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

c. Principal Investigators:

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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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<u>Progress report:</u>

As mentioned in May 2023 progress report, we have isolated RNA from the resistant and susceptible Palmer amaranth plants and sent the samples for RNA-sequencing. Briefly, to analyze the transcriptome, we sequenced RNA from 3 plants each of resistant and susceptible populations of Palmer amaranth. Third and 4th young leaf were collected from individual plant 6 hours after 2,4-D and mesotrione treatment, separately and at the same time from the non-treated plants. Total RNA was extracted, and the samples were sent to Azenta Lifesciences for RNA sequencing. Paired-end reads obtained from RNA-seq analysis were trimmed and mapped against *Amaranthus hypochondriacus* reference transcriptome., which is the closet to Palmer amaranth available in the database. A pairwise differential gene expression analysis was performed using DEseq2 package in R studio. Probable gene function for differentially expressed genes were annotated with reference to *Arabidopsis thaliana* genome. Following alignment and differential expression with DESeq2, Filtering parameters were for the adjusted *p* value = < 0.05 and log2 fold change > 2.0 and false discovery rate (FDR) was < 0.05. We identified 97 differentially expressed genes in the resistant plants when compared to susceptible without any treatment. From our previous research, the Palmer amaranth population used in this research exhibits metabolic resistance to 2,4-D and mesotrione. Therefore, we hypothesize that the genes related to herbicide metabolism may have differential regulation or be highly expressed in the non-treated samples of resistant plants compared to the susceptible plants. Our data suggest that out of 97 differentially expressed genes, there are 3 GST (glutathione-S-transferases) and 2 CYPs (cytochrome P450 enzymes) up regulated in the resistant plants compared to the susceptible plants. The GSTs and P450s are known to metabolize multiple herbicides in plants. Additionally, the data of transcriptome of resistant and susceptible plants at 6 hours after treatment with 2,4-D and mesotrione suggest that about 104 and 123 genes differentially expressed respectively in resistant plants when compare susceptible plants. Moreover, we have identified certain common GSTs and CYPs overexpressed constitutively in the resistant plants with both 2,4-D and mesotrione application and without any treatment. These results are important to assess if these specific GST or P450 enzyme activity predisposes Palmer amaranth to evolve resistance to multiple herbicides. Research is in progress to assess the differentially expressed genes between resistant and susceptible plants in response to other herbicide treatments as well.