

Herbicide metabolic resistance gene discovery in *Lolium rigidum* Gaudin using RNA-sequencing

Heping Han¹, Qin Yu¹, Susana Gonzalez², Roland Beffa² and Stephen B. Powles¹

¹ Australian Herbicide Resistance Initiative, 35 Stirling Highway, Nedlands, Western Australia 6009, Australia

² Bayer CropScience, Industriepark Höchst, Frankfurt/Main 65926, Germany (heping.han@uwa.edu.au)

Summary Evolution of multiple herbicide resistance is widespread in *Lolium rigidum* Gaudin in Australia. This resistance is often endowed by enhanced rates of herbicide metabolism involving cytochrome P450, GST or other genes. To identify the herbicide-metabolizing genes in *L. rigidum*, the subset populations with metabolic resistance mechanism to diclofop and chlorsulfuron were purified from two field-evolved resistant populations SLR31 and VLR69. No herbicide resistance endowing mutations in ALS and ACCase were detected in individual plants by DNA sequencing. The increased diclofop metabolism in purified subsets was further confirmed by HPLC compared to the susceptible population (VLR1). The F₂ populations were generated by crossing the susceptible plants (VLR1) and two purified resistant subsets. Genetic studies

showed the resistance to diclofop and chlorsulfuron in the SLR31 subset is linked but segregated in the VLR69 subset, indicating different genes conferring resistance to chlorsulfuron and diclofop in the two subsets. The most susceptible and resistant plants were individually phenotyped from the F₂ and parent subsets. RNA was extracted and RNA-sequencing performed. A few P450s and GSTs were selected as candidate genes for further validation and functional characterization by yeast and plant transformation. Herbicide-metabolizing and resistance-endowing gene discovery will greatly extend our currently limited knowledge and understanding of metabolic herbicide resistance.

Keywords Chlorsulfuron, diclofop, herbicide metabolic resistance, *Lolium rigidum*, RNA-Seq.