RNA-Seq Insights into Safener-Induced Responses in Zoysiagrass and Bermudagrass

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INTRODUCTION

Safeners are compounds designed to protect desirable plants from the potentially harmful effects of herbicides. By selectively inducing detoxification pathways, these agents enhance herbicide tolerance in crops without reducing efficacy against unwanted vegetation. Understanding the molecular basis of safener-induced responses is essential to improving herbicide selectivity and advancing sustainable agricultural practices.

This study utilizes RNA-Seq to investigate the transcriptional responses induced by the safener metcamifen, focusing on gene expression changes associated with detoxification and stress-response pathways. Key findings reveal upregulation in genes such as cytochrome P450 monooxygenases, glutathione S-transferases (GSTs), and ABC transporters—indicating an activated biochemical defense system. Insights from this work lay a foundation for future research aimed at refining safener applications and potentially broadening their use across various plant species.

MATERIALS & METHODS

Materials and Sample Collection

- ✓ Chemical Treatment: Metcamifen was applied at a concentration of 75 g ai/ha (5.13 oz/acre) using the formulation 200SC.
- ✓ Plant Species: The study involved two grass species, Bermudagrass (*Cynodon dactylon*) and Zoysiagrass (*Zoysia japonica*). 8 Replications.
- ✓ Sample Collection and RNA Extraction: Young leaves were harvested both before treatment and 24 hours after treatment. RNA was extracted using the Direct-Zol™ Miniprep Plus kit (Zymo Research).

Sequencing

•Platform: RNA sequencing was performed by Novogene.

•Parameters: Paired-end 150 (PE150) non-directional sequencing was conducted with a target of 20 million reads per sample.

RNA-Seq Analysis Methodology

1.Alignment:

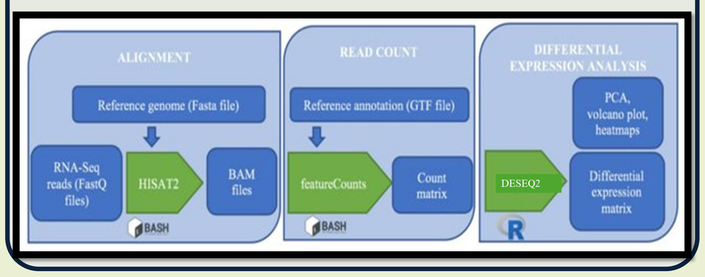
1. RNA-Seq reads in FASTQ format were aligned to the reference genome using the HISAT2 aligner, resulting in BAM files for each sample.

2.Read Counting:

1. FeatureCounts was used to generate a count matrix by quantifying reads aligned to the reference annotation (GTF file).

3.Differential Expression Analysis:

1. Differential gene expression analysis was performed using DESeq2 in R. The analysis included principal component analysis (PCA), volcano plots, and heatmaps to visualize gene expression patterns and identify significantly upregulated genes.



