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Metabolic Resistance to Acetyl-CoA Carboxylase-Inhibiting Herbicide Cyhalofop-Butyl in a Chinese *Echinochloa crus-galli* Population

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Abstract: A population of *Echinochloa crus-galli* (L.) P. Beauv obtained from direct-seeding rice fields in Jiangxi Province, China, exhibited high resistance levels (13.5-fold) to the acetyl-CoA carboxylase (*ACCase*)-inhibiting herbicide cyhalofop-butyl. Compared with the susceptible (S) population, this resistant (R) population evolved a cross-resistance to aryloxyphenoxypropionates (APPs) herbicides metamifop (2.9-fold) and fenoxapro-*p*-ethyl (4.1-fold), cyclohexanediones (CHDs) herbicide clethodim (4.7-fold), phenyl pyrazoline (DEN) herbicide pinoxaden (6.4-fold), and evolved multiple-resistance to acetolactate synthase (ALS)-inhibiting herbicide penoxsulam (3.6-fold), and auxin mimic herbicides quinclorac (>34.7-fold) and florpyrauxifen-benzyl (2.4-fold). *ACCase* gene sequencing did not reveal the existence of any known mutation point conferring with herbicide resistance. In addition, three metabolic inhibitors—one glutathione—S-transferase (GST) inhibitor (NBD-Cl), and two cytochrome P450 inhibitors (malathion and PBO)—did not reverse the cyhalofop-butyl resistance. Furthermore, enhanced metabolic rates of more than 60% 24 h after treatment with the active compound cyhalofop acid was observed in R plants compared to S plants. Hence, enhanced metabolism activity endows a non-target-site resistance to cyhalofop-butyl in the R population of *E. crus-galli*. Future research will be required to determine what metabolizing enzyme genes are responsible for cyhalofop-butyl resistance in *E. crus-galli*.

Keywords: resistance mechanisms; metabolism; cyhalofop-butyl; *Echinochloa crus-galli*; resistance pattern; multiple-resistance

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1. Introduction

Rice (*Oryza sativa* L.) is one of the main grain crops in China. In recent years, direct-seeding rice has become one of the main rice cultivation methods in China due to its high mechanization and low cost [1]. *Echinochloa crus-galli* (L.) P. Beauv infected direct-seeding rice fields widely and severely in China. Studies have shown that *E. crus-galli*, with a density of 4 plants m^{-2}, reduced the rice grain yield by 55.2%, and the rice taste quality declined significantly [2]. At present, chemical herbicides are still the main means to control *E. crus-galli* in rice fields in China, and acetyl-CoA carboxylase (*ACCase*)-inhibiting herbicides are one of most frequently used due to their high efficiency in controlling grass weeds.

ACCase-inhibiting herbicides include three different chemical classes: aryloxy phenoxy-propionates (APPs), cyclohexanediones (CHDs), and phenyl pyrazoline (DEN) [3]. Cyhalofop-butyl is the only herbicide of APPs with high safety to rice, and it has a good control effect on grass weeds, including *E. crus-galli* and *Leptochloa chinensis* in rice fields. Cyhalofop-butyl can be absorbed by the leaf and sheath of plants and translocated through the plant phloem. Afterwards, it can be rapidly degraded into the active compound cyhalofop acid [4]. Then, cyhalofop acid inhibits the activity of the *ACCase* enzyme, so that

the fatty acid synthesis stops and cell growth and division cannot proceed normally, leading to the death of the weeds. *ACCase*-inhibiting herbicides are prone to herbicide resistance evolution [5]. Several grass weeds from rice fields, including *L. chinensis*, *Diplachne fusca*, and *Eleusine indica*, have been reported to be evolving a resistance to cyhalofop-butyl [6–12]. The cases of cyhalofop-butyl-resistant *E. crus-galli* have been increasing in China over recent years [13,14].

