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Resistance patterns and molecular basis to ACCase-inhibiting herbicides

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Research Article

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Abstract

Digitaria ciliaris var. *chrysoblephara* (Fig. & De Not.) R.R. Stewart is an annual xeromorphic weed that severely infests direct-seeded rice fields in China. Herbicide resistance is emerging in *D. ciliaris* var. *chrysoblephara* owing to extensive and recurrent use of the acetyl-CoA carboxylase (ACCase)-inhibiting herbicide metamifop. In this study, a total of 53 *D. ciliaris* var. *chrysoblephara* populations randomly sampled from direct-seeded rice fields across Jiangsu Province were investigated for metamifop resistance and potential resistance-endowing mutations. Single-dose assays revealed that 17 (32.1%) populations evolved resistance to metamifop and 5 (9.4%) populations were in the process of developing resistance. The resistance index (RI) of metamifop-resistant populations ranged from 2.7 to 32.1. Amino acid substitutions (Ile-1781-Leu, Trp-2027-Cys/Ser, and Ile-2041-Asn) in *ACCase* genes were detected in resistant *D. ciliaris* var. *chrysoblephara* plants and caused various cross-resistance patterns to ACCase-inhibiting herbicides. All of four resistant populations (YC07, YZ09, SQ03, and HA06), with different *ACCase* mutations, exhibited cross-resistance to the aryloxyphenoxypropionate (APP) herbicides cyhalofop-butyl (RI values: 10.0 to 19.9), fenoxaprop-*P*-ethyl (RI values: 53.7 to 132.8), and haloxyfop-*P*-methyl (RI values: 6.2 to 62.6), and the phenylpyrazoline (DEN) pinoxaden (RI values: 2.3 to 5.4), but responded differently to the cyclohexanedione (CHD) herbicides clethodim and sethoxydim. It is noteworthy that four postemergence herbicides used for rice cropping, including bispyribac-sodium, pyraclonil, quinclorac, and anilofos, showed poor control effect against *D. ciliaris* var. *chrysoblephara*, suggesting few alternations for managing this weed in rice fields except ACCase inhibitors. In conclusion, this work demonstrated that the *D. ciliaris* var. *chrysoblephara* had developed resistance to ACCase-inhibiting herbicides in rice cultivation of China, and target-site amino acid substitutions in *ACCase* were primarily responsible for metamifop resistance.

Introduction

Digitaria spp. are annual grass weeds that are widely distributed in dryland ecosystems like roadsides, wastelands, and orchards, as well as in crops across the world (Laforest et al. 2017; Liu et al. 2023; Yu et al. 2017; Zhao et al. 2023). *Digitaria ciliaris* var. *chrysoblephara* (Fig. & De Not.) R.R. Stewart is an annual Gramineae weed that has gradually invaded dry-seeded rice fields in recent years (Guo et al. 2022). Owing to its strong capacity to adapt to different environments and its high seed production, this weed competes with rice and has become a dominant grass weed in direct-seeded rice fields of China (Cao et al. 2023; Yang et al. 2023).

Few postemergence herbicides have been registered for controlling *Digitaria* spp. weeds in rice fields, and farmers rely heavily on acetyl-CoA carboxylase (ACCase) inhibitors such as metamifop and cyhalofop-butyl. These herbicides cause plant death by binding selectively to the carboxyl transferase (CT) domain of plastidic ACCase and are allowed for grass weed control in rice fields as a selective compound (Kaundun 2014). ACCase-inhibiting herbicides can be classified within the aryloxyphenoxypropionates (APPs), cyclohexanediones (CHDs), or phenylpyrazoline (DEN) chemical families according to different chemical structures (Powles and Yu 2010). Given their high selectivity and safety to rice, ACCase-inhibiting herbicides were preferred by Chinese farmers for managing annual grass weeds postemergence in paddy fields. However, the extensive use of this mode of action (MOA) has resulted in the evolution of herbicide resistance in at least six Gramineae weed species in rice cultivation systems of China. For instance, populations of the noxious barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] (Sun et al. 2023), *Echinochloa glabrescens* Munro ex Hook. f. (Li et al. 2023; Zhao et al. 2023), rice barnyardgrass [*Echinochloa phyllopogon* (Stapf) Koso-Pol.] (Zhang et al. 2023), *D. ciliaris* var. *chrysoblephara* (Yang et al. 2023), Malabar sprangletop [*Diplachne fusca* (L.) P. Beauv. ex

