PROGRESS REPORT

Project Title: Multiple herbicide resistance in Palmer amaranth and use of gene editing for its management

c. Principal Investigators:

Dr. Mithila Jugulam, Professor (Weed Physiology), Department of Agronomy, Kansas State University, Manhattan KS 66506. Telephone: 785-532-2755; E-mail: mithila@ksu.edu.

Summary:

Development of multiple Herbicide resistance in Palmer amaranth and other major weeds of Midwestern US has been increasing rapidly, which is a serious threat for sustainable soybean production. In some scenarios growers are losing herbicide options to manage this weed. We have identified a Palmer amaranth population resistant to 6 herbicide modes of action groups. Importantly, this population can metabolize 5 herbicides group without any changes in herbicide target site. There are two major enzyme families involved in metabolism of herbicides, Cytochrome P450 and Glutathione S-transference (GST). These enzymes are important in providing selectivity for many of the herbicides used in crops. Metabolic resistance in weed can predispose them to evolve resistance to other groups of herbicides as well, which will be a serious problem for the management. In this project we intend to apply genetic and molecular methods to identify the basis for the development of metabolic resistance to multiple herbicides in Palmer amaranth. We will identify the specific enzymes involved in herbicide metabolism using RNA-sequencing technique followed by bioinformatics analyses. The outcome of this research will help in designing more sustainable strategies for weed management.

Project deliverables:

We will identify the specific metabolic enzymes i.e., cytochrome P450 or glutathione S transferases involved in the metabolism of multiple herbicides. Additionally, the RNA-sequencing data will be available for functional validation and also assess the role of other genes if any involved in herbicide resistance compared to susceptible plants.

We will also train a graduate student in weed genomics area, which is a novel field in weed science and provide hands on experience in bioinformatics.

Benefits to soybean farmers:

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Progress report:

As reported in the previous progress report, upon transcriptome analyses of the multiple herbicide-resistant Palmer amaranth resistant and susceptible samples, we identified 97 differentially expressed genes in the resistant plants when compared to susceptible without any treatment. This data is further analyzed to identify any of these differentially expressed genes belonging to cytochrome P450 or glutathione-s-transferase enzyme family. These two enzyme families are known metabolize multiple herbicides in plans. The paired end reads generated from RNA seq data, were generated were mapped to the Palmer amaranth transcriptome using HISAT. Differential gene expression analysis conducted using DEseq2, revealed up-regulation or down regulation of several genes as shown in Table 1 following treatments with chlorsulfuron, atrazine, mesotrione and 2,4-D, in the resistant plants compared to susceptible plants. Overall, more genes were differentially expressed upon chlorsulfuron, and atrazine treatment compared to 2,4-D and mesotrione. Additionally, two genes, CYP72A218 and CYP82D47 were found to be constitutively upregulated across all treatments. These two genes are known to be involved in phase-I metabolism of herbicides. Work is in progress to validate the expression levels of the two CYP genes in the resistant and susceptible Palmer amaranth plants via real time-quantitative PCR analysis. Identification and confirmation of genes involved in multiple herbicide metabolism in this Palmer amaranth will be valuable to demonstrate that metabolic resistance predisposes weed populations to evolve resistance to other herbicides without selection.

Table 1. Number of differentially expressed genes reported in KCTR (R) vs KSS (S) using *DEseq2* package upon herbicide treatments and non-treated (NT)

Condition	Treatment groups	Differentially expressed genes	Upregulated genes	Downregulated genes
Treated	R vs S – Chlorsulfuron	376	174	202
Treated	R vs S – 2,4-D	201	112	89
Treated	R vs S – Atrazine	426	182	244
Treated	R vs S – Mesotrione	216	112	104
Non-treated	R vs S – NT	457	254	203