Molecular mechanisms underlying non-target-site resistance to auxin herbicides in barnyard grass

Wannarat Jainoi[1] and Pattarasuda Chayapakdee[2*]

[1]Student special project in B.Sc. (Biology) student code 650510284, Email:wannarat_jainoil@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

Barnyard grass (*Echinochloa crus-galli*), one of the most problematic weeds in rice production worldwide, has evolved resistance to quinclorac, an auxin herbicide widely used for its control. Non–target-site resistance (NTSR), particularly metabolic resistance mediated by cytochrome P450 monooxygenases, is increasingly recognized as a key mechanism reducing herbicide efficacy and contributing to multiple herbicide resistance. This study investigated quinclorac resistance in 30 barnyard grass populations collected from major rice-growing area of Thailand and examined the resistant mechanism at molecular level. Seedlings were screened for quinclorac sensitivity using root treatment by herbicide mixture in Murashige & Skoog (MS) medium under sterile conditions. The sensitive population (MK-2D) showed complete growth inhibition at 0.01x recommended label rate of quinclorac, whereas resistant populations survived and continued growing. To evaluate multiple resistance, twenty herbicides in different modes of action were tested in the resistant populations. The results showed that PB1 and AY2 populations exhibited multiple-herbicide resistance indicating NTSR mechanism might confer resistance. To determine the involvement of metabolic resistance, malathion, a known cytochrome P450 inhibitor, was applied prior to quinclorac treatment in selected populations. Malathion pre-treatment restored quinclorac susceptibility in line R511, the quinclorac resistant line expressing high P450 level. Transcriptome analysis is being performed to elucidate all up-regulated genes involving in NTSR mechanism in these populations. Overall, quinclorac resistance in Thai barnyard grass is associated with multiple-herbicide resistance and might involve P450–based metabolic mechanisms. This research provides important implications for sustainable herbicide resistance management in rice production systems.

INTRODUCTION

- Barnyardgrass (*Echinochloa crus-galli*) is a major weed in rice fields causing serious yield losses.
- Auxin herbicides such as quinclorac are widely used but resistance has evolved due to repeated use.
- Understanding molecular mechanisms of NTSR, such as P450-mediated metabolism, is important for resistance management.

Mechanisms of herbicide resistance → Non-Target-Site Resistance (NTSR) → Enhanced herbicide metabolism

Mechanisms of herbicide resistance → Target-Site Resistance (TSR) → • Alteration of target protein structure • Target gene duplication

METHODS

1. Plate assay

1.1 Screening of quinclorac resistant population



PROVINCE	POPULATIONS
CHIANG MAI	1. CM-2A 2. CM-16 3. CM-8 4. 2CM-10
SUPHAN BURI	1. SP-1 2. SP-1B 3. SP-4A 4. SP-5B 5. 2SP-1 6. 2SP-5
KAMPHAENG PHET	1. KP-3 2. KP-1A 3. KP-4A
SARABURI	1. SB1 2. SB2
NAKHON NAYOK	1. NN1

PROVINCE	POPULATIONS
CHAI NAT	1. CHN-1 2. CHN
AYUTTHAYA	1. AY1 2. AY2
NAKHON SAWAN	1. NS-1A
CHACHOENGSAO	1. CHS-2A 2. CHS-3A
PRACHINBURI	1. PB1 2. PB1-1
MAHA SARAKHAM	1. MK-2C 2. MK-2A 3. MK-1B 4. MK-2D
ROI ET	1. RB (B37)

1/2 MS liquid media + Herbicide



QC 0.01 Rec
Rec; Recommended label rate
n=3

9 day after treatment



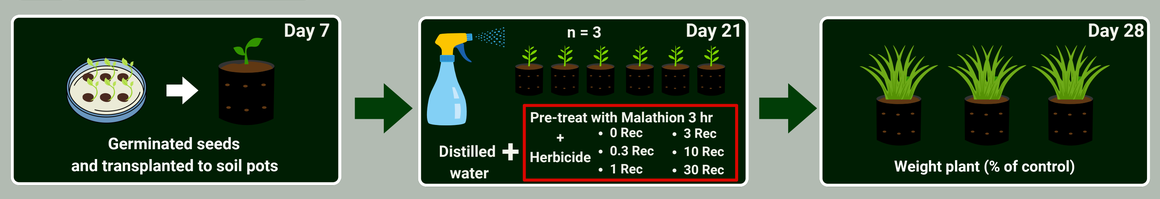
Measure
Plant height
(% of non-treated control)

