

A Palmer amaranth population with resistance to six herbicides (6-way) was identified in KS. This Palmer amaranth population was collected from a 45-year old tillage study maintained in continuous sorghum. A variety of herbicides were used in the research trial, with 2,4-D and atrazine used most frequently. However, when plants from this population were studied in the greenhouse, they survived applications of group 2 herbicides Glean (chlorsulfuron), Harmony (thifensulfuron), Beyond (imazamox), Pursuit (imazethapyr); group 14 herbicides Cobra (lactofen) and Flexstar (fomesafen); the group 27 herbicides Callisto (mesotrione) and Laudis (tembotrione), metribuzin (group 5); and glyphosate (group 9); as well as atrazine (group 5) and 2,4-D (group 4). The only herbicides in the study that provided 100% control were Liberty (glufosinate) and Gramoxone (paraquat). Such multiple herbicide resistant Palmer amaranth populations are spreading rapidly posing a serious threat for sustainable soybean production. In some scenarios growers are losing herbicide options to manage this weed. Although use of herbicides still offers an effective weed management strategy, such strategy needs to be complemented with long term sustainable biological-based strategies. The 6-way resistant Palmer amaranth converts the herbicides to inactive forms before the plant can be killed, often due to the activity of two groups of enzymes: cytochrome P450s (P450) and glutathione S-transferases (GSTs). These enzymes provide selectivity to many of the herbicides used in crops. We hypothesized that the activity of these enzymes may be elevated in the 6-way resistant Palmer amaranth. In Year-1 of this project we proposed genetic and molecular methods to assess the activity of herbicide degrading enzymes in this Palmer amaranth. Year-2 of this project is primarily focused on characterization of the herbicide degrading enzymes including P450 or GST.

Overall objective:

1. Investigate common mechanism(s) that can predispose Palmer amaranth to develop multiple herbicide resistance

Sub-objectives:

- 1a. Understand the role of cytochrome P450 enzymes (CYP) in imparting metabolic resistance to Group 2, 4, 14 and 27 herbicides in Palmer amaranth.

- 1b. Understand the role of glutathione-S-transferases (GST) in imparting metabolic resistance to Group 5 and other herbicides.

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 plants. The paired end reads generated from RNA seq data, were generated were mapped to the Palmer amaranth transcriptome using HISAT. The quality of the raw RNA-Seq data was assessed using FASTQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and adaptor sequences were trimmed using Trimmomatic. Trimmed reads were mapped again to *Amaranthus palmeri* reference transcriptome using STAR (<https://github.com/alexdobin/STAR>) and the number of mapped reads for each contig were counted using EBSeq (<https://doi.org/10.1093/bioinformatics/btt087>). A pairwise differential gene expression analysis was performed using DESeq2 package in R studio and probable gene function for differentially expressed genes were annotated. Following alignment and differential expression with DESeq2, Filtering parameters were for the adjusted p-value = < 0.05 and log2 fold change > 2.0 . We identified 130 differentially expressed genes in the resistant plants when compared to susceptible

without any treatment. Additionally, 131, 152, 530, 414, and 689 genes were differentially expressed when resistant plants were compared to susceptible plants upon 2,4-D, mesotrione, atrazine, chlorsulfuron and lactofen treatment. When the nontreated comparison were further studied two genes CYP72A219 and CYP704B1 were found to be consecutively overexpressed in the resistant plants. RT-qPCR results confirmed the overexpression of CYP72A219 and CYP704B1 genes by 6.6-7.1 and 16.7-18.8 folds respectively. These results validate our hypothesis that certain P450 genes can contribute to multiple herbicide resistance in Palmer amaranth. Current efforts are underway to conduct Arabidopsis transformation to develop transgenics that can overexpress the gene CYP72A219 for in planta confirmation of involvement of this gene in herbicide metabolism.

The outcome of this research will demonstrate the greatest threat associated with multiple resistance in weeds and importantly, that a single resistance mechanism can provide resistance to multiple herbicide groups. While mixing and rotation herbicides with multiple, effective modes of action can slow the evolution of resistance, cross-resistance associated with multiple resistance greatly reduces the effectiveness of this strategy. Minimizing the weed seed bank and adopting alternative management strategies is essential to protect the value of existing and future herbicides.