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Cross- and multiple-herbicide resistance of penoxsulam-resistant barnyardgrass in Central Thailand paddy fields

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Abstract

Background and Objective: *Echinochloa crus-galli* (L.) P. Beauv. is the most troublesome weed in paddy fields. Farmers in Central Thailand have observed poor control with the labeled rate of penoxsulam, an acetolactate synthase (ALS) inhibitor. This study was conducted to confirm and quantify barnyardgrass resistance to penoxsulam in the region and to evaluate cross- and multiple-resistance patterns against commonly used herbicides in paddy fields.

Methodology: A split-plot design with four replications was used. The main plot consisted of six penoxsulam dose rates (0, 7.03, 14.06, 28.12, 56.24, and 112.48 g a.i./ha), and the sub-plot included resistant (R) and susceptible (S) biotypes. Both biotypes were assessed for I_{50} (visual injury) and GR_{50} (plant height and fresh weight). Cross-resistance to ALS inhibitors from three chemical families and multiple-resistance to other herbicide mechanisms of action were also evaluated.

Main Results: The R-biotype showed 53.78–64.52-fold higher resistance to penoxsulam than the S-biotype. Cross-resistance was detected to bispyribac-sodium, pyribenzoxim, and triafamone, while multiple-resistance occurred to metamifop (acetyl-CoA carboxylase (ACCase) inhibitors) and quinclorac (synthetic auxin). No resistance was detected to profoxydim, propanil, or florpyrauxifen-benzyl.

Conclusions: This study provides the first confirmed and quantified case of penoxsulam-resistant barnyardgrass in Central Thailand paddy fields, revealing high-level resistance and a clear profile of cross- and multiple-herbicide resistance. These findings emphasize the need for integrated weed management strategies, including herbicide rotation across different modes of action and the incorporation of non-chemical control measures, to slow the spread and minimize the impact of ALS-resistant *E. crus-galli* populations in paddy fields.

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INTRODUCTION

Herbicide resistance has become a significant global challenge for sustainable crop production, largely due to the limited number of herbicides with alternative sites of action (Takano *et al.*, 2021). According to the international herbicide resistance survey, there are currently 534 confirmed cases of herbicide-resistant weed infestations, involving 273 species (156 dicots and 117 monocots) across 102 crops in 75 countries. Weeds have evolved resistance to 21 of the 31 known herbicide sites of action and to 168 different herbicides (Heap, 2025). Among monocotyledonous weeds, resistance to acetyl-CoA carboxylase (ACCase) inhibitors and acetolactate synthase (ALS) inhibitors has been widely documented in rice production systems worldwide.

In Thailand, several herbicide-resistant weed species have been reported in paddy fields. *Leptochloa chinensis* (L.) Nees populations with resistance to ACCase inhibitors were detected in Saphan Sung district, Bangkok (Maneechote *et al.*, 2005; Pornprom *et al.*, 2006), while populations of *Fimbristylis miliacea* (L.) Vahl were found resistant to ALS inhibitors in Bang Pla Ma district, Suphan Buri province, and Sankhaburi district, Chainat province (Phinyosak and Pornprom, 2017). Among the most troublesome rice weeds are *Echinochloa* species, particularly *Echinochloa crus-galli* (L.) P. Beauv., and weedy rice, followed by sedges (Cyperaceae) (Chen *et al.*, 2016; Kraehmer *et al.*, 2016; Zhang *et al.*, 2021). *E. crus-galli* reproduces solely by seed, exhibits rapid early growth, and is highly competitive (Bastiani *et al.*, 2015), with yield losses in rice potentially reaching 80% under season-long competition (Wilson *et al.*, 2014).

Chemical weed control remains a key strategy for managing *E. crus-galli*, and ALS inhibitors such as bispyribac-sodium, penoxsulam, pyribenzoxim, and triafamone are widely used in paddy fields of the central region of Thailand (Vasilakoglou *et al.*, 2018; Damalas and Koutroubas, 2023). However, continuous and repeated use of ALS inhibitors has led to reduced control efficacy and the emergence of resistant populations. Sripeangchan *et al.* (2019) reported decreased performance of bispyribac-sodium and penoxsulam at recommended doses against *E. crus-galli* in Ayutthaya and Chainat provinces, suggesting the spread of resistance in Central Thailand.