Weed resistance to *ACCase*-inhibiting herbicides is mainly caused by *ACCase* gene mutations and/or enhanced herbicide metabolism [15,16]. The Ile-1781-Leu and Asp-2078-Glu mutation in the *ACCase* gene of *E. crus-galli* have been demonstrated to confer target-site resistance (TSR) to *ACCase* inhibitors [13,17]. In addition, some studies found that *ACCase* mutations were not detected in herbicide-resistant *E. crus-galli* populations, and non-target-site resistance (NTSR) mechanisms may be involved in these populations [14,18,19]. NTSR mainly involves herbicide absorption, translocation, and metabolism [19]. In particular, the NTSR mechanism by enhanced metabolism is associated with plant detoxifying enzymes, such as cytochrome P450, glutathione-S-transferase (GST), and glucosyltransferases [20]. Compared to TSR mechanisms, very little genes were identified in NTSR mechanisms in *Echinochloa* spp. species [21–24]. In addition, potential NTSR in weeds can be due to multiple resistances to widespread herbicides, causing more difficulties to control resistant weeds.

In this study, we found an *E. crus-galli* population exhibiting high resistance level to cyhalofop-butyl. Therefore, we aimed to: (1) determine the cross- or multiple-resistance patterns to *ACCase*-inhibiting, ALS-inhibiting, and auxin mimic herbicides; (2) investigate the potential resistance mechanisms; (3) confirm the effect of three metabolic enzymes inhibitors—malathion, PBO, and NBD-Cl—on the resistance to cyhalofop-butyl.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The resistant (R) population of *E. crus-galli* was obtained in 2019 from a direct-seeding rice field in Jiangxi Province, China (116.09° E, 28.99° N), which was severely infected by this weed. The susceptible (S) population was from an area where herbicides have not been used (116.15° E, 28.98° N). Seeds of each population were collected from each field with at least 20 plants pooled.

E. crus-galli seeds were germinated at 30 °C. Then, germinated seedlings were transplanted to a mixture of organic matter and vermiculite (1:1 *v*:*v*) loaded in 7 cm-diameter pots, and grown in a greenhouse at 30 ± 2 °C with a 14 h: 10 h day: night photoperiod, and 65–75% relative humidity. When grown to a 2-to 3-leaf stage, the seedlings of each pot were thinned to 8 plants.

2.2. Dose-Response to Cyhalofop-Butyl

E. crus-galli plants with 3 true leaves were treated with cyhalofop-butyl using a sprayer delivering a water volume of 450 L ha^{-1} at 300 KPa with a fan nozzle positioned 58 cm above the plants. The cyhalofop-butyl rates for the S population were 0, 1.30, 3.89, 11.67, 35, 105 g ai ha^{-1}, and for the R population were 0, 3.89, 11.67, 35, 105, 315 g ai ha^{-1}. At 15 days after treatment, the aboveground fresh weights of S and R plants were measured. Three replications were set for each herbicide rate, and each experiment was carried out twice.

2.3. Response to ACCase Inhibitors

E. crus-galli seedlings were grown and treated by herbicide solutions at 3-leaf stage under the conditions described above. Four herbicides that have the same mechanism of action as cyhalofop-butyl were used to determine the cross-resistance to *ACCase* inhibitors, namely metamifop and fenoxaprop-*p*-ethyl (APPs), clethodim (CHDs), and pinoxaden (DEN). The herbicides' commercial information and application rates for S and R populations are listed in Table 1. The fresh shoot weights were recorded 15 days later, and the experiment was conducted twice with three replicates.

Table 1. Herbicides' information and treatment doses used for dose-response experiments of S and R populations.

