Title : Elucidation of target-site resistance mechanisms in ALS-inhibitor-resistant *Echinochloa crus-galli*

Author(s) :
1. Sangtawan Boonsombat — **Student ID :** 650510306
2. **Student ID :**
3. **Student ID :**

Major : Biology

Advisor(s) :
1. Lecturer Dr. Pattarasuda Chayapakdee
2.
3.

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ABSTRACT

Barnyard grass (*Echinochloa crus*-galli) is a major weed in rice paddies and has developing resistance due to repeated herbicide use. One of the most resistances is to bispyribac-sodium (BS), an acetolactate synthase (ALS) inhibitor which inhibit amino acid biosynthesis. This study aimed to screen BS-resistant populations collected in Thailand, investigate sequence variations in the ALS gene, and examine the involvement of cytochrome P450 enzymes on metabolic resistance. To evaluate herbicide resistance, seedlings from 40 populations were transplanted into sterile plates containing Murashige and Skoog (MS) liquid medium supplemented with BS at six concentrations, applied according to recommended label rates, over a 13-day period. The result indicated that a BS at approximately 0.1x recommended label rates effectively distinguished resistant from susceptible line, whereas the resistant line maintained growth even at higher concentrations. Additionally, multiple herbicide sensitivity assays were performed by using 20 herbicides representing different modes of action. The resistant line (SP-4A) exhibited resistance exclusively to ALS-inhibiting herbicides. However, molecular analysis of ALS gene sequences was conducted to detect genetic mutations. Genomic DNA was extracted, and three copies of ALS gene were amplified for sequencing. The results of ALS gene sequence analysis for all tested barnyard grass lines revealed none of amino acid substitutions were detected in the three ALS genes. In addition, the potential

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involvement of cytochrome P450 metabolic resistance was investigated to further clarify the resistance mechanism. These findings suggest that non–target-site resistance mechanisms may play a major role in BS resistance in Thai populations of *E. crus-galli*, providing important insights for sustainable weed management strategies.

Keywords: *Echinochloa crus*-galli, Barnyard grass, bispyribac-sodium, ALS inhibitor, herbicide resistance, cytochrome P450

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