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Preliminary study of cross-resistance barnyardgrass (*Echinochloa crus-galli*) to penoxsulam and bispyribac-sodium in Malaysia

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ABSTRACT

The increasing failure of acetolactate synthase-inhibiting herbicides, particularly penoxsulam, to control *Echinochloa crus-galli* in Malaysian rice fields has raised concerns of herbicide resistance. This study investigated the resistance to penoxsulam in *E. crus-galli* populations from Alor Setar, Kedah, and evaluated alternative herbicides with different modes of action. Seeds were collected from suspected resistant and known susceptible fields. After dormancy breaking, seedling bioassays and dose-response trials were conducted under glasshouse conditions. Resistance screening showed 100% survival of the resistant (R) biotype with penoxsulam and bispyribac-sodium, while the susceptible (S) biotype had complete mortality. The R biotype required 32 times the recommended rate of penoxsulam (800 g a.i. ha^{-1}) for control. GR_{50} and LD_{50} values indicated 480-fold and 535-fold resistance, confirming high resistance. To find alternatives, pretilachlor, quinclorac, and florpyrauxifen-benzyl were tested. All achieved 100% mortality in both R and S biotypes, indicating no multiple resistance. These results confirm ALS-inhibitor resistance in *E. crus-galli*, while other herbicides remain effective. This study provides the first report of cross-resistance to penoxsulam and bispyribac-sodium in Malaysia. The continued susceptibility of the resistant biotype to quinclorac, pretilachlor, and florpyrauxifen-benzyl offers an opportunity for herbicide rotation and mixtures in direct-seeded rice systems.

INTRODUCTION

Echinochloa crus-galli, a species among over 250 in the genus *Echinochloa*, is globally regarded as one of the most problematic weeds in agriculture, especially in rice cultivation (Damalas & Koutroubas, 2023). Its substantial morphological variation complicates taxonomic classification, posing challenges for accurate identification. As a C4, short-day, annual grass originally from Europe, *E. crus-galli* has demonstrated

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significant ecological plasticity, successfully adapting to climates across Asia, Australia, and the Americas (Juliano et al., 2010). It is believed that contaminated seed introduced *Echinochloa crus-galli*, which was first documented in Malaysia in 1925 (Rao et al., 2021). It has since become the dominant *Echinochloa* species in Malaysian rice ecosystems.

E. crus-galli possesses numerous traits that enhance its competitive ability such as fast growth, prolific seed production (1,000,000 seeds per plant), and adaptability to both moist and flooded environments (Tahir & Roma-Burgos, 2021). Morphologically, it has a fibrous root system, tough culms, variable leaf surfaces, and spikelets that may or may not bear awns (Maun & Barrett, 1986). Genetically, it is hexaploid with 52 chromosomes and primarily self-pollinates via cleistogamy, though occasional wind-mediated outcrossing occurs (Rudell et al., 2025; Mollaee & Maity, 2025).

The species persists through seed dormancy and can remain viable in the soil for up to twelve years (Pisoni et al., 2024). It exhibits hypogeal germination and can sprout under low-oxygen conditions, thriving in flooded systems like rice paddies (Maun & Barrett, 1986; Bajwa et al., 2015). Photoperiod sensitivity influences its year-round flowering and its ability to emerge concurrently with rice intensifies competition, often causing yield losses above 50% (Ziska et al., 2015). As few as nine plants per square meter can reduce rice yields by 57% (Maun & Barrett, 1986). Its shorter life cycle (85–95 days) compared to rice (115–120 days) allows early resource monopolization (Ho, 2003; Azmi, 1988). Morphological resemblance to rice at the seedling stage further hinders timely detection (Bajwa et al., 2015).

