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

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Abstract

This study aimed to screen Bispyribac-sodium (BS) herbicide resistant populations of barnyardgrass collected in Thailand, investigate sequence variations in the Acetolactate synthase (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 MS liquid medium supplemented with BS at six concentrations according to recommended label rates, over a 9-day period. The result indicated that BS at 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 using 20 herbicides from different modes of action. The resistant line (SP-4A) exhibited resistance exclusively to ALS-inhibiting herbicides, suggesting target-site resistance as a major mechanism. However, ALS gene sequence analysis revealed none of amino acid substitutions in the three ALS genes of the resistant line. In addition, the potential involvement of cytochrome P450 metabolic resistance was observed. 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.

Introduction

- Barnyard grass (*Echinochloa crus-galli*) is a major weed in rice cultivation around the world [1]. Its resistance to herbicides causes a 21% - 79% decrease in rice yield worldwide [2].
- One of the most widespread resistances is to BS, an ALS inhibitor which inhibits amino acid biosynthesis.



Herbicide resistance machanism

- Target-site resistance (TSR)
  - alteration, mutation of herbicide-target protein
- Non-target-site resistance (NTSR)
  - blocking of herbicide access to the target site
  - ⚠️ e.g. herbicide metabolism

Methods

Seed collection



Table 1. Location of seed collection used in this study

Location	Population	Location	Population
Kamphaeng phet	KP-1A	Saraburi	SB-1
	KP-4A		SB-2
	KP-2	Chiang Mai	CM15
	KP-3		CM17
Suphan Buri	SP-1		2-CM-10
	SP-1A	Maha Sarakham	MK-2A
	SP-4A		MK-1B
	SP-5B		MK-2C
	2SP-1		MK-2D
	2SP-5	Chachoengsao	CH1
Nakhon Sawan	NS-1A		CHS-2A
Ayutthaya	AY-2		CHS-3A
Chai Nat	CHN-1	Prachinburi	PB1-1

1 Herbicide sensitivity assays > Liquid medium

- Screening of BS resistant populations — BS 6 concentrations

4 d-old seedlings → MS Liquid medium + BS herbicide



Rec = Recommended label rate



Measure shoot lenght 9 days after treatment (% of control)

- Multiple herbicides sensitivity assays — 20 types of herbicides

A Herbicide concentration selection of sensitive line — 6 concentrations

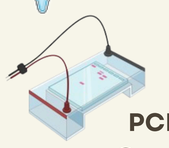
B Multiple herbicides test in resistance line — ⚠️ Resistance lines tested with herbicide concentration from A

2 Herbicide resistance machanism

- TSR evaluation

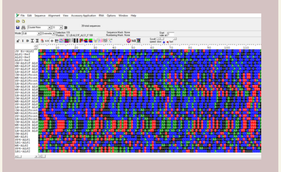


ALS genes amplification by PCR



PCR product Quantification and Qualification

Gene sequencing → Gene alignment



BioEdit program

- NTSR evaluation



1 Malathion (P450 inhibitor)

2 BS herbicide 6 concentrations

14 d-old plants



Measure fresh weight 14 days after treatment

Dose response curve: R line vs S line



Results and Discussion

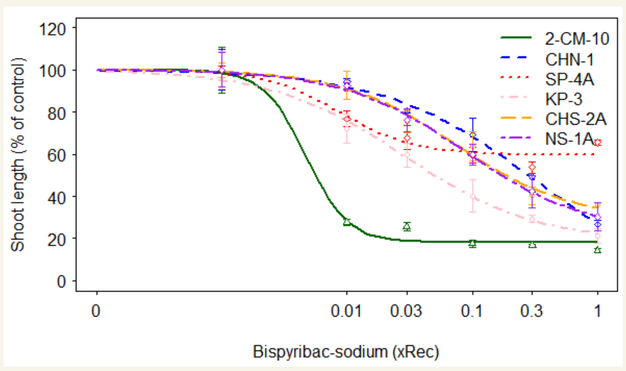


Fig 1. Dose-response curves of BS herbicides in six barnyard grass populations represented by shoot length (% of control). The solid line and dashed line represent S and R population, respectively BS rec rate is 37.5 g.a.i ha^{-1}

Table 2. Plant survival rate at 1 Rec of BS herbicide (n=12). *1 Classification of herbicide sensitivity; S for susceptible and R for resistance population.

Line	Plant survival (%)	Classification *1	Line	Plant survival (%)	Classification *1
SB-1	66.67	R	KP-1A	66.67	R
SB-2	0.00	S	KP-4A	100.00	R
CM-15	0.00	S	KP-2	0.00	S
CM-17	100.00	R	KP-3	66.67	R
2-CM-10	0.00	S	NS-1A	66.67	R
MK-2A	66.67	R	CHN-1	100.00	R
MK-1B	0.00	S	SP-1	100.00	R
MK-2C	100.00	R	SP-1A	66.67	R
MK-2D	0.00	S	SP-5B	100.00	R
CHS-2A	100.00	R	SP-4A	100.00	R
CHS-3A	100.00	R	2SP-1	0.00	S
AY-2	66.67	R	2SP-5	0.00	S
PB1	0.00	S	CH1	0.00	S