Penoxsulam, a triazolopyrimidine ALS inhibitor, was previously highly effective in controlling barnyardgrass, but farmers in Central Thailand have increasingly reported unsatisfactory control despite using labeled rates. The central region is a major rice-growing area characterized by intensive, continuous rice cultivation and heavy reliance on herbicides, creating strong selection pressure for resistance evolution. While penoxsulam resistance has been documented in other countries (Chen *et al.*, 2016; Choudhary *et al.*, 2023), its current extent, resistance levels, and cross-resistance patterns in *E. crus-galli* populations of Central Thailand remain insufficiently documented.

Therefore, this study aimed to (1) confirm suspected field resistance of *E. crus-galli* to penoxsulam in Central Thailand, (2) determine cross-resistance to ALS inhibitors from three chemical families, and (3) assess multiple-herbicide resistance to other mechanisms of action commonly used in paddy fields. Findings from this work will provide essential information for designing effective herbicide rotation strategies and slowing the spread of resistant barnyardgrass populations in the region.

MATERIALS AND METHODS

Seed Collection

Based on a survey from the paddy field in 2021, penoxsulam resistance in barnyardgrass has been studied using information collected from 9 sites: 3 sites from Ban Sa sub-district, 4 sites from Samchuk sub-district, and 2 sites from Yan Yao sub-district, at Samchuk district, Suphanburi province, Thailand. The resistant barnyardgrass (R-biotype) was found in seven rice fields, whereas the susceptible barnyardgrass (S-biotype) was found in only two rice fields. The S-biotype was collected from a rice field area where ALS inhibitors can be used to control it. Mature seed samples of barnyardgrass were collected from intensive rice cultivation in Sam Chuk district, Suphan Buri province, in Central Thailand. Seeds were picked from barnyardgrass plants suspected to be herbicide-resistant because of their survival in rice fields after herbicide application. The mature seeds of barnyardgrass, susceptible and resistant biotypes, were planted in 8-inch diameter pots. Prepared soil and vermiculin planting material were added to the pot to maintain soil humidity. Plants were grown at 25–38/21–27 °C day/night, 12 hours photoperiod, and 50–70% of relative humidity. The experiment was conducted at the field laboratory, Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand, from May 2022 to February 2023.

Growth Response Experiment

The experiment was arranged in a split-plot design in CRD with four replications. The main plot was penoxsulam at different rates (0, 7.03, 14.06 (recommended dose), 28.12, 56.24, and 112.48 g a.i./ha). The sub-plot was S- and R-biotypes. Herbicides were applied at 12 days after sowing (DAS), when weed seedlings reached the 2–4 leaf stage. Herbicides were applied using a backpack sprayer equipped with a flat-fan nozzle. It was set at 7 bars pressure, which had a capacity of 15 liters by mixing in a solution totaling 250 liters of water per hectare.

Data Collection

Visual injury of percentage control was taken at 14 and 21 days after application (DAA) on a scale of 0 (no reduction or injury), 50 (moderate injury), and 100 (complete destruction) (Burrill *et al.*, 1976). The plant height (cm) from the soil surface to the top of the leaves and the fresh weight (g) of the whole plant of S- and R-biotypes were measured. On average, there were 10 plants per replication, at 21 and 30 DAA. Growth response of barnyardgrass to penoxsulam was assessed by determining the dose of herbicide required to kill 50% of plants (I_{50}) and 50% plant growth reduction (GR_{50}), and compared to that of the untreated control for each biotype. The determination of the resistance index was compared as the I_{50} (or GR_{50}) value of the resistant biotype versus the I_{50} (or GR_{50}) value of the susceptible biotype (Valverde *et al.*, 2000).

Statistical Analysis

The variances of the data were analyzed by analysis of variance (ANOVA) with the R program version 4.0.2. (R Development Core Team, 2014). Least significant difference (LSD) was used to evaluate the significance of the intervention at a probability ($P < 0.05$) to consider the I_{50} value (the amount of herbicide that kills 50% of the population) and GR_{50} (the amount of herbicide that causes a 50% reduction in plant by 50%). Analyses of the I_{50} and GR_{50} values of treated herbicide compared to untreated control were performed using the formula as described by Collett (2002).

$$P = \Phi(ß_0 + ß_1 X)$$

where P is the effective dose at 50%, Φ is the cumulative distribution, $ß_0$ is the constant value, $ß_1$ is the coefficient of independent variables, and X is the herbicide dosage.

