

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

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Glyphosate is the most widely used herbicide in the world. It is used to control perennial grasses and weeds having broad leaves. Glyphosate works by inhibiting the plant specific enzyme 5-enolpyruvylshikimate-3-phosphate synthase that catalyzes the conversion of shikimic acid to chorismate, which serves as the precursor to production of aromatic amino acids, namely tyrosine, phenylalanine and tryptophan. Conventional glyphosate resistance in plants is induced by the insertion of a bacterial EPSPS gene that results in the overexpression of the EPSPS enzyme resulting in sustained production of the enzyme and thereby overcoming the bottleneck caused by the glyphosate application. However, of late several weed species have naturally acquired resistance to glyphosate. Resistance to abiotic stress in plants may be, in some cases, contributed by amino acid metabolism. Accumulation of amino acids may help in producing an osmolytic balance that helps in clearing the stressing causing agents or acts as a reservoir pool of building blocks that help in the production of stress releasing or detoxifying compounds or enzymes. Metabolomic profiling helps in analyzing small molecules/metabolites. These procedures, carried out by either using GC-MS or LC-MS, can reveal a wealth of information on the physiology of the plant. The objective of the present study was to study the free amino acids levels in glyphosate susceptible and resistant *Palmer amaranthus* upon glyphosate application using Reverse phase HPLC-MS. The plants were analyzed at different time periods to capture the effect of glyphosate on amino acids at increasing exposure time. Univariate and multivariate analysis showed significant difference in levels of certain amino acids between the susceptible and resistant plants at different time points of harvest. Univariate analysis within susceptible plants showed that as the duration of exposure to glyphosate increased, there were significant changes in the level of certain amino acids. After 24 hours of spraying, there were significant difference among several amino acids between control and sprayed treatments. As expectedly, there was significant decrease in aromatic amino acids in the sprayed plants compared to the control group. Also decrease in the levels of Aspartic acid, Alanine and Threonine were also seen. However, an increase in Asparagine, Glutamine, Isoleucine and Leucine were seen in the sprayed plants. In the control group between susceptible and resistant plants, only aspartic acid was found to have significant difference among all the amino acids tested. However at the same time period, univariate analysis showed that in the sprayed group, 11 amino acids and 2 non amino acid nitrogen compounds had significant difference between the susceptible and resistant plants. Multivariate analysis using Principle Component Analysis (PCA) also showed a similar trend indicating that glyphosate spraying has a significant effect on not just the aromatic acids, but also on other nitrogen compounds including non-aromatic amino acids.