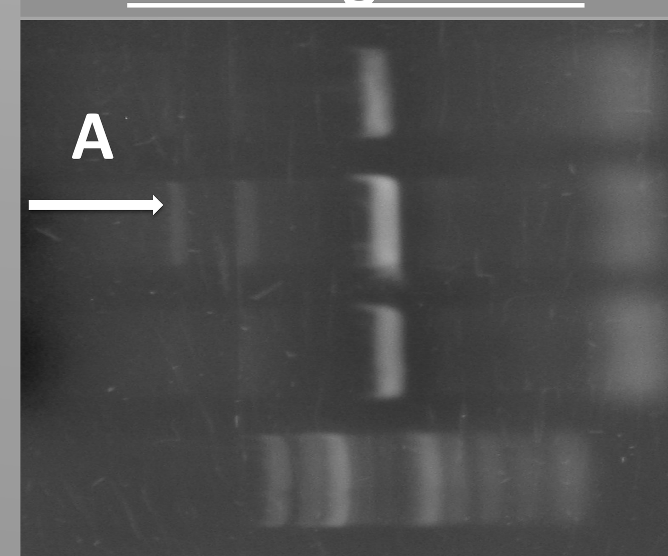
Figure 4

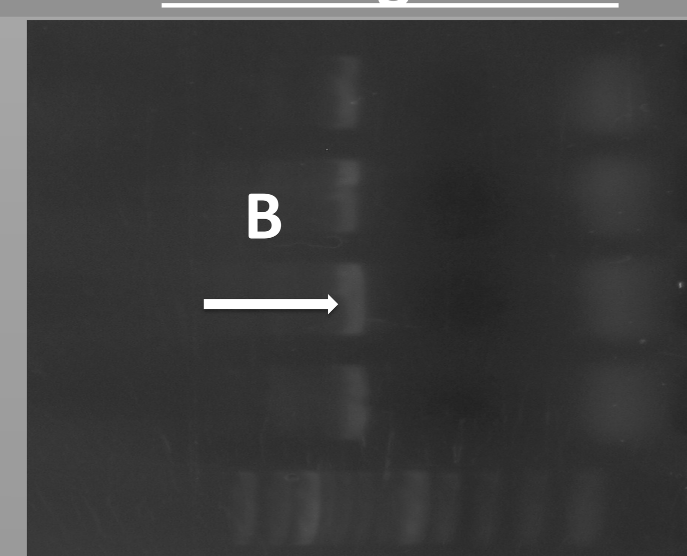
Sanger Sequencing Alignment

	Ala-122	Pro-197	Ala-205
Arabidopsis thaliana	GTGCATCA	TCCCTCGT	ATGCGTTT
Protein Sequence	G A S	V P R	D A F
Euphorbia maculata	GCGCTTCA	TGCCTCGC	ACGCGTTT
Protein Sequence	R A S	V P R	D A F

Figure 5

ALS Fragment 1 **ALS Fragment 2**





Results and Discussion

DNA extraction techniques had to be modified after several failures due to the high polysaccharide content of the milky, latex sap that is found in spotted spurge. A DNA concentration of approximately 55ng/uL was used to run PCR with all primers listed in Figure 2. Of the primers listed, only 2 pairs have proven to amplify portions of the ALS gene. Primer pair A (Figure 3) successfully amplified base pairs (approximate) 455 to 2012 (Figure 5-A), but at a concentration too low to sequence. Primer pair B (Figure 3) successfully amplified base pairs 295 to 1170 (Figure 5-B). The concentration of this amplification was purified to 27ng/uL of DNA and was sequenced. Figure 4 shows sections of the alignment of the nucleotide sequence and the translated protein sequence for both spotted spurge and *A. thaliana* to identify if a substitution was present in the amino acid sequence. The three segments shown illustrate the most common mutation sites that confer resistance. The alignment revealed no missense mutations in the resistance conferring codons. More primers are being developed to continue efforts in sequencing the final portion of the ALS gene.

Future Research

- Successful amplification of the remainder of the ALS gene of spotted spurge is to be accomplished through further PCR primer design to determine if a mutation is present.
- If amplification of the second half of the ALS gene continues to fail, transcriptome Next Generation Sequencing (Illumina platform) may be conducted. This will allow for analysis and juxtaposition of the gene expression in both the susceptible and resistant population of spotted spurge. If varied gene expression is found, then a possible mechanism of resistance could be deduced.

Literature

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