Analyses of the dose-response curves were performed using a linear equation in the Priprobit program version 1.63 (Sakuma, 1998) with a log-logistic model (Finney, 1971).

$$\text{Probit}(P) = a + b \log(Dose)$$

where P is 5 (constant value), a is the constant value or line intersection point, and b is the slope of the lines.

The S- and R-biotypes population was determined by calculating the resistance index according to Valverde *et al.* (2000) from the equation as follows:

Resistance index = I_{50} value of resistant biotype/ I_{50} value of susceptible biotype or

= GR_{50} value of resistant biotype/ GR_{50} value of susceptible biotype

Cross-Resistance

Cross-resistance of the R-biotype to ALS inhibitors from three different chemical families was evaluated. The experiment was designed in a completely randomized design (CRD) with four replications. Herbicides were applied at 12 DAS (2–4 leaf stage of barnyardgrass). All herbicide treatments were at the recommended field rate as shown in Table 1. Visual assessments of percentage control were taken 7 and 14 DAA on a scale of 0 (no reduction or injury), 50 (moderate injury), and 100 (complete destruction) (Burrill *et al.*, 1976). The plant height (cm) and fresh weight (g) of the R-biotype were recorded as mentioned earlier. On average, there were 10 plants per replication at 21 and 30 DAA. The data was gathered for analysis of statistical variance according to the CRD experiment. Mean differences were compared using Fisher's protected LSD test at P < 0.05 by using the R-Version 4.0.2 program (R Development Core Team, 2014).

Multiple-Resistance

Multiple resistance to other herbicide mechanisms of action were evaluated to alternate the herbicide currently labelled in rice. Seeds of R-biotype were placed in pots prepared in greenhouse conditions. The experiment was designed in CRD with four replications and applied herbicides at 12 DAS (2–4 leaf stage of barnyardgrass). The labelled rate of herbicide application was determined with R-biotype (Table 2). The experiments and data collection were conducted simultaneously, using the same methodology, as cross-resistance was

Table 1 Cross-resistance herbicide treatments used during the experiment

Herbicide	a.i. (%)	Dose (g a.i./ha)	Chemical family (group)	Application timing (DAS)
Control	-	-	-	-
Bispyribac-sodium	10% SC	25.00	Triazolopyrimidine	15
Penoxsulam	2.5% OD	14.06	Pyrimidinylthio-benzoate	7–12
Pyribenzoxim	5% EC	31.25	Pyrimidinylthio-benzoate	9
Triafamone	20% SC	62.50	Sulfonanilide	15

Note: a.i. (%) = percent active ingredient, g a.i./ha = grams active ingredient per hectare, DAS = days after sowing, SC = suspension concentrate, OD = oil dispersion, and EC = emulsifiable concentrate.

investigated. The data was gathered for analysis of statistical variance according to the CRD experiment. Mean differences were compared using Fisher's protected LSD test at $P < 0.05$ by using the R-Version 4.0.2 program (R Development Core Team, 2014).

Table 2 Multiple-resistance herbicide treatments used during the experiment

Herbicide	Mechanisms of action	a.i. (%)	Dose (g a.i./ha)	Application timing (DAS)
Control	-	-	-	-
Penoxsulam	ALS inhibitors	2.5% OD	14.06	7–12
Metamifop	ACCase inhibitors	10% EC	100.00	10
Profoxydim	ACCase inhibitors	7.5% EC	121.88	15
Propanil	PS II inhibitors	36% EC	2,250.00	15
Quinclorac	Synthetic auxins	25% SC	750.00	10
Florpyrauxifen-benzyl	Synthetic auxins	2.5% EC	25.00	10–14

Note: a.i. (%) = percent active ingredient, g a.i./ha = grams active ingredient per hectare, DAS = days after sowing, SC = suspension concentrate, OD = oil dispersion, and EC = emulsifiable concentrate.