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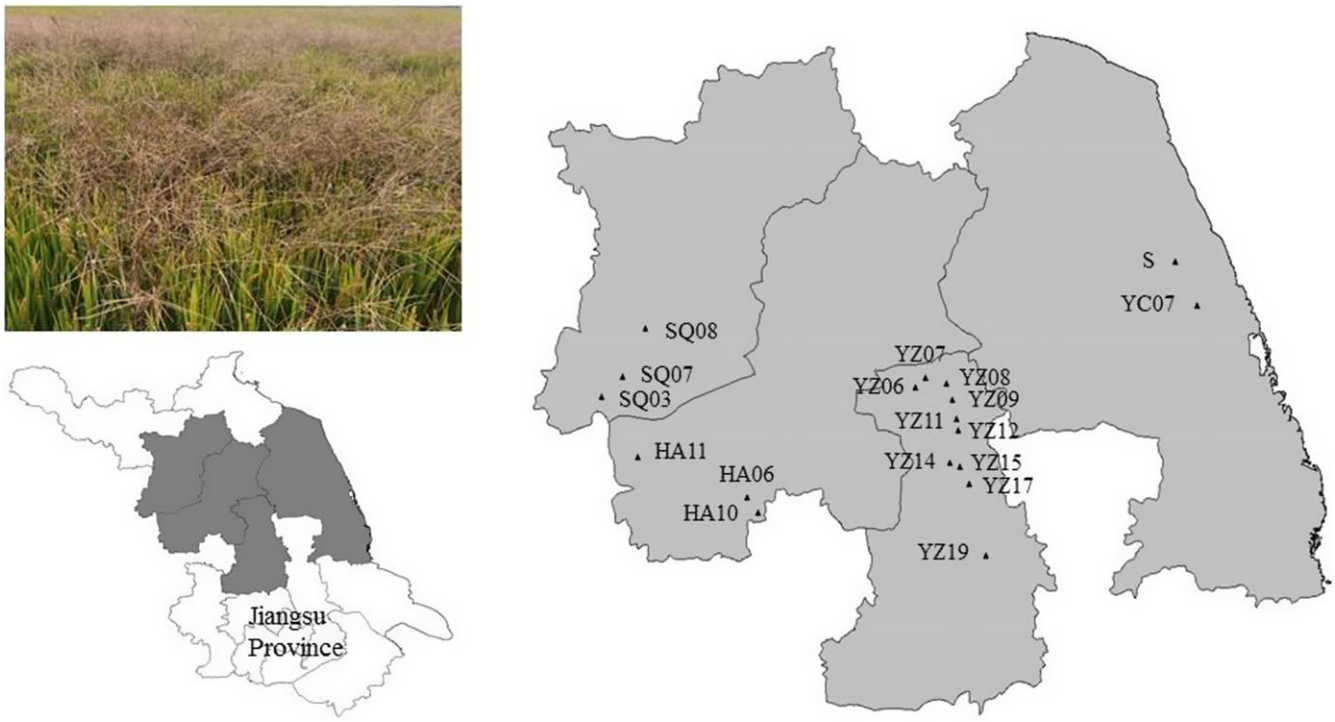


Figure 1. The occurrence of *Digitaria ciliaris* var. *chrysoblephara* in direct-seeded rice fields and location of a susceptible and 17 metamifop-resistant populations across Jiangsu Province, China, tested in this study.

Roem. & Schult.] (Yuan et al. 2019), goosegrass [*Eleusine indica* (L.) Gaertn.] (Deng et al. 2023a), and Chinese sprangletop [*Leptochloa chinensis* (L.) Nees ex.] (Deng et al. 2023b) have been confirmed resistant to ACCase-inhibiting herbicides.

Resistance to ACCase-inhibiting herbicides is caused by a non–target site based resistance (NTSR) mechanism and/or a target site–based resistance (TSR) mechanism (Délye et al. 2013). NTSR to ACCase inhibitors is mainly involved in enhanced metabolism due to a change in the metabolic enzyme activity, such as cytochrome P450 monooxygenase and glutathione transferase. (Deng et al. 2021; Yang et al. 2022). TSR to ACCase inhibitors involves alterations of the target-site protein structure owing to specific amino acid changes in the CT domain or/and increased abundance of the target protein resulting from *ACCase* gene duplication or overexpression (Laforest et al. 2017). Thus far, a total of 17 resistance-endowing mutations at *ACCase* codon positions Ile-1781 (substituted by Leu/Thr/Val), Leu-1818 (Phe), Trp-1999 (Cys/Leu/Ser), Trp-2027 (Cys/Leu/Ser), Ile-2041 (Asn/Val), Asn-2078 (Glu/Gly), Cys-2088 (Arg), and Gly-2096 (Ala/Ser) have been reported in grass weeds, and they usually confer cross-resistance to some of the ACCase inhibitors (Gaines et al. 2020; Jin et al. 2022; Zhang et al. 2022).