Type	Herbicides	Formulation [1] and Company	Treatment Doses (g ai ha^{-1})	Field Recommended Dose (g ai ha^{-1})
ACCase inhibitors	Cyhalofop-butyl	100 g L^{-1} EC, Dow AgroSciences	S: 0, 1.30 3.89, 11.67, 35, 105 R: 0, 3.89, 11.67, 35, 105, 315	105
	Metamifop	10% EC, FMC	S: 0, 1.48, 4.4, 13.33, 40, 120 R: 0, 4.44, 13.33, 40, 120, 360	120
	Fenoxaprop-*p*-ethyl	69 g L^{-1} EW, Bayer CropScience	S: 0, 0.26, 0.77, 2.3, 6.9, 20.7 R: 0, 0.77, 2.3, 6.9, 20.7, 62.1	62.1
	Clethodim	120 g L^{-1} EC, Qingdao Modern Agrochemical	S: 0, 0.3, 0.89, 2.67, 8, 24 R: 0, 0.89, 2.67, 8, 24, 72	72
	Pinoxaden	5% EC, Syngenta	S: 0, 0.56, 1.67, 5, 15, 45 R: 0, 1.67, 5, 15, 45, 135	45
ALS inhibitors	Penoxsulam	25 g L^{-1} OD, Dow AgroSciences	S: 0, 0.37, 1.11, 3.33, 10, 30 R: 0, 1.11, 3.33, 10, 30, 90	30
	Bispyribac-sodium	10% SC, Jiangsu Hormone Research Institute	S: 0, 0.56, 1.67, 5, 15, 45 R: 0, 1.67, 5, 15, 45, 135	45
Auxin mimic herbicides	Quinclorac	75% WG, Jiangsu Hormone Research Institute	S: 0, 5.56, 16.67, 50, 150, 450 R: 0, 50, 150, 450, 1350, 4050	450
	Florpyrauxifen-benzyl	3% EC, Dow AgroSciences	S: 0, 0.23, 0.67, 2, 6, 18 R: 0, 0.67, 2, 6, 18, 54	18

[1] EC, emulsifiable concentrate; EW, emulsion in water; OD, oil dispersion; SC, suspension concentrate; WG, water dispersible granule.

2.4. Response to Other Herbicides Used in Direct-Seeding Rice Fields

Four herbicides used for controlling grass weeds with post-emergence in direct-seeding rice fields were chosen to test the susceptibility of *E. crus-galli*. Commercial formulations of two ALS inhibitors, penoxsulam and bispyribac-sodium, and two auxin mimic herbicides, quinclorac and florpyrauxifen-benzyl, were applied at the 3-leaf stage with the dose gradients listed in Table 1. The fresh shoot weights were measured 15 days later. Each experiment was conducted twice with three replicates.

2.5. ACCase Genes Isolation and Sequencing

Genomic DNA was extracted from leaf tissues according to the instruction of the DNAsecure Plant Kit (Tiangen Biotech, Beijing, China). The *ACCase* gene fragments of *E. crus-galli* were amplified with primers, as reported by Yang et al. [14], which could cover all known mutation sites (Ile-1781, Trp-1999, Trp-2027, Ile-2041, Asp-2078, Cys-2088, and Gly-2096) responsible for resistance to *ACCase* inhibitors. The PCR mixture contained 25 µL of 2 × Taq PCR Mix, 1 µL template DNA, 1 µL of forward primer, 1 µL of reverse primer, and 22 µL ddH_2O. The PCR profile was carried out as described by Yang et al. [14]. Afterwards, the amplified PCR products were purified, cloned into the pLB vector, and transformed into TOP 10 chemical competent cells (Tiangen Biotech, Beijing, China). In total, 5 S and 10 R plants were analyzed, and at least 10 transformed clones of each sample were selected and sequenced by Sangon Biotechnology (Shanghai, China) to obtain all of the six *ACCase* gene copies.

