Study of the Molecular Mechanisms of Bispyribac-Sodium Resistance in Barnyard Grass (*Echinochloa crus-galli*)

Chanoknad Lomkate[1] and Pattarasuda Chayapakdee[2*]

[1]Student special project in B.Sc. (Biology) student code 640510318, Email: chanoknad_l@cmu.ac.th
[2*]Lecturer Dr., Department of Biology, Faculty of science, Chiang Mai University, Email: pattarasuda.c@cmu.ac.th
239 Huay Kaew Road, Mueang District, Chiang Mai, Thailand, 52000

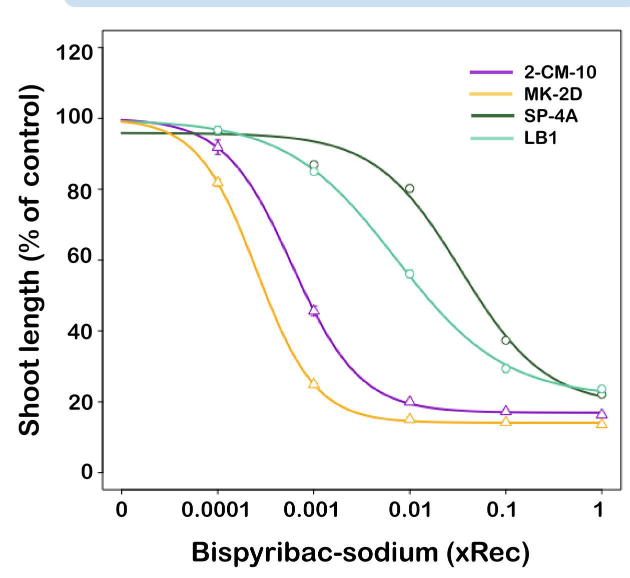
Abstract

This study investigates the mechanisms behind bispyribac-sodium (BS) resistance in barnyard grass populations in Thailand to support better weed management strategies. To evaluate herbicide resistance, a dose-response assay was conducted using both susceptible (2-CM-10 and MK-2D) and resistant (SP-4A and LB1) barnyard grass lines. Seedlings were cultured on MS medium supplemented with BS at six concentrations, applied according to recommended label rates, over a 9-day period. The results indicated that a BS concentration of 500 g a.i./ha effectively distinguished resistant from susceptible lines. Additionally, multiple herbicide sensitivity assays were performed by culturing seedlings on MS medium supplemented with seven herbicides with different modes of action. The SP-4A line exhibited resistance exclusively to ALS-inhibiting herbicides, whereas the LB1 line showed resistance to both ALS inhibitors and quinclorac. Furthermore, molecular analysis of ALS gene sequences was conducted to investigate potential genetic mutations associated with resistance. Genomic DNA was extracted, and three copies of the ALS gene were amplified for sequencing. The results of the ALS gene sequence analysis for all tested barnyard grass lines will be presented in subsequent sections. These findings enhance our understanding of herbicide resistance mechanisms in *E. crus-galli* and provide a foundation for developing targeted weed control strategies in rice production systems.

Introduction

- Barnyard grass is a major weed in rice paddies, reducing crop yields and developing resistance due to repeated herbicide use. [1]
- One of the most widespread resistances is to BS, an acetolactate synthase (ALS)-inhibiting herbicide that disrupts protein synthesis. [2]
- To address this issue, understanding the molecular mechanisms of herbicide resistance in weeds is essential.