RESULTS AND DISCUSSION

Growth Response

Physiological responses of both biotypes were evaluated with the toxicity scale at 14 and 21 DAA, and plant height and fresh weight were evaluated at 21 and 30 DAA. It was found that both biotypes and substance rate were significantly different at $P < 0.01$. The biotype and herbicide dosage interacted with each other. As a result, both S- and R-biotypes showed higher toxicity symptoms in parallel with higher rates of substance use. Based on the toxicity levels of both biotypes at 14 and 21 DAA compared to control treatment, the recommended rate of the herbicide was 14.06 g a.i./ha. The S-biotype had a toxicity level at 100%, while the R-biotype had a toxicity level at 10% and 27.50%, respectively (Figures 1A–1B). Then, the toxicity data of both biotypes were evaluated for the I_{50} value to further consider the resistance. The physiological response to plant height of both biotypes at 21 and 30 DAA compared to the control treatment was observed. A recommended rate of 14.06 g a.i./ha was applied. It could control the S-biotype with 100% efficiency (dead weed). However, the R-biotype was controlled at rates of up to 60.90% and 69.80%, respectively (Figures 2A–2B). Similar to Malik *et al.* (2014), when considering the physiological response in terms of fresh weight of both biotypes at 21 and 30 days after application, it was observed that the R-biotype had fresh weights of 50% and 57.80%, respectively. A recommended rate of 14.06 g a.i./ha was applied. This finding is consistent with a study on *Echinochloa crus-galli* resistance to bispyribac-sodium in India (Choudhary *et al.*, 2023), where no fresh weight of the S-biotype was observed (Figures 3A–3B). Then, the data on height and fresh weight of both biotypes were used for the analysis of the GR_{50} value, which was further used to determine the resistance index.

Based on the resistance index analysis of herbicide toxicity symptoms at 14 DAA, the S- and R-biotypes had the I_{50} value of 4.05 and 236.01, respectively, which accounted for a resistance index of 58.27 times. At 21 DAA, the S- and R-biotypes had the I_{50} value of 4.82 and 294.54, respectively, which accounted for a resistance index of 61.11 times. When the resistance index was analyzed for the physiological response in plant height at 21 DAA, the S- and R-biotypes had GR_{50} values of 7.82 and 420.53, respectively, which accounted for a resistance index that was 53.78 times greater. At 30 DAA, the S- and R-biotypes had GR_{50} values of 7.97 and 451.31, respectively, which corresponded to a resistance index of 56.62 times. When the resistance index was analyzed for the physiological response in terms of fresh weight at 21 DAA, the S- and R-biotypes had GR_{50} values of 6.94 and 420.53, respectively, which accounted for a resistance index that was 60.60 times greater. At 30 DAA, the S- and R-biotypes had GR_{50} values of 7.73 and 498.72, respectively, which resulted in a resistance index that was 64.52 times higher (Table 3). When considering the physiological response of barnyardgrass to penoxsulam, the R-biotype was up to 57.25 times higher than the S-biotype. With the frequent and intense use of penoxsulam in paddy fields of the Central region of Thailand, the continuous rice cultivation year after year, and a limited number of available herbicides, it is not surprising that barnyardgrass has developed resistance to penoxsulam. This study revealed that the suspected R-biotype had a high level of resistance to penoxsulam. Therefore, the R-biotype would be considered for cross-resistance to ALS inhibitors and multiple-resistance herbicides, which have different mechanisms of action, to manage this weed using cultural and chemical tools in paddy fields.