2.6. Cyhalofop-Butyl Metabolism in S and R Plants of E. crus-galli

When the plants had grown to the 3- to 4-leaf stage, they were foliar treated with cyhalofop-butyl at 1/3 of the field recommended application rate (35 g ai ha^{-1}); 97% cyhalofop-butyl technical (Jiangsu Fenghua Chemical Industrial Co., Ltd., Yancheng, China) was dissolved in 1 mL acetone, diluted to the application rate with 0.2% Tween-80

aqueous solution, and sprayed under the conditions described above. Aboveground tissue samples of S and R plants were harvested at 2, 24, and 48 h after treatment (HAT), and frozen using liquid nitrogen. Five plants were pooled as a replicate, and each harvest time point was conducted with four replications.

Cyhalofop-butyl and its active metabolite cyhalofop acid were extracted from S and R samples according to the methods of Deng et al. [25]. The final supernatant obtained was analyzed by UPLC-MS/MS (Agilent Technologies 1200 Triple Quad 6460, Santa Clara, CA, USA) after being filtered through a 0.22 μm membrane. The extraction was separated with an Agilent C_{18} column (5 μm, 2.1 × 150 mm). A 0.01% formamide aqueous solution-acetonitrile mixture (*v*:*v* 20:80) was used as the mobile phase. The fragment ions of cyhalofop-butyl and cyhalofop acid are 256.2/120.2 m/z and 228.1/208.0 m/z, respectively.

2.7. Metabolic Inhibitors Effect on Resistance to Cyhalofop-Butyl

To preliminary evaluate the involvement of a metabolic enzyme in the metabolic resistance mechanism, plants at the 2- to 3-leaf stage from S and R populations were each pre-sprayed with malathion (J&K Scientific, Beijing, China), piperonylbutoxide (PBO, Shanghai Aladdin Biochemical Technology, Shanghai, China), and 4-chloro-7-nitrobenzofurazan (NBD-Cl, Shanghai Aladdin Biochemical Technology, Shanghai, China). The pre-experiment applications of malathion, PBO, and NBD-Cl at rates of 1000, 2000, and 300 g ai ha^{-1}, respectively, displayed no visual effects on the growth of *E. crus-galli*. Malathion was prepared in deionized water. PBO and NBD-Cl were dissolved in 1 mL acetone, and diluted to the application rates with 0.2% Tween-80 aqueous solution. Cyhalofop-butyl was sprayed with the same doses as indicated by the dose-response curve. Malathion, PBO and NBD-Cl were sprayed 2 h, 4 h, and 48 h, before cyhalofop-butyl application [25,26]. At 21 days, the fresh shoot weights were measured, and the GR_{50} of S and R population to cyhalofop-butyl in the presence and absence of metabolic inhibitors was determined.

2.8. Statistical Analysis

The dose-response data of two repeated experiments were pooled as they showed no significance with the *t*-test ($p < 0.05$) (SPSS 20.0, IBM, Chicago, IL, USA). The herbicide dose causing 50% growth reduction (GR_{50}) of plants was calculated using Equation (1) and SigmaPlot software (version 12.0, Systat Software, San Jose, CA, USA) [27].

$$y = C + (D - C)/[1 + (x/GR_{50})^b] \quad (1)$$

In this equation, C is the lower limit, D is the upper limit, x is the herbicide rate, y is the fresh above-ground weight relative to non-treated control (%) response to x, and b is the slope around GR_{50}. The GR_{50} ratio of the R population divided by the S population was calculated as the resistance index (RI). Data on cyhalofop-butyl metabolism were analyzed using SPSS 20.0 software and the analysis of variance (ANOVA).

3. Results

3.1. Resistance level to Cyhalofop-Butyl

The resistance level to cyhalofop-butyl of the R population was confirmed by the dose-response experiment (Figure 1). The S population was completely killed by 1/3 of the field recommended application dose (35 g ai ha^{-1}), and the GR_{50} was calculated to be 5.6 g ai ha^{-1}. The R population was still all alive at the highest rate (315 g ai ha^{-1}) and exhibited > 20% growth relative to the untreated control at that rate. The GR_{50} value of the R population was 75.8 g ai ha^{-1} and the resistance index (RI) was 13.5.