BS dose response curves



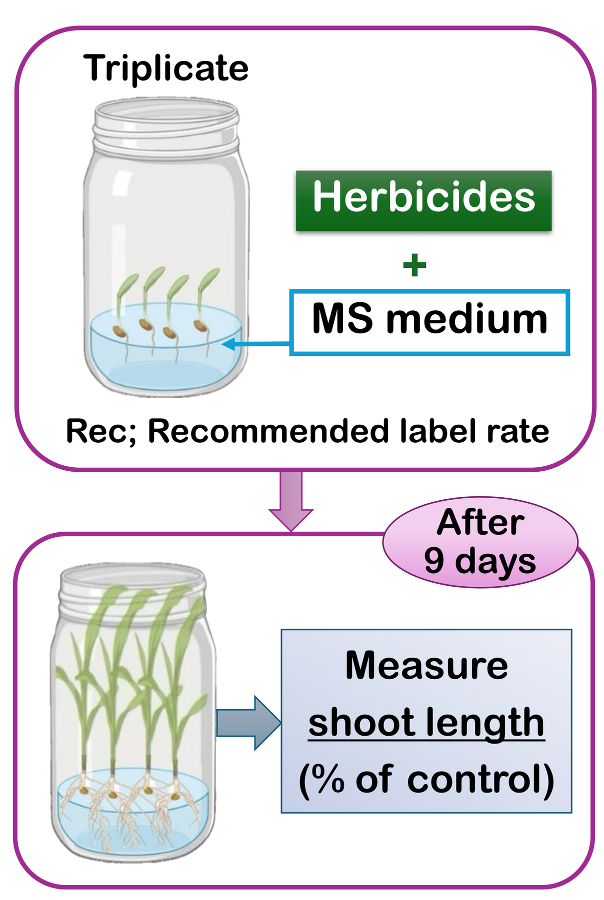
Mechanisms of herbicide resistance → Non-Target site resistant (NTSR)

Mechanisms of herbicide resistance → Target site resistant (TSR) → alteration, mutation of herbicide-target protein