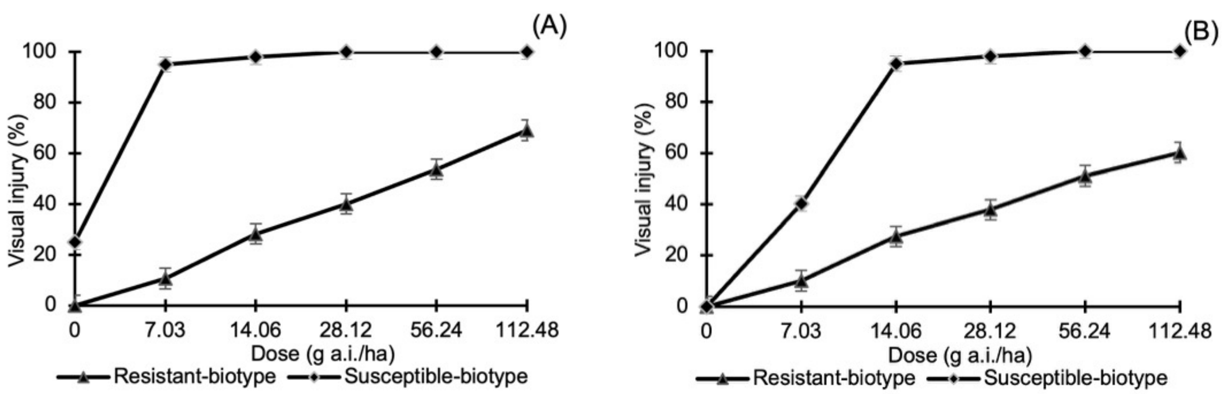

Figure 1 Penoxsulam dose-response assays on susceptible- and resistant-biotypes at 14 (A) and 21 days after application (B). Vertical bars represent mean ± standard error (n = 10).

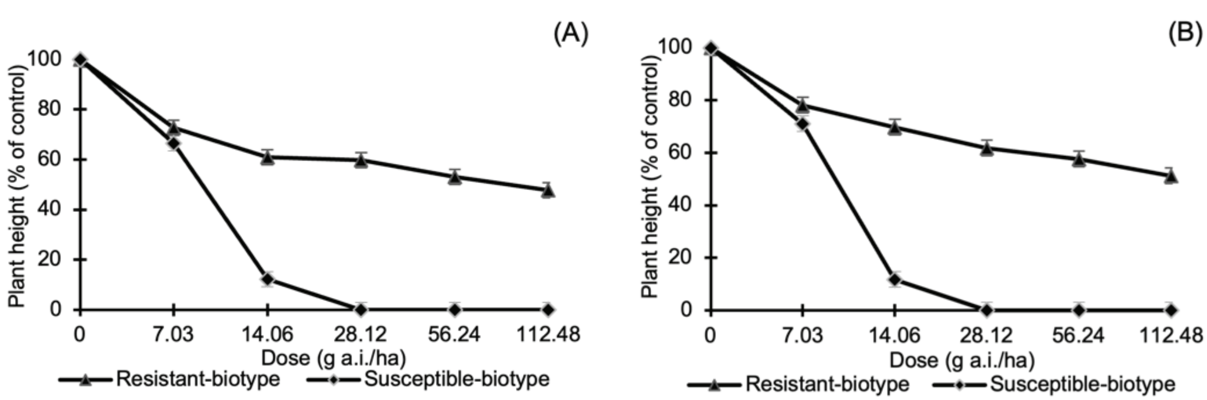

Figure 2 Effect of penoxsulam on plant height of susceptible- and resistant-biotypes at 21 (A) and 30 days after application (B). Vertical bars represent mean ± standard error (n = 10).

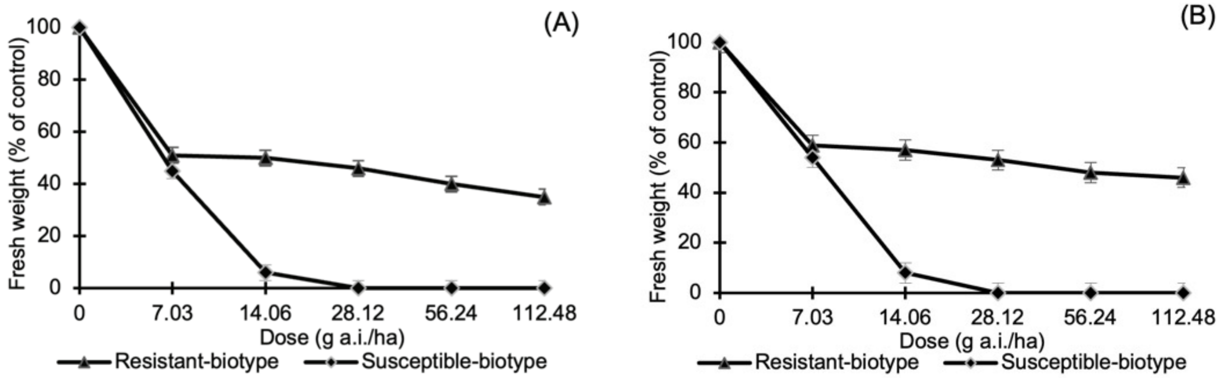

Figure 3 Effect of penoxsulam on fresh weight of susceptible- and resistant-biotypes at 21 (A) and 30 days after application (B). Vertical bars represent mean ± standard error (n = 10).