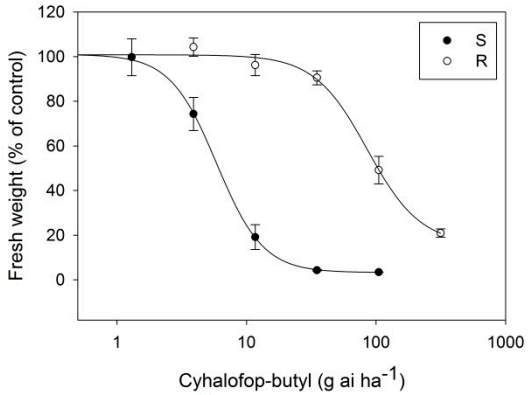

Figure 1. Cyhalofopbutyl dose-response curves of S and R *E. crus-galli* populations.

3.2. Resistance Pattern to Different Herbicides

The R population exhibited cross-resistance to three classes of *ACCase* inhibitors (Table 2). All the four *ACCase* inhibitors tested could kill the S plants completely under the field recommended rate. Compared to the S population, the R population was moderately resistant to fenoxapro-*p*-ethyl (APPs, RI = 4.1), clethodim (CHDs, RI = 4.7) and pinoxaden (DEN, RI = 6.4), and lowly resistant to metamifop (APPs, RI = 2.9) (Table 2).

Table 2. GR_{50} and resistance index (RI) to *ACCase*-inhibiting herbicides and other herbicides used for direct-seeding rice of *E. crus-galli* populations.

Herbicides	S		R		RI [2]
	$GR_{50} \pm SE$ [1]	*p*	$GR_{50} \pm SE$	*p*	
Cyhalofop-butyl	5.6 ± 0.6	<0.01	75.8 ± 8.7	<0.01	13.5
Metamifop	8.1 ± 0.5	<0.01	23.7 ± 1.3	<0.01	2.9
Fenoxaprop-*p*-ethyl	1.2 ± 0.1	<0.01	4.9 ± 0.9	<0.01	4.1
Clethodim	2.1 ± 0.2	<0.01	9.8 ± 0.8	<0.01	4.7
Pinoxaden	3.7 ± 0.5	<0.01	23.7 ± 3.2	<0.01	6.4
Penoxsulam	3.2 ± 1.1	0.02	28.6 ± 2.19	<0.01	8.9
Bispyribac-sodium	11.64 ± 2.67	0.02	14.18 ± 0.94	<0.01	1.2
Quinclorac	116.63 ± 17.17	0.01	>4050.00	0.03	>34.7
Florpyrauxifen-benzyl	3.4 ± 0.2	<0.01	10.1 ± 0.6	<0.01	3.0

[1] GR_{50}, herbicide dose causing 50% growth reduction. [2] RI = GR_{50} resistant biotype (R)/GR_{50} susceptible biotype (S).

The susceptibility of herbicides with different modes of action was also determined with the whole plant experiments. The ALS inhibitor bispyribac-sodium completely controlled both the S and R plants at the field rate (45 g ai ha^{-1}). On the contrary, the other three herbicides exhibited poor control effects on the R plants. The R population exhibited high-level resistance to auxin mimic herbicide quinclorac (RI > 34.7), moderate-level resistance to ALS inhibitor penoxsulam (RI = 8.9), and low-level resistance to auxin mimic herbicide florpyrauxifen-benzyl (RI = 2.4) (Table 2).

3.3. ACCase Gene Sequencing

Partial sequences of the carboxyltransferase domain of *ACCase* genes were amplified and examined. As *E. crus-galli* is hexaploid, all six transcripts were amplified. Sequence alignment results showed that *ACCase* 1 to *ACCase* 6 have more than 99% similarity in the nucleotide sequence. However, none of the mutation sites in *ACCase* genes conferring resistance were detected in the R population.

