

**STUDY ON THE METABOLISM-BASED RESISTANCE IN A MULTIPLE
HERBICIDE RESISTANT LINE OF *Echinochloa phyllopogon* (Stapf) Koss.**

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Herbicide resistance endowed by enhanced metabolism of herbicides is a major threat to the effectiveness and sustainability of weed control by herbicides, as it is unpredictable and could extend resistance to different chemical groups and modes of action encompassing existing, novel and to-be-discovered herbicides. The molecular mechanisms of metabolism-based herbicide resistance remain to be inadequately understood due to its complexity and limited information on the molecular key players and genes involved in the resistance. Consequently, this has hindered the prediction of cross-resistance patterns in weeds. A multiple herbicide resistant (MHR) line of *Echinochloa phyllopogon* originally collected from Sacramento Valley, California has been found to be resistant to at least 12 herbicides from nine different chemical groups, including acetyl-CoA carboxylase inhibitor, fenoxaprop-*P*-ethyl (FE) and very long chain fatty acid elongase inhibitor, thiobencarb (TB). Recently, two cytochrome P450 genes (*CYP81A12* and *CYP81A21*) belonging to CYP81A subfamily were identified to be involved in concomitant cross-resistance to six unrelated herbicide classes in MHR *E. phyllopogon*. This suggests a critical role of CYP81As in endowing unpredictable cross-resistances in this weed species. Thus, elucidation of the metabolizing functions of CYP81As to a larger group of herbicides needs to be realized. Further, the mechanisms of FE and TB resistance in the MHR line of *E. phyllopogon* remain unknown, while previous studies hinted that CYP81As are not involved in the resistance

to these herbicides. To further understand the mechanisms involved in metabolism-based herbicide resistance in *E. phyllopogon*, three research studies were conducted as follows:

Chapter 1: Functional Characterization of CYP81A P450s in *Echinochloa phyllopogon*

Members of CYP81A subfamily in *E. phyllopogon* were previously reported for conferring cross-resistance to six unrelated herbicide classes. The metabolism functions of all nine putative functional *CYP81A* genes of *E. phyllopogon* to 33 herbicides from 24 distinct chemical groups were characterized via ectopic expression in *Arabidopsis thaliana* and in *Escherichia coli*. CYP81A12, CYP81A15, CYP81A18, CYP81A21 and CYP81A24 exhibited wide substrate and distinct specificity when expressed in *A. thaliana*; and produced either hydroxylated, *N*-/*O*-demethylated or both metabolites when expressed in *E. coli*. The pattern of resistance conferred by these CYP81As is geared towards all chemical groups of acetolactate synthase inhibitors and is expanded to herbicides inhibiting photosystem II, phytoene desaturase, protoporphyrinogen oxidase, 4-hydroxyphenylpyruvate dioxygenase, and 1-deoxy-D-xylulose 5-phosphate synthase. Based on the metabolism profile, cross-resistance in MHR *E. phyllopogon* to novel herbicides pyrimisulfan and propyrisulfuron, and to mesotrione was predicted and confirmed. These results emphasized the risk of cross-resistance caused by up-regulated genes with wide substrate specificity; underpinned the perilous role of CYP81As in herbicide metabolism; predicted unexpected cross-resistances to novel herbicides; and identified suitable herbicides to control the MHR line of *E. phyllopogon*.

Chapter 2: Elucidation of Fenoxaprop-*P*-Ethyl Resistance in *Echinochloa phyllopogon*

Previous research suggested that FE resistance in MHR *E. phyllopogon* from California is under the control of a single locus, and is associated with the six herbicides in which the resistance mechanism is due to the overexpression of CYP81A12 and CYP81A21 P450s. In this study, the involvement of P450s in FE resistance was tested by analyzing the sensitivity of rice transformed with the P450 genes. No FE resistance was observed in rice calli expressing the P450s. Previous report revealed an enhanced accumulation of glutathione-conjugated FE in MHR *E. phyllopogon*, suggesting the involvement of glutathione S-transferases (GSTs) in FE resistance. GST contigs that were constitutively highly expressed in the MHR line were analyzed by real-time qPCR for association of higher transcript levels with the resistance in the F₆ progenies of MHR and sensitive lines. Next, the candidate GSTs were isolated, cloned and expressed in rice calli, followed by FE sensitivity assay. One GST gene was identified to be conferring slight tolerance to FE when expressed in rice calli, thus suggesting its involvement in FE resistance in MHR *E. phyllopogon*.

Chapter 3: Inheritance of Thiobencarb Resistance in *Echinochloa phyllopogon*

MHR *E. phyllopogon* is 19-fold more resistant to TB compared to the sensitive line. However, the mechanism of TB resistance in the MHR line remains to be unidentified. To elucidate the genetic control and inheritance of TB resistance, field-dose sensitivity assay in F₂ progenies from a cross between MHR and sensitive line was performed. Result suggests that the mechanism of TB resistance is under the control of a major locus. Progeny testing in the F₆ lines revealed that TB resistance is

not perfectly associated with the other herbicides in which the resistance mechanism is due to the overexpression of *CYP81A12* and *CYP81A21* P450s. The distinct sensitivities of some F₆ lines imply that the mechanism of TB resistance in MHR *E. phyllopogon* is different from that of the other herbicides metabolized by P450s. The GST gene identified for FE resistance also conferred slight tolerance to TB when expressed in rice calli, although its expression was not associated with TB resistance in the F₆ generation.

Based on the metabolism profile of CY81As, cross-resistance to novel herbicides was successfully predicted in an *E. phyllopogon* population in which two CYP81A genes are overexpressed. This study implies that the profiling strategy to characterize the key enzymes for herbicide metabolism could help predict the cross-resistance pattern of metabolism-based herbicide resistance in weeds. In FE resistance study, one GST gene was identified to be conferring slight tolerance to FE when ectopically expressed in rice, thus suggesting its involvement in FE resistance in the MHR line. The indisputable role of the GST gene in FE conjugation needs to be further investigated. Inheritance of TB resistance in the F₆ progenies of MHR *E. phyllopogon* suggests that an unknown additional mechanism may be present for TB resistance. Inevitably, identification of the key players involved in TB resistance will be the future direction of this research.