In Malaysia, weed-related yield losses in rice range from 5% to 85%, influenced by factors such as season, location, and management methods (Dilipkumar et al., 2020). In direct-seeded rice (DSR), the absence of continuous flooding and wider row spacing contribute to higher weed pressure. For instance, *E. crus-galli* density was 120% greater in 38 cm row spacing compared to 13 cm (Reed et al., 2024). Since the 1970s, DSR systems have facilitated the weed's dominance due to synchronous germination with rice (Matloob, 2015). The weed competes heavily for light, water, and nutrients, reducing rice yields by up to 41% and depleting soil nitrogen by as much as 80% (Tosiah et al., 2009; Hamza et al., 2012).

In rice production systems, acetolactate synthase (ALS)-inhibiting herbicides such as penoxsulam and bispyribac-sodium are widely used to control *Echinochloa crus-galli* due to their high efficacy and crop selectivity, particularly in Malaysian direct-seeded rice systems (Rahman et al., 2010). However, extensive reliance on herbicide-based technologies and repeated use of a limited number of active ingredients have accelerated the evolution of herbicide resistance in weed populations worldwide (Perotti et al., 2020). For instance, herbicide resistance in *E. crus-galli* was first recorded in Greece in 1986 (Giannopolitis & Vassiliou, 1989) and in Malaysia, *E. crus-galli* has shown resistance to herbicides like propanil, cyhalofop-butyl, quinclorac, and bispyribac-sodium (Rahman et al., 2010; Gobi et al., 2025). The increasing prevalence of herbicide resistance, coupled with the continued reliance on chemical control in DSR systems, underscores the importance of evaluating herbicides with novel modes of action. This study was initiated to verify the first documented case of cross-resistance in *E. crus-galli* to bispyribac-sodium and penoxsulam. Additionally, it aimed to quantify the resistance level of the resistant biotype relative to a known susceptible biotype and to assess the efficacy of alternative herbicides with different modes of action for managing the resistant population.

MATERIALS AND METHODS

Seed collection and preparation

Freshly matured seeds of *E. crus-galli* were collected from a rice field suspected of containing herbicide-resistant biotypes and another field 10 km away with susceptible biotypes. Both sites were in Alor Setar, Kedah, Malaysia. Seeds used in this study were from the same populations described by Gobi et al. (2025)

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who documented bispyribac-sodium resistance in *E. crus-galli*. Recently, farmers have reported reduced effectiveness of penoxsulam for controlling *E. crus-galli*, limiting herbicide options (Nasir, personal communication, 2025). This species typically matures just before the rice crop, allowing a narrow window for seed collection (Burgos et al., 2013). Mature seeds were collected from plants, dried at room temperature (25 ± 2°C), and stored in paper envelopes in a refrigerator.

Seeds were treated with concentrated sulfuric acid (H_2SO_4) for 12–15 minutes to break dormancy (Paudyal et al., 2021). Germination tests were conducted in a growth chamber set at 35/20°C (day/night) with a 12-hour light cycle. Petri dishes (9 cm) containing filter paper and 5 ml distilled water were used. Twenty seeds from each sample were placed in separate dishes, and germination was recorded upon radicle emergence. Samples with germination rates above 70% were selected for herbicide tests.

Herbicides

The response of plant samples to bispyribac-sodium, penoxsulam, quinclorac, pretilachlor and florpyrauxifen-benzyl was evaluated and the information of each herbicide is listed in Table 1.

Table 1. List of herbicide common names, trade names, mode of action and manufacturers

Common name	Recommended rate (g ai ha^{-1})	Trade name	Formulation type	Mode of action	Manufacturers
Bispyribac-sodium	9.7	Nominee 100 SC	Suspension concentrates	Inhibition of acetolactate synthase	Agricultural Chemicals (M) Sdn Bhd
Penoxsulam	6.68	Rainbow	Oil dispersible	Inhibition of acetolactate synthase	Syngenta Crop Protection Sdn Bhd,
Quinclorac	21.9	Synapse 21.9 SC	Suspension concentrates	Auxin mimics	Hextar Chemicals Sdn. Bhd.
Pretilachlor	28.7	Sofit N 300EC	Emulsifiable concentrate	Inhibition of very long chain fatty acid	Syngenta Crop Protection Sdn Bhd
Florpyrauxifen-benzyl	2.7	Loyant	Emulsifiable concentrate	Auxin mimics	Corteva Agriscience (Malaysia) Sdn. Bhd.