RESULTS

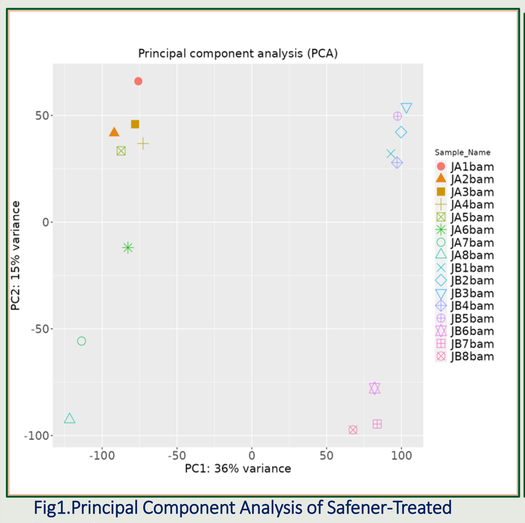


Fig1.Principal Component Analysis of Safener-Treated Bermudagrass

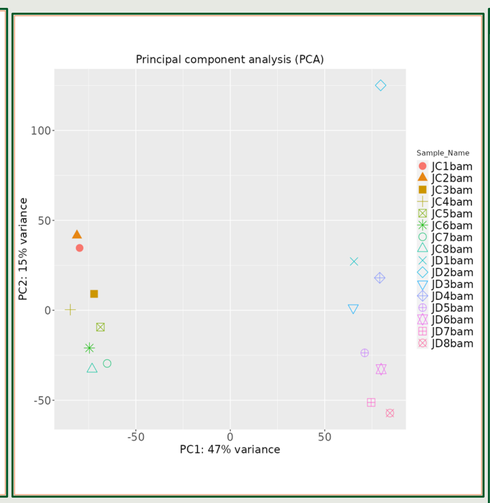


Fig2.Principal Component Analysis of Safener-Treated Zoysiagrass

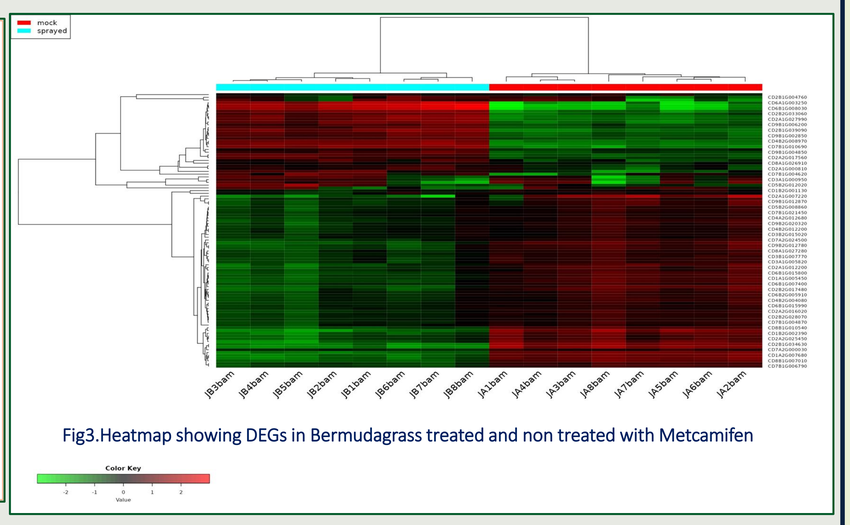
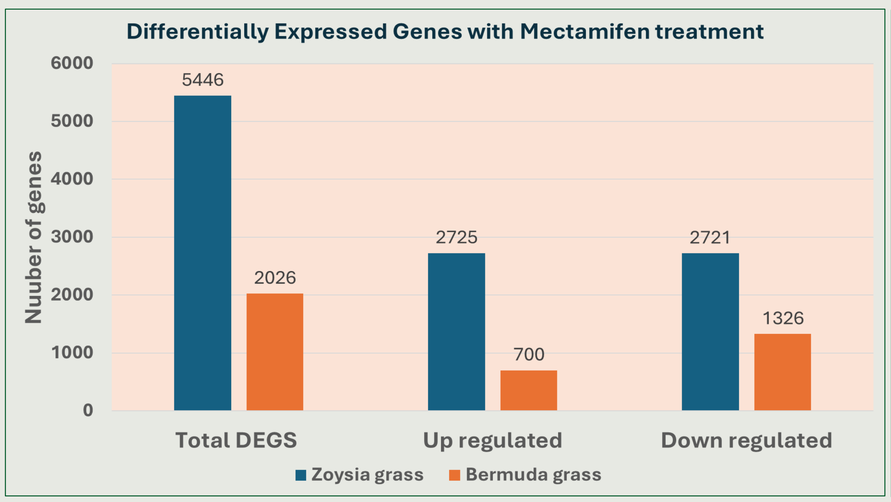


Fig3.Heatmap showing DEGs in Bermudagrass treated and non treated with Metcamifen