3.4. Enhanced Cyhalofop-Butyl Metabolism in R Plants

According to the results shown in Table 3, the transformation rate of cyhalofop-butyl into cyhalofop acid was similar in the two biotypes, because there was no significant

difference in its content between S and R plants. The cyhalofop acid in S was 2.7- and 3-fold richer than that in R plants at 24 and 48 HAT, respectively, which means that the *E. crus-galli* plants of the R population could detoxify cyhalofop acid faster than that of S population.

Table 3. Content of cyhalofop-butyl and cyhalofop acid in *E. crus-galli* plants from S and R populations after cyhalofop-butyl treatment.

Time (h)	Cyhalofop-Butyl (μg kg^{-1})		Cyhalofop Acid (μg kg^{-1})	
	S	R	S	R
2	0.85 ± 0.10	0.94 ± 0.12	0.71 ± 0.08	0.67 ± 0.07
24	0.42 ± 0.05	0.48 ± 0.06	0.57 ± 0.09	0.21 ± 0.05 *
48	0.027 ± 0.008	0.024 ± 0.004	0.24 ± 0.07	0.08 ± 0.02 **

*, ** significant difference at $p < 0.05$, 0.01, respectively.

3.5. Metabolic Inhibitors Eeffect on Cyhalofop-Butyl Resistance

To investigate the involvement of metabolic enzymes in the metabolic resistance, two P450 inhibitors (malathion and PBO) and a GST inhibitor (NBD-Cl) were applied prior to cyhalofop-butyl treatment. For the S population, there were no significant differences on plant growth between inhibitors pre-treatment and cyhalofop-butyl treatment alone, with the GR_{50} ranging from 2.7 to 4.3 g ai ha^{-1} (Figure 2). For the R population, the GR_{50} values to cyhalofop-butyl in the presence of malathion, PBO, and NBD-Cl were 37.5, 44.1, and 32.7 g ai ha^{-1}, respectively, and showed no significant difference to that in the absence of inhibitors (33.2 g ai ha^{-1}) (Figure 2). Hence, the three metabolic enzyme inhibitors did not weaken the resistance of the R *E. crus-galli* population to cyhalofop-butyl.

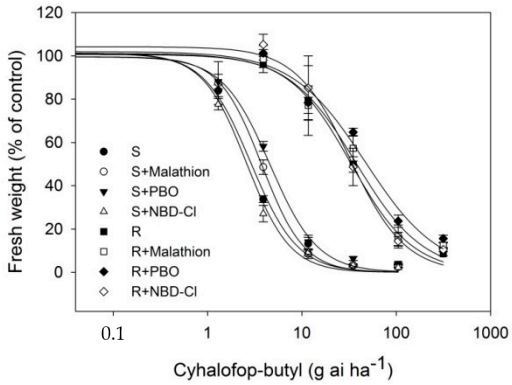

Figure 2. Metabolic enzyme inhibitor effect on cyhalofop-butyl resistance in the S and R populations of *E. crus-galli*.

4. Discussion

This study demonstrated that a population of *E. crus-galli*, which was collected from direct-seeding rice fields in Jiangxi Province, China, induced a high resistance level to *ACCase*-inhibiting herbicide cyhalofop-butyl, with a RI value of 13.5. Dose-response experiments confirmed that the R population at the whole-plant level was resistant to three groups of *ACCase* inhibitors—metamifop and fenoxapro-*p*-ethyl (APPs), clethodim (CHDs), and pinoxaden (DEN). Furthermore, the R population also displayed multiple-resistances to herbicides with other modes of action used in rice fields, especially more than 34.7-fold resistance to the auxin mimic herbicide quinclorac and 8.9-fold resistance to the ALS-inhibiting herbicide penoxsulam, making it very challenging to control the population of *E. crus-galli*.