Herbicide-resistance screening test in *E. crus-galli*

A screening experiment was conducted to evaluate the response of *E. crus-galli* biotypes to bispyribac-sodium and penoxsulam. One hundred seeds from each biotype were pre-germinated under controlled conditions as previously described. Twenty germinated seeds were transplanted into plastic pots (19 cm diameter × 26 cm height) filled with rice field soil and arranged in a completely randomized block design (CRBD) within a glasshouse environment. The pots were irrigated as needed throughout the experiment. Herbicide treatments were applied at the recommended label rates when seedlings reached the 3 to 4-leaf growth stage. Subsequently, pots were flooded to a depth of 3 cm one day after treatment. Emergence data were collected, and plant survivability was evaluated at 21 days after treatment (DAT). Survival rates were calculated based on the following formula:

$$Survival\ rate\ (\%) = \frac{(Number\ of\ surviving\ in\ treated\ pots)}{(Number\ of\ surviving\ plants\ in\ control\ pots)}\ \mathrm{x}\ 100$$

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Dose response of *E. crus-galli* to Penoxsulam

A dose-response experiment was undertaken to evaluate the level of resistance of *E. crus-galli* to penoxsulam based on the methods of Gobi et al. (2025) with slight modifications. Assessing the level of resistance is essential for elucidating underlying resistance mechanisms and informing the development of effective control strategies. The study was conducted under glasshouse conditions using a completely randomized block design (CRBD) with six replications.

A dose-response experiment was conducted to evaluate the herbicidal activity of penoxsulam on *E. crus-galli* biotypes exhibiting resistance (R) and susceptible (S). For penoxsulam, R biotypes (0, 3.34, 6.68, 13.35, 26.7, 53.4, 106.8, 213.6, and 427.2 g a.i. ha^{-1}), and susceptible (S) biotypes with 0, 0.03, 0.05, 0.10, 0.21, 0.42, 0.83, 1.67, 3.34, and 6.68 g a.i. ha^{-1}), where 13.35 g a.i. ha^{-1} is the recommended field rate. The selected doses ranged from causing minimal effects to complete plant death. For biotypes with high resistance, the dose range was extended until the plants no longer responded to higher doses.

Each treatment consisted of six replicates with 20 seeds of *E. crus-galli* sown in individual plastic cups. The trial was carried out under a glasshouse and initial screenings were utilized to refine the dose range to 8–9 concentrations per biotype. Plant survival was recorded at 21 days after treatment (DAT). The number of surviving plants was recorded; healthy plants were harvested, washed and weighed to obtain shoot biomass. The plant samples were then air-dried, enclosed in paper envelopes, and oven-dried at 70 °C to determine their shoot dry weight.

Herbicide efficacy tests

The resistant and susceptible *E. crus-galli* biotypes were treated with pretilachlor (28.7 g a.i. ha^{-1}), quinclorac (21.9 g a.i. ha^{-1}) and florpyrauxifen-benzyl (2.7 g a.i. ha^{-1}) at their recommended field rates (Table 3). Pretilachlor were used as pre-emergence treatments (0 to 1-leaf stage), while quinclorac and florpyrauxifen-benzyl were applied post-emergence (3 to 4-leaf stage). A total of 100 seedlings were sprayed for each herbicide. Untreated controls were also included. Planting, herbicide application, and maintenance were conducted following previously established protocols. Plant survival was assessed 21 days after treatment.

Statistical analysis

For the herbicide-resistant screening test, a chi-square test was conducted to analyze and compare the frequency of *E. crus-galli* seedlings which survived after being treated with selected herbicides at 5% of significance level. For the dose-response assay shoot dry weight and survival data were analyzed using a log-logistic regression model, as detailed below:

$$Y = d/ (1 + [x/x0]\ b)$$

Where Y = the percentage of shoot dry weight/plant survival, a = the coefficients corresponding to the upper asymptotes, b = the slope of the line, x0 = the herbicide rate required to inhibit the shoot growth (GR_{50}) or provide plant survival (LD_{50}) by 50%, and x = the herbicide rate.