Table 3 Resistance levels of penoxsulam in barnyardgrass at Sam Chuk district, Suphan Buri

Barnyardgrass	Visual injury (I_{50})		Plant height (GR_{50})		Fresh weight (GR_{50})	
biotypes	14 DAA	21 DAA	21 DAA	30 DAA	21 DAA	30 DAA
S-biotype	4.05	4.82	7.82	7.97	6.94	7.73
R-biotype	236.01	294.54	420.53	451.31	420.53	498.72
Resistance index	58.27	61.11	53.78	56.62	60.60	64.52

Note: I_{50} = herbicide rate required to cause 50% injury, GR_{50} = herbicide rate to reduce plant growth by 50% relative to untreated control, DAA = days after application.

Cross-Resistance

Cross-resistance of the R-biotype to ALS-inhibiting herbicides from three different chemical families: triazolopyrimidine (penoxsulam), pyrimidinylthio-benzoate (bispyribac-sodium and pyribenzoxim), and sulfonanilide (triafamone) was evaluated. It was found that cross-resistance of R-biotype to ALS-inhibiting herbicides from three different chemical families was not different at $P < 0.05$ (Table 4). Based on the level of toxicity at 7 and 14 DAA, the R-biotype exhibited slight stunting, accompanied by a slight reduction in growth, when treated with bispyribac-sodium, pyribenzoxim, and triafamone at the labeled rate. The percentage of height and fresh weight at 21 and 30 DAA showed that plant height and fresh weight of the R-biotype differed at $P < 0.05$. It was revealed that after application of ALS inhibitors from three different chemical families, the growth of the resistant biotype was halted compared to the control treatment. The results indicated that the R-biotype showed cross–resistance to ALS-inhibiting herbicides across three distinct chemical groups: triazolopyrimidine, pyrimidinylthio-benzoate, and sulfonanilide. Thus, the use of herbicides that inhibit the activity of the ALS enzyme in this list (Table 1) failed to control the R-biotype.

Long-term use of ALS inhibitors might cause barnyardgrass to develop cross-resistance. It had been reported that barnyardgrass in California rice production that was resistant to thiobencarb, benzobicyclon, halosulfuron, and penoxsulam

Table 4 Cross-resistance of penoxsulam resistant barnyardgrass to ALS-inhibiting herbicides from three different chemical families

Herbicide	Dose (g a.i./ha)	Visual injury (%)		Plant height (cm)		Fresh weight (g)	
		7 DAA	14 DAA	21 DAA	30 DAA	21 DAA	30 DAA
Control	0.00	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	75.18 ± 0.54^{a}	82.95 ± 0.59^{a}	55.30 ± 0.36^{a}	66.85 ± 0.53^{a}
Penoxsulam	14.06	0.00 ± 0.00^{b}	20.00 ± 0.00^{b}	60.60 ± 0.36^{bc}	67.85 ± 0.39^{bc}	51.35 ± 0.4^{bc}	57.93 ± 0.57^{d}
Bispyribac-sodium	25.00	15.00 ± 5.77^{a}	37.50 ± 5.00^{a}	59.73 ± 0.44^{c}	67.00 ± 0.63^{c}	50.18 ± 0.38^{d}	57.40 ± 0.69^{d}
Pyribenzoxim	31.25	12.50 ± 5.00^{a}	32.50 ± 5.00^{a}	56.93 ± 0.34^{d}	64.08 ± 0.41^{d}	51.10 ± 0.47^{c}	58.85 ± 0.59^{c}
Triafamone	62.50	0.00 ± 0.00^{b}	22.50 ± 5.00^{b}	61.10 ± 0.61^{b}	68.88 ± 0.44^{b}	51.88 ± 0.38^{b}	60.33 ± 0.41^{b}
$LSD_{(0.05)}$		5.1479	5.8372	0.7050	0.7560	0.6009	0.8539
P-value		0.0010	0.0010	0.0010	0.0010	0.0010	0.0010

Note: Data are means ± standard deviation. Mean within the same column with the same letter are not significantly different between treatments of each factor based on LSD at a 5% level of significance. a.i. = active ingredient, DAA = days after application. Visual assessments on a scale of 0 (no reduction or injury), 50 (moderate injury), and 100 (complete destruction).