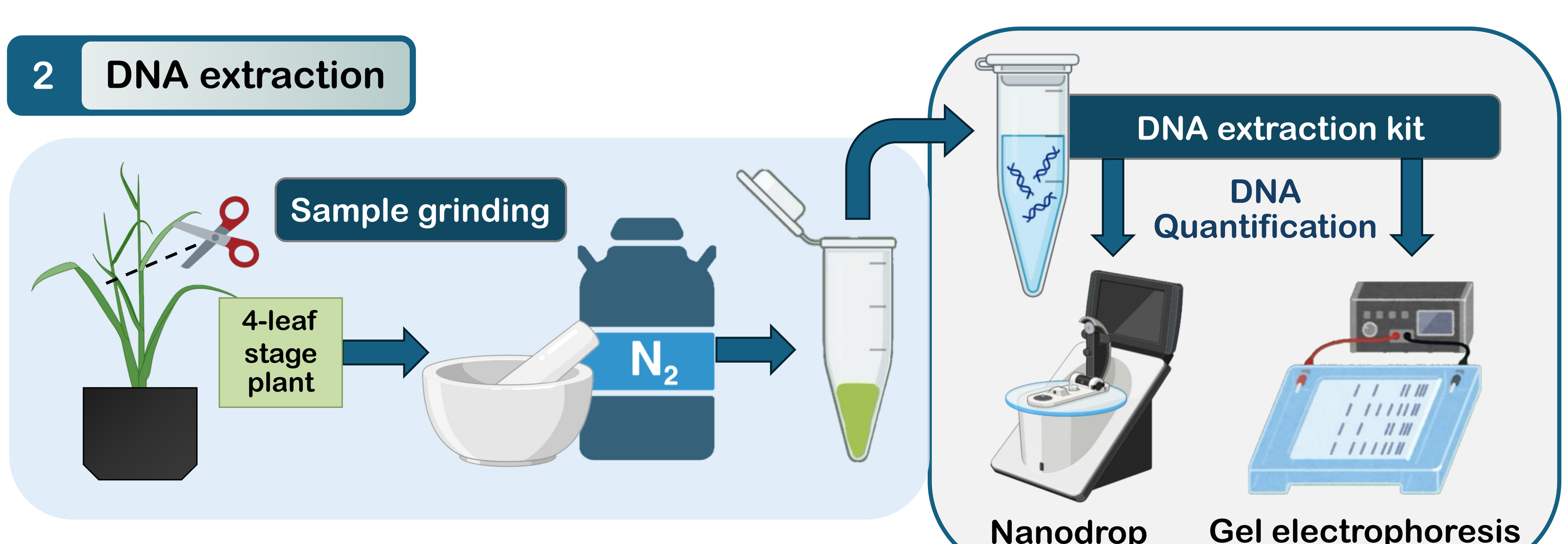
Methods

1 Multiple herbicide sensitivity assays

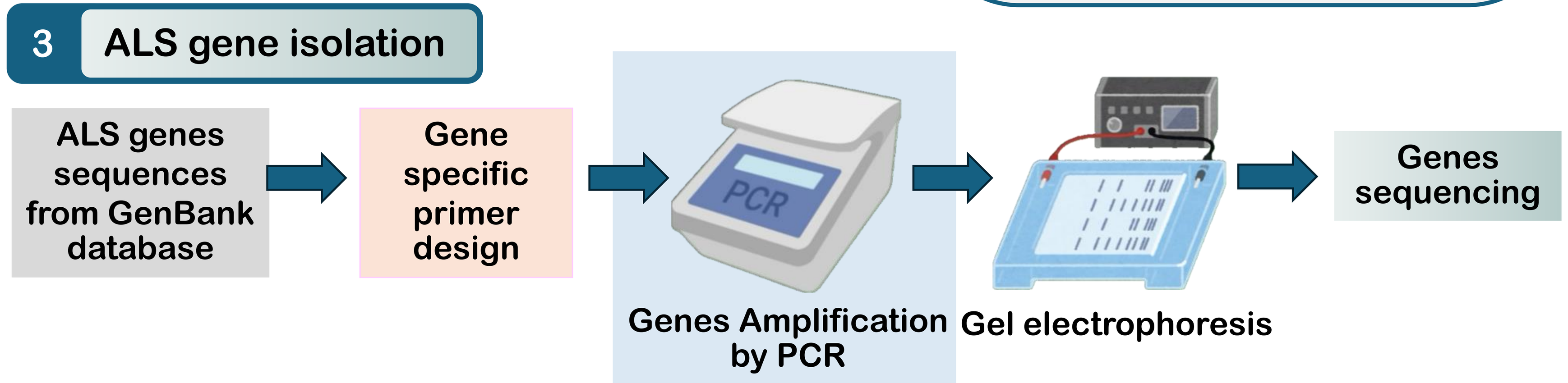
Herbicide	Mode of action	Rec (mg ai L^{-1})
Bispyribac-sodium (BS)	ALS inhibitor	37.5
Bensulfuron-methyl (BSM)	ALS inhibitor	62.5
Penoxsulam (PN)	ALS inhibitor	37.5
Clomazone (CZ)	DOXP-synthase inhibitor	600
Quinclorac (QC)	Synthetic auxin	750
Profoxydim (PD)	ACCase inhibitor	225
Propanil (PP)	PS-II inhibitor	4050



2 DNA extraction



3 ALS gene isolation



Primer	Forward primer	Reverse primer
EcALS1[3]	5'- CAATCCCCCATCCTCTCC -3'	5'- CAGAACAAGGGAGAACATCAGAC -3'
EcALS2[3]	5'- ATCCCCCTTCCTCCTTGC -3'	5'- ATAGACAGAACAAAGGAGAACATCG -3'
EcALS3[3]	5'- CCCCAATCCCCCATCCAT -3'	5'- GCACCGCTCGCTGAATAC -3'