Pilot trials were conducted to refine the herbicide dose–response experiments. Each subsequent dose–response experiment was repeated at least twice, yielding consistent results; hence, data from one representative experiment are presented for each dose–response analysis. Resistance levels were quantified by calculating the GR_{50} and LD_{50} values for the resistant (R) biotype relative to the susceptible (S) biotype.

RESULTS AND DISCUSSION

Confirmation of cross-resistant *E. crus-galli*

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The Chi-square test revealed a statistically significant difference ($p < 0.05$) in the survival frequency of *E. crus-galli* seedlings treated with penoxsulam and bispyribac-sodium, indicating varying levels of herbicide susceptibility among the tested populations. All putative resistant seedlings survived treatment with the ALS-inhibiting herbicide penoxsulam and bispyribac-sodium, whereas complete mortality was observed with the putative susceptible biotype. These results confirm the occurrence of cross-resistant *E. crus-galli* biotype to both penoxsulam and bispyribac-sodium.

In the penoxsulam dose–response experiment, increasing herbicide rates led to a marked reduction in both survival and shoot dry weight of the resistant (R) and susceptible (S) *E. crus-galli* biotypes (Figure 1 and 2). The S biotype showed 100% mortality at the recommended field rate of penoxsulam (13.35 g a.i. ha^{-1}), whereas the R biotype survived even at much higher doses and was only completely controlled at 800 g a.i. ha^{-1} approximately 32 times the recommended rate. The LD_{50} for the S biotype is 0.26 g a.i. ha^{-1}, while the R biotype requires 139 g a.i. ha^{-1}, indicating a resistance level of 535-fold (Table 2). The GR_{50} values are 0.25 g a.i. ha^{-1} for the S biotype and 120 g a.i. ha^{-1} for the R biotype, indicating a resistance level of 480-fold (Table 2).

Table 2. Estimated penoxsulam LD_{50}, GR_{50} and resistance level (RL) values for resistant (R) *E. crus-galli* biotype compared to susceptible (S) *E. crus-galli* biotype

S	**R**	**RL**
LD_{50} (g ai ha^{-1})		
0.26 (0.01)	139 (7)	535
GR_{50} (g ai ha^{-1})		
0.25 (0.01)	120 (10)	480

LD_{50} is the herbicide dose that causes 50% mortality. GR_{50} is the herbicide dose that causes 50% shoot growth reduction. The values in parentheses are the standard error of the mean.

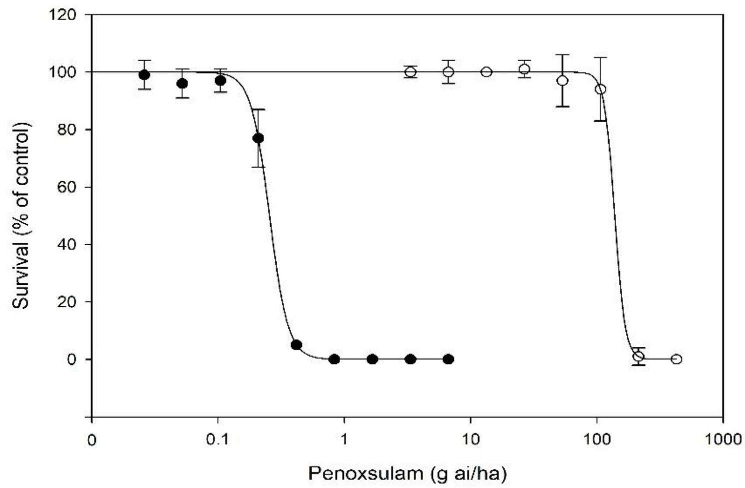

Fig. 1. Survival of the susceptible (●) and resistant (○) biotypes of *E. crus-galli*, as affected by penoxsulam. Each value is the mean of six replicates with the vertical bars representing the standard deviation of the mean.