1.2 Multiple herbicide resistance test

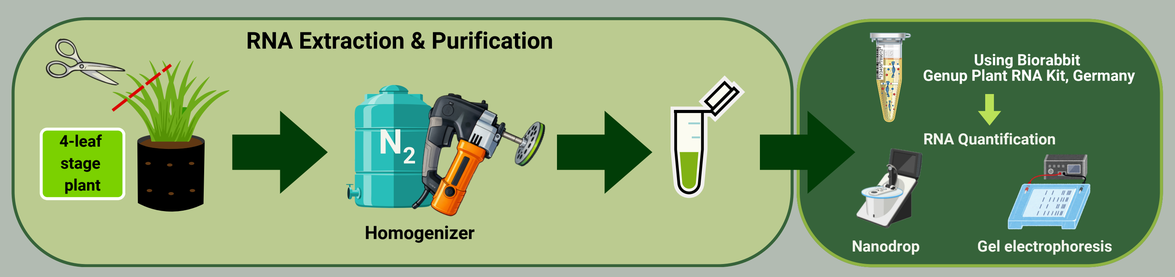
Herbicides	Mode of action	Recommended rate (g a.i./rai)	Application rate (x Rate)
Quinclorac (QC)	Auxin mimic	375	0.01
Florpyrauxifen-benzyl (FPX)	Auxin mimic	54	0.1
Bispyribac-sodium (BS)	ALS inhibitor	19	0.1
Bensulfuron-methyl (BM)	ALS inhibitor	18.75	1
Bispyribac-sodium(BS)	ALS inhibitor	15.625	1
Metsulfuron-methyl (MM)	ALS inhibitor	37.5	1
Pyrazosulfuron-ethyl (PE)	ALS inhibitor	43.75	1
Penoxsulam (PNX)	ALS inhibitor	18.75	1
Profoxydim (PFD)	ACCase inhibitor	112.5	0.01
Metamifop (MF)	ACCase inhibitor	100	0.1

Herbicides	Mode of action	Recommended rate (g a.i./rai)	Application rate (x Rec)
Feoxaprop-P-ethyi (FPE)	ACCase inhibitor	40.625	0.01
Quizalofop-P-ethyi (QPE)	ACCase inhibitor	75	0.01
Cyhalofop-butyl (CB)	ACCase inhibitor	100	0.01
Pyribenzoxim (PX)	ACCase inhibitor	31.25	1
Butachlor (BTC)	Inhibitor of very-long-chain fatty acid (VLCFA)	900	0.001
Anilofos (AF)	Inhibitor of very-long-chain fatty acid (VLCFA)	375	0.1
Pretilachlor (PC)	Inhibitor of very-long-chain fatty acid (VLCFA)	656.25	0.1
Clomazone (CZ)	Carotenoid biosynthesis inhibitor	300	0.001
Propanil (PPN)	Photosystem II inhibitor	2025	0.1
Oxadiazon (OX)	PPO inhibitor	500	0.01

2. Spray treatment



3. Sample preparation for transcriptome and gene expression analysis



cDNA synthesis (ReverTraAceRT, Toyobo, Japan) → qPCR (Thunderbird NextSYBR, Toyobo, Japan) → P450 gene expression → Transcriptome Analysis

qPCR primer
Internal control gene *EIF4B*
Target gene *CYP81A12/21*

Primer sequences of target genes
F1 5'-ATGGCGCCAAGGTGGACT-3'
R1 5'-TGAAGAACATCATGCAGAGCT-3

RESULTS AND DISCUSSION

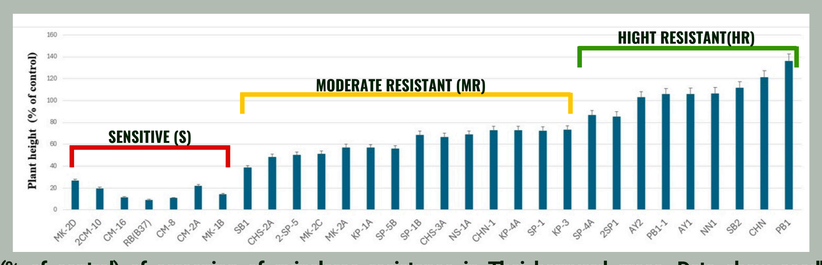


Fig 1. Plant height (% of control) of screening of quinclorac resistance in Thai barnyard grass. Data show seedlings after treated with quinclorac (0.01 rec) for 9 days. Based on plant height response, populations were classified into three groups: sensitive (<35% of control), moderately resistant (35–80%), and highly resistant (>80%).

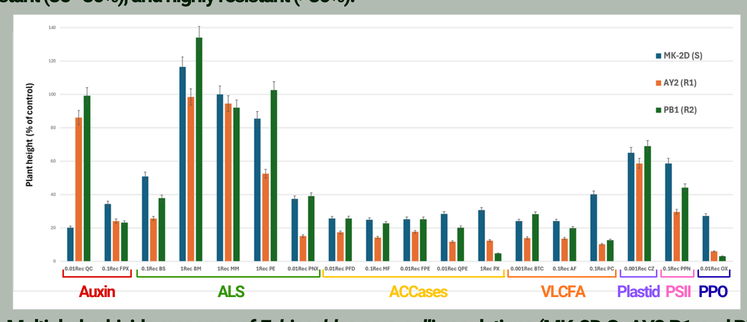
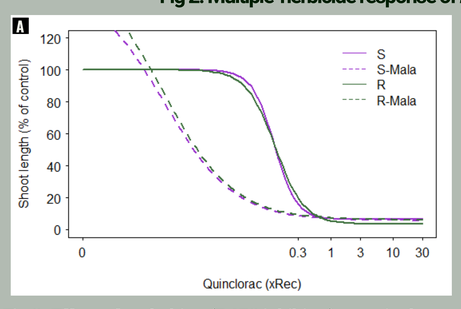


Fig 2. Multiple-herbicide response of *Echinochloa crus-galli* populations (MK-2D:S, AY2:R1, and PB1:R2).