Table 5 Multiple-resistance of penoxsulam resistant barnyardgrass to other mechanisms of action

Herbicide	Dose (g a.i./ha)	Visual injury (%)		Plant height (cm)		Fresh weight (g)	
		7 DAA	14 DAA	21 DAA	30 DAA	21 DAA	30 DAA
Control	0.00	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	75.18 ± 0.54^{a}	82.95 ± 0.59^{a}	55.30 ± 0.36^{b}	66.85 ± 0.53^{a}
Penoxsulam	14.06	0.00 ± 0.00^{e}	25.00 ± 5.77^{c}	60.60 ± 0.36^{c}	67.85 ± 0.39^{d}	51.35 ± 0.4^{d}	57.93 ± 0.57^{d}
Metamifop	100.00	15.00 ± 5.77^{d}	37.50 ± 5.00^{b}	60.30 ± 0.36^{c}	68.85 ± 0.59^{c}	53.25 ± 0.59^{c}	61.95 ± 0.59^{c}
Profoxydim	121.88	35.00 ± 5.77^{c}	95.00 ± 5.77^{a}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
Propanil	2,250.00	55.00 ± 5.77^{b}	100.00 ± 0.00^{a}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
Quinclorac	750.00	17.50 ± 5.00^{d}	42.50 ± 5.00^{b}	62.88 ± 0.57^{b}	71.15 ± 0.72^{b}	56.45 ± 0.59^{a}	64.23 ± 0.69^{b}
Florpyrauxifen-benzyl	25.00	75.00 ± 5.77^{a}	100.00 ± 0.00^{a}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
$LSD_{(0.05)}$		6.9936	6.0033	0.5189	0.6497	0.5530	0.6665
P-value		0.0010	0.0010	0.0010	0.0010	0.0010	0.0010

Note: Data are means ± standard deviation. Means within the same column with the same letter are not significantly different between treatments of each factor based on LSD at a 5% level of significance. a.i. = active ingredient, DAA = days after application. Visual assessments on a scale of 0 (no reduction or injury), 50 (moderate injury), and 100 (complete destruction).

developed cross-resistance to ALS inhibitors (Vulchi *et al.*, 2024). In recent years, Thai farmers have become heavily dependent on ALS inhibitors for weed control in rice, which may lead to the development of herbicide-resistant weed populations. However, the incidence of barnyardgrass resistance to ALS inhibitors continues to increase in rice fields. Better control of resistant populations with ALS inhibitors might be the result of using a new herbicide with a different mode of action than ALS inhibitors. Therefore, appropriate prevention and control measures should be taken to avoid the increasing occurrence of uncontrollable barnyardgrass resistance to herbicides. Furthermore, multiple-resistance in a population of the R-biotype in ALS inhibitors would be studied, which might result in multiple-herbicide resistance to the ALS inhibitors and/or other groups with different inhibitory reaction sites in plants.

Multiple-Resistance

Multiple-herbicide resistance of the R-biotype to other mechanisms of action, including metamifop and profoxydim (ACCase inhibitors), propanil (photosystem II inhibitors), quinclorac, and florpyrauxifen-benzyl (synthetic auxins) were evaluated. Multiple-resistance of R-biotype to other mechanisms of action was significantly different at $P < 0.05$ (Table 5). Based on the level of toxicity at 7 and 14 DAA, the R-biotype showed moderate toxicity with a slight reduction in growth to metamifop and quinclorac at the labeled rate. However, after application of profoxydim, propanil, and florpyrauxifen-benzyl at the labeled rate, the R-biotype growth was halted compared to the control treatment. Herbicide response evaluation in the plant height and fresh weight of the resistant biotype at 21 and 30 DAA followed a trend similar to the level of toxicity. Therefore, the use of metamifop and quinclorac in controlling the R-biotype might impair its control effectiveness. The R-biotype showed signs of toxicity, where they developed dried yellow leaves and eventually died after application to profoxydim, propanil, and florpyrauxifen-benzyl. Farmers should alternate the use of herbicides with different mechanisms of action, as mentioned above, to effectively inhibit the proliferation of the R-biotype.