Resistance to acetolactate synthase (ALS)-inhibiting herbicides in *E. crus-galli* is a widespread issue affecting rice cultivation globally. In comparison, Gobi et al. (2025) found that *E. crus-galli* from the same geographical region exhibited strong resistance to bispyribac-sodium. The susceptible (S) biotype showed 100% mortality at the recommended rate of 9.7 g a.i. ha^{-1}, while the resistant (R) biotype was only fully controlled at 1,241 g a.i. ha^{-1}, while having a 128-fold increase in LD_{50}. Additionally, the herbicide rates required to reduce shoot growth by 50% (GR_{50}) were 0.5 g a.i. ha^{-1} for the S biotype and 35 g a.i. ha^{-1} for the R biotype, reflecting a 70-fold resistance level. The results indicate that *E. crus-galli* shows stronger resistance to penoxsulam than bispyribac-sodium, emphasizing the urgent need for effective ALS resistance management in Malaysian rice fields.

Damalas and Koutroubas (2023) noted that *E. crus-galli* is among the most frequently reported herbicide-resistant weed species, particularly against ALS inhibitors. Documented resistance levels vary by region with resistance evaluated based on shoot dry weight reduction. In India, the bispyribac-sodium resistant biotype of *E. crus-galli* showed a 2–4-fold increase in GR_{50} compared to susceptible biotypes (Choudhary et al., 2023). Riar et al. (2012) reported that the penoxsulam- resistant AR1 biotype of *E. crus-galli* from the United States had LD_{50} of > 94-fold higher than the susceptible biotype. Furthermore, the same study demonstrated that penoxsulam resistance was facilitated by the enhanced metabolic detoxification via cytochrome P450 monooxygenase enzyme. Another study by Panozzo et al. (2017) has revealed that the cross-resistance of *E. crus-galli* to bispyribac-sodium and penoxsulam was due to a target-site mutation in the ALS gene. The mutation resulted in an Ala-122-Asn amino acid substitution, which modified the herbicide binding site and reduced herbicide efficacy. Herbicide resistance in *E. crus-galli* arises from multiple mechanisms, requiring integrated weed management approaches such as the strategic use of optimized herbicide mixtures for effective and sustainable control in rice cultivation.

Efficacy of herbicides with other modes of action

Pre-emergence and post-emergence herbicide treatments using pretilachlor, quinclorac, and florpyrauxifen-benzyl with different modes of action were applied to both resistant (R) and susceptible (S) biotypes of *E. crus-galli* under controlled glasshouse conditions (Table 3). All three herbicides resulted in 100% mortality across both biotypes, with no survivors observed. These findings strongly suggest that the tested *E. crus-galli* populations remain highly susceptible to herbicides with alternative modes of action. Pretilachlor, quinclorac, and florpyrauxifen-benzyl have demonstrated high efficacy and serve as essential components in diversified weed management strategies aimed at reducing the risk of herbicide resistance development.

In contrast to acetolactate synthase (ALS) inhibitors such as bispyribac-sodium and penoxsulam, which exert their herbicidal effect by inhibiting the biosynthesis of branched-chain amino acids, herbicides with alternative modes of action have maintained consistent efficacy against *E. crus-galli*. A notable example is pretilachlor, a chloroacetanilide herbicide, which controls weed growth by inhibiting the biosynthesis of very-long-chain fatty acids, a critical component for membrane formation during early seedling development. Field-based research conducted by Tahir et al. (2021) showed that pretilachlor, when applied at 1500 g a.i. ha^{-1} three days after sowing (DAS), significantly extended the critical period for weed removal up to 33 DAS and effectively reduced *E. crus-galli* density and biomass compared to vsuppression of *E. crus-galli* at the 5-leaf stage when applied at 35 g a.i. ha^{-1}. These results underscore the herbicide's broad-spectrum activity and its potential utility across diverse agroecological conditions.