Results and Discussion

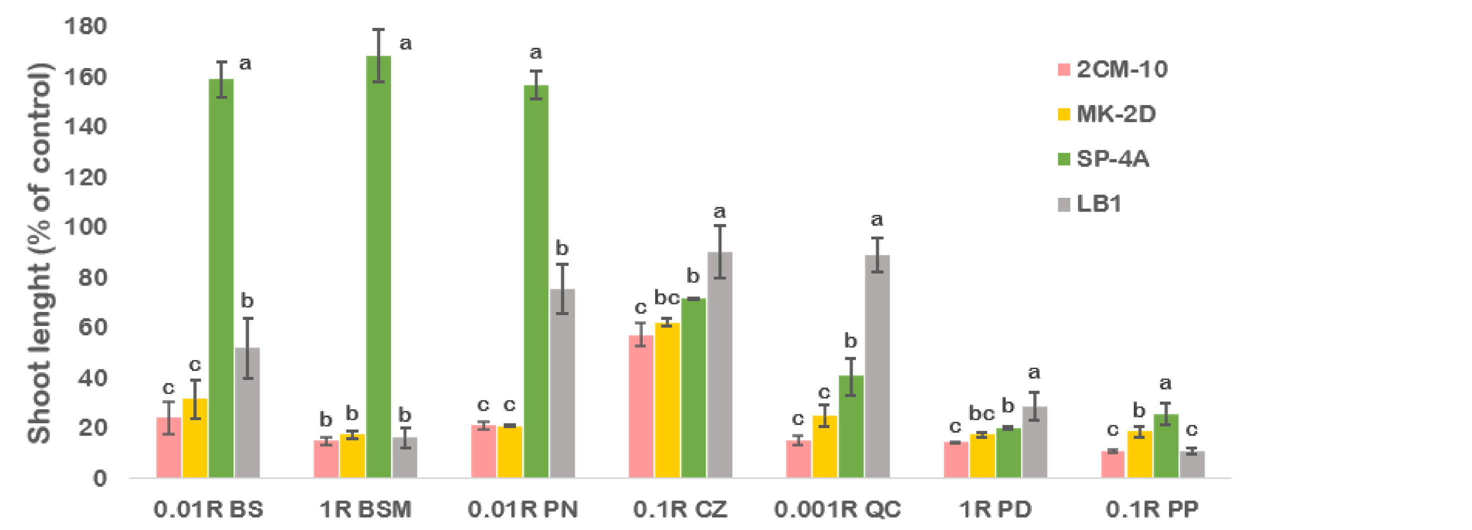
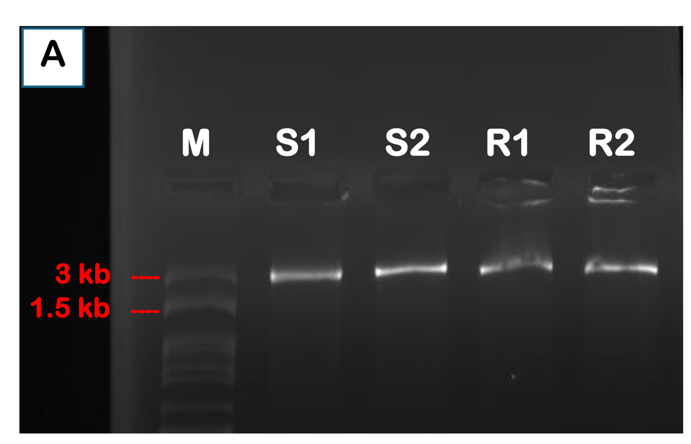


Fig 1. Shoot length (% of control) of *E. crus-galli* (2-CM-10, MK-2D, SP-4A and LB1) after treated with different types of herbicides for 9 days. SP-4A line from Suphan Buri exhibited resistant to all ALS inhibitors (PN, BS and BSM), whereas LB1 from Lopburi showed resistant to both ALS inhibitors (BS, PN) and auxin mimics (QC). Significant differences of means were tested by DMRT at $p<0.05$.



B

Lane	Line	DNA concentration (ng/μL)	$A_{260/280}$
S1	2-CM-10	49.3	1.74
S2	MK-2D	33.0	1.70
R1	SP-4A	38.2	1.77
R2	LB1	19.9	1.70

Fig 2. Genomic DNA extracted from leaves of *E. crus-galli*. (A) Agarose gel electrophoresis showed solid DNA band with low fragmentation. (B) DNA concentration and purity measured by Nanodrop spectrophotometer. The result suggested that genomic DNA isolated by this method have high quantity and quality enough for further experiments.