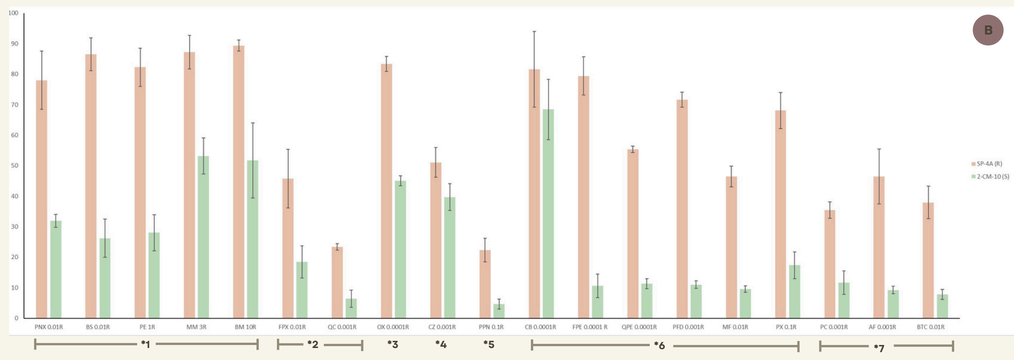


Fig 2. Multiple herbicides-resistance test of sensitive (S) and resistant (R) lines. (A) Mode of action of herbicide used in this study. (B) Response of each group of herbicide. Plant shoot length was measured at 9 days after treatment (n=6). SP-4A (R) line exhibited resistance to all ALS inhibitors Bensulfuron-methyl(BM), Bispyribac-sodium(BS), Metsulfuron-methyl(MM), Pyrazosulfuron-ethyl(PE), Penoxsulam(PNX) and other groups of herbicide.

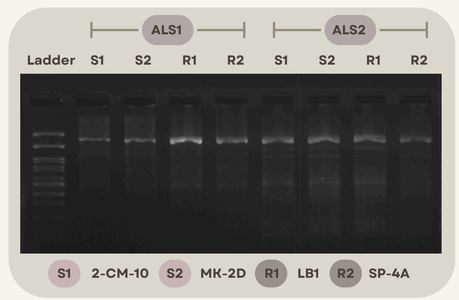


Fig 3. Isolation of ALS genes using specific primers.

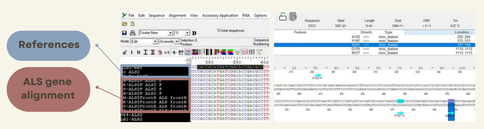


Table 3. Comparison of ALS genes sequences of S and R lines at important positions that usually mutated and cause TSR to ALS inhibitors. No amino acid substitution was observed in these populations

[3]	Line				
	WT	CM (S1)	MK (S2)	LB1 (R1)	SP4 (R2)
Gene ALS1					
A122	Ala	Ala	Ala	Ala	Ala
P197	Pro	Pro	Pro	Pro	Pro
A205	Ala	Ala	Ala	Ala	Ala
D376	Asp	Asp	Asp	Asp	Asp
R377	Arg	Arg	Arg	Arg	Arg
W574	Trp	Trp	Trp	Trp	Trp
S653	Ser	Ser	Ser	Ser	Ser
G653	Gly	Gly	Gly	Gly	Gly
Gene ALS2					
A122	Ala	Ala	Ala	Ala	Ala
P197	Pro	Pro	Pro	Pro	Pro
A205	Ala	Ala	Ala	Ala	Ala
D376	Asp	Asp	Asp	Asp	Asp
R377	Arg	Arg	Arg	Arg	Arg
W574	Trp	Trp	Trp	Trp	Trp
S653	Ser	Ser	Ser	Ser	Ser
G654	Gly	Gly	Gly	Gly	Gly
Gene ALS3					
A122	Ala	Ala	Ala	Ala	Ala
P197	Pro	Pro	Pro	Pro	Pro
A205	Ala	Ala	Ala	Ala	Ala
D376	Asp	Asp	Asp	Asp	Asp
R377	Arg	Arg	Arg	Arg	Arg
W574	Trp	Trp	Trp	Trp	Trp
S653	Ser	Ser	Ser	Ser	Ser
G654	Gly	Gly	Gly	Gly	Gly

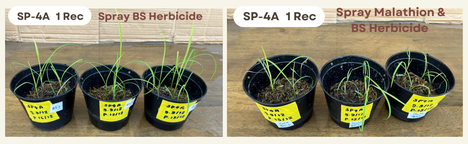


Fig 4. Effect of malathion (P450 inhibitor) on BS sensitivity of R line. Malathion pre-treatment reduced BS resistance in R line.

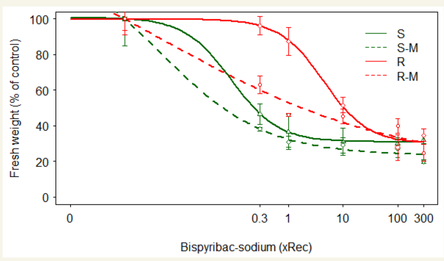


Fig 5. Dose-response curves of BS in S (2-CM-10) and R (SP-4A) line comparing pre-treat with malathion (dashed line) and spray herbicide only (solid line), Data shows mean ± SE of triplications

Conclusion

- There is no amino acid substitutions in three ALS genes of R lines.
- P450 inhibitor increased herbicide sensitivity in the resistant line suggesting that cytochrome P450 may involve in resistant mechanism.
- Further studies are required to examine the expression of cytochrome P450 and other related genes using RNA-sequencing analysis.

Acknowledgement

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References

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