Quinclorac, another synthetic auxin with a unique chemical structure has also proven effective in managing *E. crus-galli*. Its mechanism of action involves mimicking indole-3-acetic acid, which induces excessive ethylene production and ultimately inhibits plant growth and survival. While many synthetic auxins are limited to controlling broadleaf weeds, quinclorac is notable for its effectiveness against both broadleaf and grass weed species. According to Grossmann (2010), quinclorac is effective against several problematic grass species, including *Echinochloa*, *Digitaria*, *Setaria*, and *Brachiaria*, thereby making it a valuable component of selective weed control strategies in direct-seeded rice systems.

Table 3. Response of resistant (R) and susceptible (S) *E. crus-galli* seeds collected from paddy field with selected herbicides with other modes of action at the recommended rate at the 0 to 1-leaf stage and 3- to 4-leaf stage under glasshouse conditions

Herbicide treatment	Rate (g a.i. ha^{-1})	Number of seedlings				Survivability (%)	
		Screened		Survival			
		R	S	R	S	R	S
Pre emergence application (0 to 1-leaf)							
Pretilachlor	28.7	100	100	0	0	0	0
Post emergence application (3 to 4-leaves)							
Quinclorac	21.9	100	100	0	0	0	0
Florpyrauxifen-benzyl	2.7	100	100	0	0	0	0

Collectively, these findings demonstrate that ALS-resistant *E. crus-galli* biotypes retain full susceptibility to herbicides with alternative modes of action. The consistent performance of pretilachlor, florpyrauxifen-benzyl, and quinclorac across both resistant and susceptible populations highlights their potential as tank mixtures in herbicide resistance management. In an effort to manage resistance in *E. crus-galli*, Gobi et al. (2025) evaluated the efficacy of herbicide tank mixtures as a control strategy. The study revealed that combining propanil, a photosystem II (PSII) inhibitor, with quinclorac, a synthetic auxin, at specific ratios significantly enhanced control of the bispyribac-sodium-resistant biotype. The most effective combination was a 40:60 ratio of propanil to quinclorac, which demonstrated strong synergistic activity and allowed substantial dose reductions at 2.2-fold for propanil and 4.7-fold for quinclorac compared to single applications. However, the efficacy of the tank mixtures was influenced by the proportion of herbicides used; notably, the 80:20 propanil to quinclorac ratio exhibited antagonistic effects, which may be attributed to reduced herbicide uptake and impaired translocation within the plant system.

In addition, *E. crus-galli* was synergistically inhibited through pre-emergence applications of sunflower leaf extract and pretilachlor mixtures at a 90:10 extract-to-herbicide ratio under laboratory conditions (Dilipkumar & Chuah, 2013). Another study demonstrated that in Marang sandy loam soil, the application of a pretilachlor–sunflower leaf extract mixture at 180 g a.i. ha^{-1} and 200% (w/v), respectively, applied immediately after sowing resulted in up to 95% inhibition of *E. crus-galli* emergence and shoot growth when applied as pre-emergence (Dilipkumar et al., 2012).

CONCLUSIONS

In summary, this study provides evidence of resistance in *E. crus-galli* to the herbicide penoxsulam. The resistant biotype exhibited significantly lower control efficacy and markedly higher GR_{50} (480-fold) and LD_{50} (535-fold) values compared to the susceptible biotype. These results indicate that repeated application of herbicides with similar modes of action can accelerate resistance development. Therefore, integrating diverse weed management strategies through rotating herbicides with different sites of action such as pretilachlor, quinclorac and florpyrauxifen-benzyl, using mixtures and incorporating non-chemical control methods is essential to delay resistance evolution.

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CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefit, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

AUTHORS' CONTRIBUTIONS

NAFH conducted the research and was responsible for writing and revising the manuscript. CTS conceptualized the study, provided the theoretical framework, designed the methodology, supervised research progress, and approved the final submission. Dilipkumar Masilamany co-supervised the work, provided guidance, critical feedback, and support during revisions. Khairun Nisa Kamaruddin contributed as a team member in supporting the research activities.

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