No known mutation sites responsible for herbicide resistance were detected in any of the six *ACCase* transcripts, which indicates the possible participation of NTSR mechanisms. In *Echinochloa* spp., there are only two reports of *ACCase* mutations, namely, Ile-1781-Leu and Asp-2078-Glu substitution [13,17,28]. In contrast, more resistant cases are involved in

NTSR mechanisms [18,19,29]. A similar result was reported in a *Lolium rigidum* population from Spain, which showed multiple- and cross-resistance to ALS and *ACCase* inhibiting herbicides, and without mutations in the *ACCase* gene [30]. Our results also support this phenomenon. This may be associated with the dilution effect of multiple *ACCase* genes. Yu et al. [31] reported that *Avena fatua* carrying one mutant allele in the three *ACCase* copies only exhibited low-level resistance to *ACCase*-inhibiting herbicides. Therefore, NTSR may be more likely to evolve in weed species with multiple copies of target-site genes.

As a typical APP herbicide, cyhalofop-butyl is rapidly converted into its active metabolite, cyhalofop acid, after being absorbed by plants [4]. In our study, similar contents of cyhalofop-butyl were observed in S and R plants, indicating the same absorption and transformation rate of cyhalofop-butyl in the two populations. However, the content of cyhalofop acid in R plants was significantly reduced by 63% and 67% at 24 and 48 HAT, respectively, compared to the S plants, suggesting enhanced metabolism was the prime reason conferring resistance to cyhalofop-butyl. Our results are in accordance with the researches of other *ACCase* inhibitor resistant weeds [32–37]. Similar results were also observed in resistance to the auxin mimic herbicide florpyrauxifen-benzyl in this species, in which significant herbicide absorption and metabolism both contribute to the inability to control *E. crus-galli* [38].

Nevertheless, none of the tested metabolic enzyme inhibitors significantly affected resistance to cyhalofop-butyl in the R population. Similar results were reported in *Echinochloa colona* resistance to fenoxaprop-*p*-ethyl, which was not reversed by any of three metabolic inhibitors [26]. This is in contrast to some types of grass weeds, such as *Bromus rigidus*, *L. rigidum*, and *Polypogon fugax*, in which resistance could be greatly reduced by malathion or PBO treatment [39–42]. In *L. chinensis*, where a resistance mechanism with an enhanced herbicide metabolism has been identified, none of malathion, PBO, or NBD-Cl inhibited cyhalofop-butyl resistance [25]. This suggests that these three metabolic inhibitors may not inhibit the activity of all enzymes involved in weed resistance. Although one cytochrome P450 enzyme, CYP81A68, has been identified in *E. crus-galli* to endow metabolic resistance to *ACCase*-inhibiting herbicides [24], there are still many enzymes responsible for NTSR need to be verified. Therefore, additional work, such as RNA-sequence analyses and gene expression assays, will be required to determine what metabolizing enzyme genes are responsible for cyhalofop-butyl resistance in *E. crus-galli*.

In conclusion, the *E. crus-galli* population collected from direct-seeding rice fields in China exhibited a high resistance level to cyhalofop-butyl, and the enhanced metabolism conferred an NTSR in this population. It should be noted that the R population had also evolved cross-resistance to three classes of *ACCase* inhibitors and multiple-resistance to ALS inhibitors and auxin mimic herbicides, which highlights the difficulties in selecting alternative post-emergence herbicides to control the resistant weeds. The emergence of multiple-resistance *E. crus-galli* populations were reported in several regions of China in recent years, including Anhui, Jiangsu, Hunan, and Ningxia Province [13,14,24,43], and should remind Chinese growers of the evolving multiple herbicide resistance risk in *E. crus-galli* populations to most available herbicides used in rice fields of China. Therefore, non-chemical methods, such as reducing the seed bank and using bioherbicides, and integrated management strategies should be adopted to reduce the pressure of herbicide selection and dependence, particularly with regard to dealing with this species that is highly prone to herbicide resistance.

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