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Amith Sadananda Maroli
Clemson University

Nishanth Tharayil
Clemson University

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Glyphosate Application Causes Physiological Perturbations in Amino Acid Profiles of Palmer amaranth- A Study of Susceptible and Resistant Biotypes of *Amaranthus palmeri*

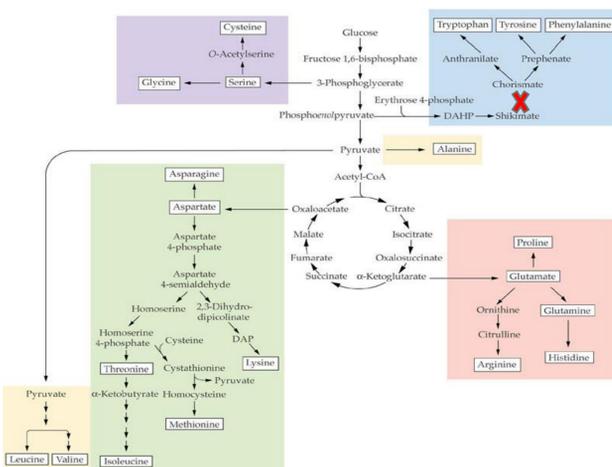


Amith Sadananda Maroli¹ and Nishanth Tharayil¹

¹ Department of Plant and Environmental Science, SAFES, CAFLS, Clemson University, Clemson, South Carolina, 29634

INTRODUCTION

Lethality of most systemic herbicide could be mainly attributed to the disruption of essential metabolic pathways in plants, Glyphosate is broad spectrum, non selective herbicide that acts as an amino acid synthesis inhibitor. Glyphosate inhibits the synthesis of aromatic amino acids by acting as competitive inhibitor to the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). It was considered that plants would be unable to develop resistance to glyphosate

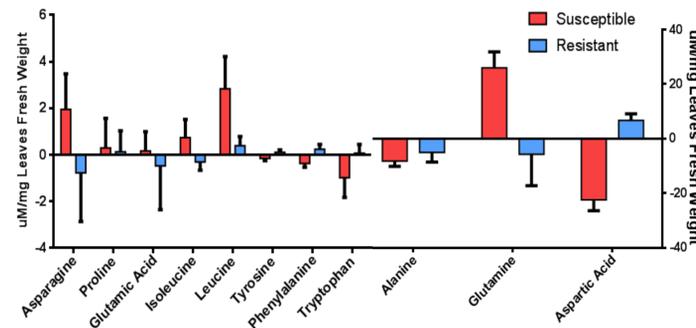


However, recently several weed species including *Amaranthus sps* have shown to resist glyphosate applications at higher than normal field rates. This ability to tolerate high levels of glyphosate could be due to the alterations in the metabolome of the plant.

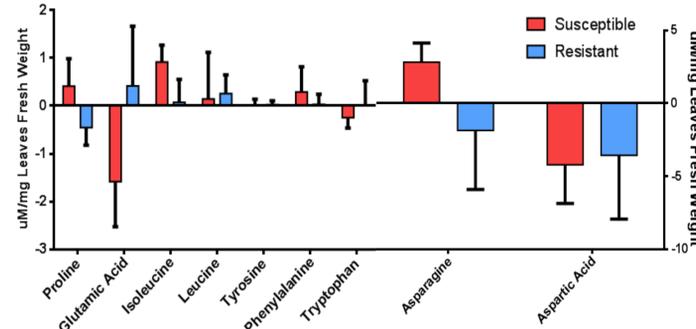
Hence metabolomic approach presents a unique opportunity to gain detailed understanding not only on the mode of action of herbicides, but also to outline the possible cellular-level mechanism that confers tolerance/resistance to some biotypes.

RESULTS

Amino acid profiling of plants showed significant changes. In experimental units harvested 24 hours after glyphosate application, concentrations of aromatic amino acids (Tyrosine, Phenylalanine, Tryptophan) decreased in the susceptible plants as compared to the resistant biotypes. However the levels of Asparagine, Isoleucine, Leucine and Proline increased in susceptible plants compared to the resistant biotypes.



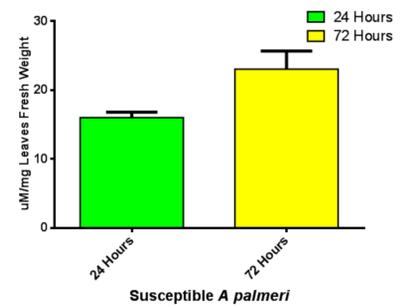
However, after 72 hours of application, the susceptible plants started showing wilting symptoms in contrast to the resistant biotypes which did not show such signs of wilting. Amino acid analysis of these plants showed that levels of most amino acids including the nitrogen donating amino acids in the susceptible plants increased where as the levels of the nitrogen donating amino acids decreased in the resistant plants.