The mechanisms of barnyardgrass resistance to herbicides used in rice. The resistance cases concerning *Echinochloa crus-galli* included TSR caused by amino acid substitutions in the ALS enzyme (Panozzo *et al.*, 2021; Yang *et al.*, 2021; Fang *et al.*, 2022), as well as NTSR related to various causes (Hwang *et al.*, 2022; Pan *et al.*, 2022). Although several resistance cases in barnyardgrass are due to mutations in the target enzyme (e.g., ALS), most resistance cases refer to improvement of the herbicide detoxification ability. In the present study, the R-biotype has developed multiple herbicide resistance to metamifop (ACCase inhibitors) and quinclorac (synthetic auxins). Post-emergence applications of metamifop and quinclorac will likely provide early-season residual control of the R-biotype in paddy fields. However, the R-biotype did not develop multiple resistance to other mechanisms of action: profoxydim (ACCase inhibitors), propanil (photosystem II inhibitors), and florpyrauxifen-benzyl (synthetic auxins). Therefore, these results suggest that the use of herbicides with different inhibitory action sites, as mentioned above, was not effective in controlling the R-biotype. According to Iwakami *et al.* (2015), multiple herbicide resistance has been reported in some ACCase-inhibitor-resistant weeds such as barnyardgrass biotypes in Okayama, Japan. In contrast, *Echinochloa phyllopogon* (Stapf.) was resistant to bispyribac-sodium, imazamox, and penoxsulam (Papapanagiotou *et al.*, 2025). It has also been found that barnyardgrass that is resistant to penoxsulam could develop multiple-resistance to cyhalofop-butyl, fenoxaprop-P-ethyl and metamifop, ACCase inhibitors (Chen *et al.*, 2016). Similar to our findings, Qiong *et al.* (2019) reported that populations of barnyardgrass that had resistance to ALS inhibitors also became resistant to quinclorac, which significantly impaired their control efficiency. Thus, it is evident that weeds resistant to a particular herbicide can also become resistant to multiple substances. This study confirmed that the use of substances with the exact invasive mechanism in a group can lead to multiple resistance to other herbicides with different sites of action in plants. Therefore, farmers should alternate or rotate the use of herbicides in each group for effective barnyardgrass control to prevent and avoid the spread of penoxsulam-resistant barnyardgrass in paddy fields.

Farmers have reported that rice grain yield loss due to the presence of barnyardgrass depends on weed density, rice cultivar, and growing season. They should conduct regular surveys and sampling of resistant weeds to inform the selection of herbicides. Diversification of crop and weed management practices, emphasizing non-chemical weed control tactics, is an essential tool for proactive management of herbicide-resistant weeds. Irrigation water, soil tillage tools, and other pathways of contamination must be eliminated to prevent seed dispersal from paddy fields infested with resistant barnyardgrass to other fields. Additionally, proper management methods should be employed to minimize the reserve of resistant weed seeds in the soil. The data obtained from this study can be used as a guideline for farmers to choose the right herbicide and to alternate the use of herbicides with different mechanisms of action to prevent the further spread or slow down the problem of resistant barnyardgrass in paddy fields in Central Thailand. Therefore, the farmers should change the herbicide used from ALS-inhibiting herbicides to profoxydim (ACCase inhibitors), propanil (photosystem II inhibitors), and florpyrauxifen-benzyl (synthetic auxins) to manage this weed using cultural and chemical tools in paddy fields.

CONCLUSIONS

This study confirmed that barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) populations in Central Thailand paddy fields have evolved a high level of resistance to ALS-inhibiting herbicides, particularly penoxsulam, with resistance indices exceeding 50-fold compared to susceptible biotypes. The resistant biotype also exhibited cross-resistance to bispyribac-sodium, pyribenzoxim, and triafamone, and developed multiple resistance to metamifop (ACCase inhibitors) and quinclorac (synthetic auxins). In contrast, it remained susceptible to profoxydim (ACCase inhibitors), propanil (PS II inhibitors), and florpyrauxifen-benzyl (synthetic auxin). These findings provide evidence-based recommendations for herbicide rotation strategies in Central Thailand, highlighting the need to alternate herbicides with different modes of action, such as profoxydim, propanil, and florpyrauxifen-benzyl, in conjunction with integrated weed management practices. Adoption of these measures will be crucial in slowing the spread of herbicide-resistant barnyardgrass, safeguarding rice yields, and maintaining long-term weed control efficacy in paddy fields.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this research.

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