A



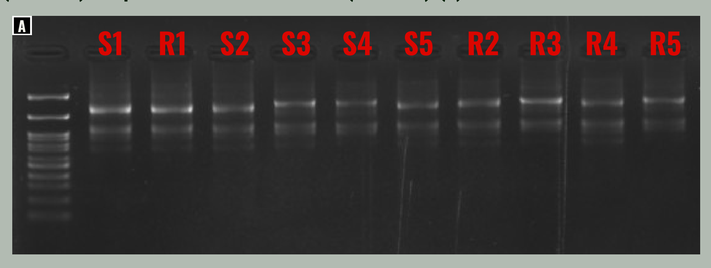
B

Lines	QC GR50 (xRec)	QC+M GR50 (xRec)	Resistance Ratio (GR50)
R	0.126655	0.002017	0.015928937
S	0.122134	0.002094	0.017148924

GR50 ; is the herbicide dose that causes 50% growth reduction.

Fig 3. Effect of malathion (P450 inhibitor) on quinclorac sensitivity of S and R lines. (A) Does-response curves comparing quinclorac spray only (solid line) and pre-treated with malathion (dash line) (B) GR50 and resistance ratio between with and without malathion treatment.

A



B

Line	Sample Name	Nucleic Acid (ng/uL)	A260/A280
S1	S401 4L 1P	215.762	2
R1	R511 4L 1P	222.436	1.922
S2	S401 2L 1P	148.405	1.885
S3	S401 2L 2P	150.514	1.896
S4	S401 1L 1P	80.342	1.744
S5	S401 1L 2P	99.798	1.798
R2	R511 2L 1P	103.676	1.78
R3	R511 2L 2P	204.233	1.927
R4	R511 1L 1P	67.688	1.677
R5	R511 1L 2P	109.765	1.853

Fig 4. Evaluation of RNA quality and quantity extracted from S and R lines. (A) Observation of RNA fragmentation using gel electrophoresis (B) Quantity of RNA extracted from each plant growth stage (1,2 and 4 leaf- stage) using Nano-drop spectrophotometer.

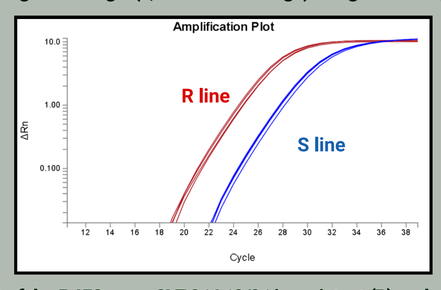


Fig 5. Quantitative (qPCR) amplification plot of the P450 gene *CYP81A12/21* in resistant (R) and sensitive (S) lines. The resistant line showed earlier amplification, suggesting higher expression of the target gene. *EIF4B* was used as the Internal control gene for normalization.

CONCLUSION

- Populations of *Echinochloa crus-galli* in Thailand exhibit resistance to quinclorac, and populations PB1 and AY2 show multiple herbicide resistance, suggesting a non-target-site resistance (NTSR) mechanism.
- Malathion treatment did not significantly restore quinclorac sensitivity in the sensitive line, suggesting that P450 may not be the main mechanism.
- RNA quality was suitable for transcriptome analysis, and qPCR confirmed expression of the target gene in the resistant line

ACKNOWLEDGEMENT

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

References

[1] Ghanizadeh, H., & Harrington, K. C. (2017). Non-target site mechanisms of resistance to herbicides. Critical Reviews in Plant Sciences, 36, 24–34. https://doi.org/10.1080/07352689.2017.1316134

[2] Grossmann, K. (2010). Auxin herbicides: Current status of mechanism and mode of action. Pest Management Science, 66(2), 113–120. https://doi.org/10.1002/ps.1860

[3] Iwakami, S., Endo, M., Saika, H., Okuno, J., Nakamura, N., Yokoyama, M., Watanabe, H., Toki, S., Uchino, A., & Inamura, T. (2014). Cytochrome P450 *CYP81A12* and *CYP81A21* are associated with resistance to two acetolactate synthase inhibitors in *Echinochloa phyllopogon*. Plant Physiology, 165, 618–629. https://doi.org/10.1104/pp.113.232843

[4] Malik, M. S., Burgos, N. R., & Talbert, R. E. (2010). Confirmation and control of propanil-resistant and quinclorac-resistant barnyardgrass (*Echinochloa crus-galli*) in rice. Weed Technology, 24, 226–233. https://doi.org/10.1614/WT-09-053.1