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EPSPS variability, gene expression, and enzymatic activity in glyphosate-resistant biotypes of *Digitaria insularis*

E. Galeano[1]*, A.A.M. Barroso[1]*, T.S. Vasconcelos[1], A. López-Rubio[2], A.J.P. Albrecht[1], R. Victoria Filho[1] and H. Carrer[1]

[1]Departamento de Ciências Biológicas,
Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, SP, Brasil
[2]Grupo Investigación Ciencias Forenses y Salud,
Tecnológico de Antioquia Institución Universitaria, Medellín, Antioquia, Colombia

*These authors contributed equally to this study.
Corresponding authors: E. Galeano / H. Carrer
E-mail: estebangg18@hotmail.com / hecarrer@usp.br

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ABSTRACT. Weed resistance to herbicides is a natural phenomenon that exerts selection on individuals in a population. In Brazil, glyphosate resistance was recently detected in *Digitaria insularis*. The objective of this study was to elucidate mechanisms of weed resistance in this plant, including genetic variability, allelism, amino acid substitutions, gene expression, and enzymatic activity levels. Most of these have not previously been studied in this species. *D. insularis* DNA sequences were used to analyze genetic variability. cDNA from resistant and susceptible plants was used to identify

mutations, alleles, and *5-enolpyruvylshikimate-3-phosphate synthase* (*EPSPS*) expression, using real-time quantitative reverse transcription-polymerase chain reaction. In addition, EPSPS activity was measured. We found a decrease in genetic variability between populations related to glyphosate application. Substitutions from proline to threonine and tyrosine to cysteine led to a decrease in EPSPS affinity for the glyphosate. In addition, the EPSPS enzymatic activity was slightly higher in resistant plants, whereas *EPSPS* gene expression was almost identical in both biotypes, suggesting feedback regulation at different levels. To conclude, our results suggest new molecular mechanisms used by *D. insularis* to increase glyphosate resistance.

Key words: Weed resistance; Amino acid substitution; Genetic variability; Gene expression; Enzymatic activity