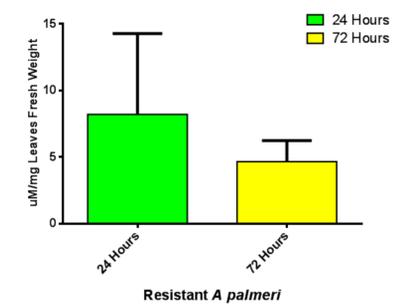
The increase in the stress response amino acids shows that susceptible plants are unable to tolerate glyphosate as compared to that of resistant biotypes

RESULTS

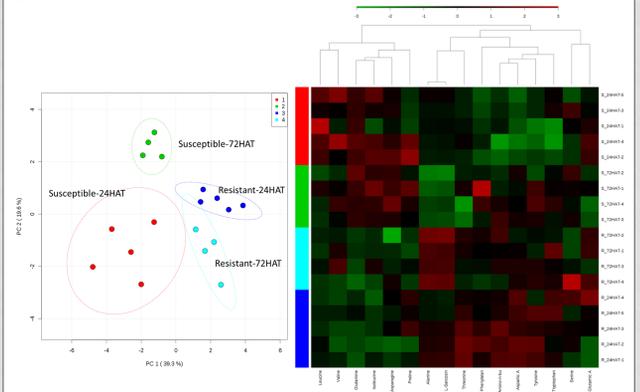
Since glyphosate act as a competitive inhibitor to PEP by binding to EPSPS, accumulation of shikimic acid, is seen. In susceptible plants, application of 0.75x glyphosate caused a 17 fold increase in shikimic acid concentration in within 24 hours after treatment. After 72 hours of treatment, the levels further increased to 24 fold indicating the disruption of shikimate pathway.



Interestingly, glyphosate application to resistant plants also resulted in an 11 fold accumulation in shikimic acid levels within 24 hours. However, the level of shikimic acid decreased to 5 fold after 72 hours

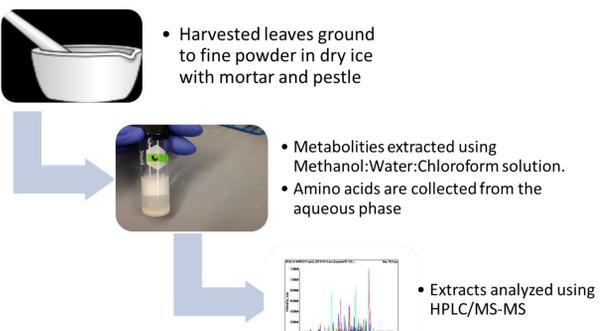


Principal Component analysis showed a similar trend such that there was a significant difference between the amino acid profiles of susceptible and resistant biotypes. Moreover, there was also change in the profiles due to the time of exposure to glyphosate between and within the two biotype.



MATERIALS AND METHODS

Susceptible and Resistant *A palmeri* seeds were propagated and transplanted into pots and grown under green house conditions. Mature plants (8 to 10 cm) were sprayed with 0.645 kg ae/HA (0.75X) of RoundUp® PowerMax™ Glyphosate solution. Plants were sprayed with deionized water served as control units. Leaves of both set of plants were harvested 24 hours and 72 hours after treatment and stored in -80°C for metabolomic analysis.



Statistical significance of the data were evaluated by univariate analysis using ANOVA and Tukey's Test and Multivariate analysis using Principle Component Analysis (PCA)

CONCLUSIONS

Unlike genes that are subjected to epigenetic regulations and proteins that are influenced by post translational modifications, the metabolome of a biological entity is not subjected to modifications. It provides unaltered snapshot of the physiological system. By directly measuring the metabolic state of a cell, metabolomics offers a powerful augmenting approach for molecular phenotyping. In our study, application of 0.75X glyphosate caused significant changes in the nitrogen pool of the *Amaranthus* plants. The changes in the amino acids profile could be attributed to the increase in the C flow through EMP pathway and the TCA cycle, due to the blockage of shikimate pathway by glyphosate, which in turn would up-regulate the biosynthesis of other amino acids. Interestingly, even though the glyphosate treatment resulted in shikimate accumulation in resistant biotypes, the amino acid profile was similar to the control, indicating a less physiological response of resistant biotypes to glyphosate treatment. This observation is similar to the studies done on *Amaranthus* seeds collected from North Carolina^[1] and Tennessee^[2], which indicates that *A palmeri* could have a different mechanism of glyphosate resistance other than amplification and overexpression of the EPSPS gene

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 Gaines, Todd A., et al Proceedings of the National Academy of Sciences 107.3 (2010): 1029-1034.

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