A

PCR Condition	T(°C)	Time	Cycle
Pre-denaturation	94	2 m	1
Denaturation	98	10 s	40
Annealing	59	30 s	40
Extension	68	1.2 m	40
Final Extension	68	3 m	1

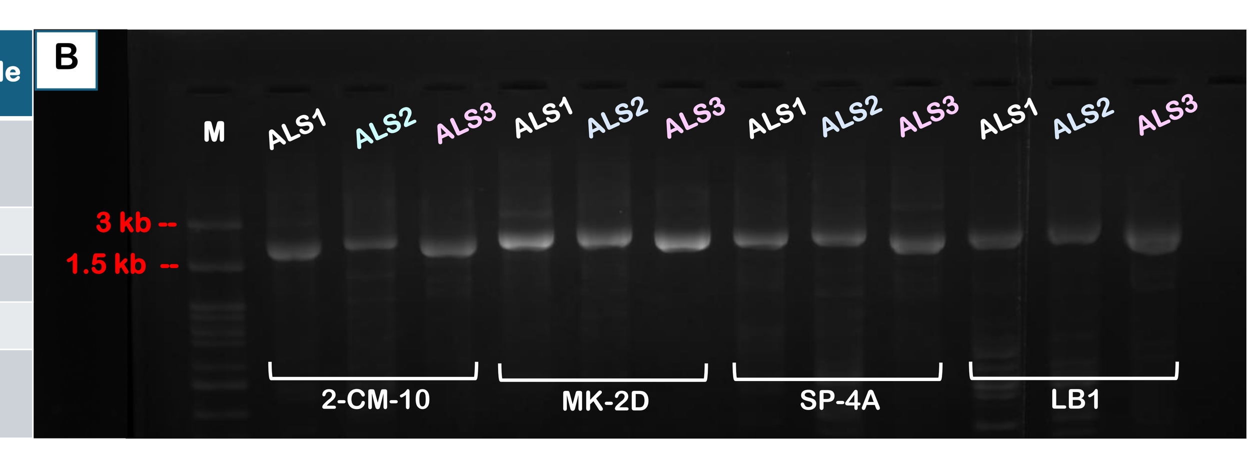


Fig 3. PCR products of ALS genes of *E. crus-galli.* (A) PCR condition. (B) Gel electrophoresis Lane 1: DNA marker; Lanes 2-13: PCR product amplified by EcALS1, EcALS2 and EcALS3 primer pairs with 2-CM-10, MK-2D, SP-4A and LB1 DNA templates, respectively. Expected length of PCR products of ALS1, 2 and 3 were 2107, 2104 and 2034 bp, respectively.

Conclusion

- SP-4A line exhibited resistance exclusively to ALS-inhibiting herbicides, indicating that it is likely a TSR mechanism.
- LB1 line showed resistance to herbicides with multiple modes of action, suggesting both NTSR and TSR mechanisms may involved.
- ALS genes sequence alignment is being analyzed to elucidate genetic mutations related to TSR mechanisms

Acknowledgement

This research was funded by Chiang Mai University. Plant samples were partially received from Dr. Jarunya Pinsuda, Plant Protection Research and Development Office.

References

[1] Damalas, C. A., & Koutroubas, S. D. (2023). Herbicide-resistant barnyardgrass (Echinochloa crus-galli) in global rice production. *Weed Biology and Management*, *23*(1), 23–33. https://doi.org/10.1111/wbm.12262
[2] Endo, M., Shimizu, T., Fujimori, T., Yanagisawa, S., Toki, S., (2013). Herbicide-resistant mutations in acetolactate synthasecan reduce feedback inhibition and lead to accumulation of branched-chain amino acids. Food and NutritionSciences, 4:522-528
[3] Iwakami, S., Hashimoto, M., Matsushima, K., Watanabe, H., Hamamura, K., & Uchino, A. (2015). Multiple-herbicide resistance in Echinochloa crus-galli var. formosensis, an allohexaploid weed species, in dry-seeded rice. Pesticide Biochemistry and Physiology, 119, 1–8. https://doi.org/10.1016/j.pestbp.2015.02.007