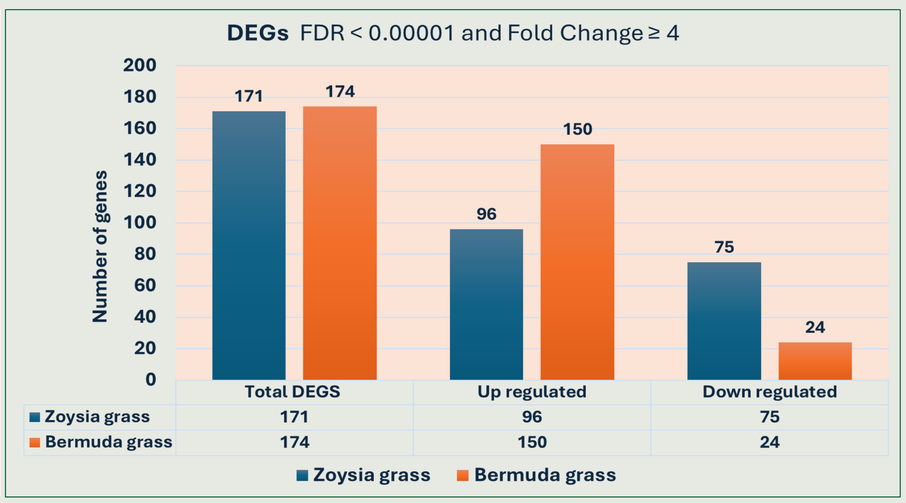
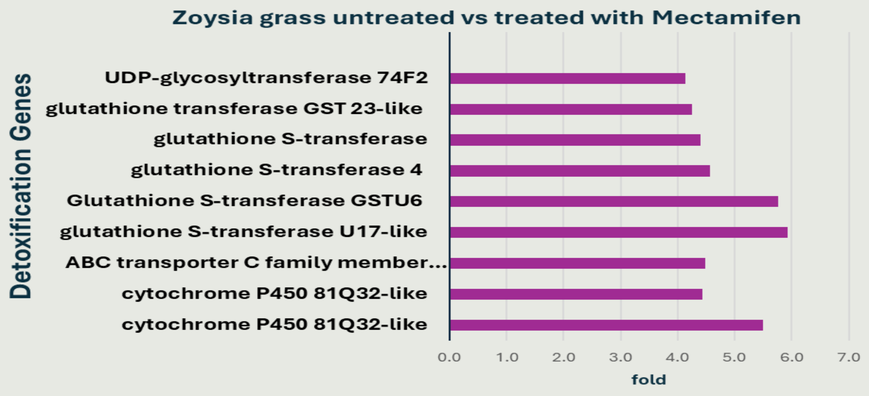
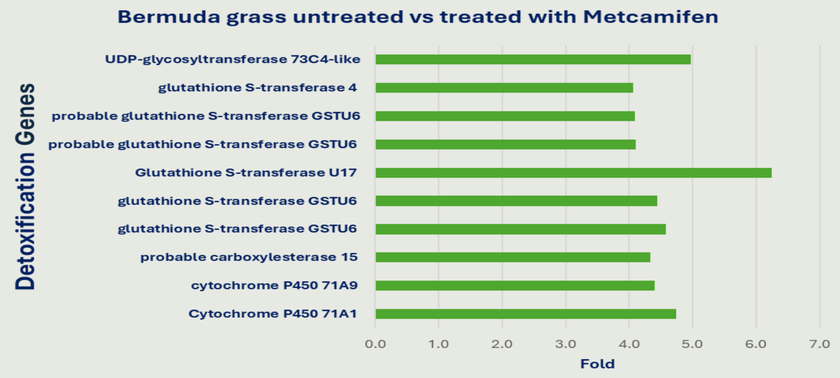


Fig4. Differentaillly Expressed Genes with Mectamifen treatment in Zoysiagrass and Bermudagrass at different fold changes and fdr





CONCLUSIONS

- ✓ The RNA-Seq analysis of *zoysiagrass* and *bermudagrass* treated with the safener metcamifen has revealed significant insights into the molecular mechanisms underlying safener-induced herbicide tolerance.
- ✓ Key findings include the upregulation of detoxification-related genes, such as cytochrome P450 monooxygenases, glutathione S-transferases (GSTs), and ABC transporters. Additionally, several genes within the jasmonic acid signaling pathway were activated, suggesting a broader stress response that may contribute to enhanced physiological resilience.
- ✓ These results indicate that safeners like metcamifen may not only promote herbicide detoxification but also trigger additional defense pathways. This dual effect enhances the plant's ability to withstand herbicidal stress, highlighting potential molecular targets for developing improved safener formulations.

FUTURE RESEARCH

This study lays the groundwork for identifying molecular targets associated with safener-induced detoxification pathways. Future research will delve into characterizing specific cytochrome P450 enzymes involved in these responses to elucidate their roles in safener activity. Additionally, expanding these investigations to St. Augustinegrass, as well as weed species like goosegrass and crabgrass, will provide insights into safener-mediated selectivity across diverse species, potentially enhancing herbicide tolerance in turfgrass and improving integrated weed management strategies.

REFERENCES

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- Brazier-Hicks, M., Franco-Ortega, S., Watson, P., Rougemont, B., Cohn, J., Dale, R., Hawkes, T. R., Goldberg-Cavalleri, A., Onkokesung, N., & Edwards, R. (2022). Characterization of cytochrome P450s with key roles in determining herbicide selectivity in maize. *ACS Omega*, 7(19), 17416–17431.

SPECIAL THANKS: Syngenta Professional Solutions, Syngenta Crop Protection ,Greensboro, NC