Metamifop is an APP herbicide that was introduced to China in 2010 and showed high herbicidal activity against grass weeds in rice-cropping systems, including *Digitaria* spp., *Echinochloa* spp., and *L. chinensis* (Cao et al. 2023). In recent years, it has been increasingly observed that metamifop has had poor effect in controlling *Digitaria* spp. weeds in direct-seeded rice fields of China, especially in Jiangsu Province. Moreover, two allelic variants, Ile-1781-Leu and Trp-2027-Cys, have been identified in *D. ciliaris* var. *chrysoblephara* as conferring high-level resistance to metamifop (Cao et al. 2023; Yang et al. 2023). Accordingly, it is necessary to investigate the resistance of *D. ciliaris* var. *chrysoblephara* to metamifop and cross-resistance patterns of different mutations in order to develop efficient and proactive weed management strategies for controlling this troublesome weed.

In this research, 53 *D. ciliaris* var. *chrysoblephara* populations that severely invaded direct-seeded rice fields were collected from Jiangsu Province in China. The objectives of the following study were to: (1) confirm and quantify the degree of metamifop resistance in different populations, (2) characterize the resistance mechanisms by surveying target-site mutations in *ACCase* genes of resistance-evolved populations, and (3) evaluate the cross-resistance patterns to herbicides with different MOAs in the resistant populations with specific *ACCase* mutations.

Materials and Methods

Sample Collection

Fifty-three *D. ciliaris* var. *chrysoblephara* populations severely infesting direct-seeded rice fields were sampled across Jiangsu Province in China in October 2022. Plants were sampled from fields with severe infestation of *D. ciliaris* var. *chrysoblephara*, and seeds from at least 30 mature plants from each field were pooled as a single population. Seeds of each population were stored in separate envelopes at room temperature until the end of seed dormancy. The susceptible (S) population was collected in a noncultivated area with no herbicide-use history in Yancheng, Jiangsu Province, China (33.71°N, 120.35°E) (Figure 1).

Single-Dose Resistance Testing

Metamifop resistance of different *D. ciliaris* var. *chrysoblephara* populations was detected by a discriminating dosage of 60 g ai ha^{-1} (1/2-fold of the recommended dose), which can completely kill the S plants. Seeds were germinated at 28 to 30 C for 3 d, sown in square pots (7 by 7 by 8 cm) containing potting mix (50% organic matter and

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50% vermiculite), and transplanted in a greenhouse with a temperature of 30 ± 2 C and a light/dark photoperiod of 14 h/10 h. The commercial formulation of metamifop was sprayed using a fan-nozzle laboratory sprayer cabinet delivering 300 L water ha^{-1} at 0.2 MPa on 3-leaf-stage plants. Approximately 12 to 14 seedlings per pot and four replicates were conducted for each population.

Seedling mortality of each population was calculated at 21 d after treatment (DAT). Populations of *D. ciliaris* var. *chrysoblephara* were defined as resistant (R) if more than 20% of plants in the population survived metamifop application, developing resistance (DR) if 1% to 20% of individuals survived metamifop, and susceptible (S) if less than 1% of individuals survived metamifop (Khammassi et al. 2019).

Dose Response to Metamifop

A total of 17 R populations and the S population were used for the whole-plant dose–response experiment on the basis of the initial single-dose resistance-screening results. The seedlings were foliar-treated with gradient doses of metamifop as described earlier when they reached the 2- to 3-leaf stage. The application dosages were 0X, 1/81X, 1/27X, 1/9X, 1/3X, and 1X doses of the recommended rate (120 g ai ha^{-1}) for the S population and 0X, 1/9X, 1/3X, 1X, 3X, and 9X doses of the recommended rate for the R populations. At 21 DAT, the fresh weight of aboveground parts in each pot was measured. The experiment was conducted twice with three replicates for each treatment.

ACCase Partial Gene Sequencing

Survivors from metamifop resistance identification in the single-dose resistance-screening assays were frozen at −80 C and used for *ACCase* gene sequencing. Approximately 2 to 10 individual plants of each population were sequenced due to difference in survival rates (Supplementary Table S1). DNA was extracted using a Plant Genomic DNA Kit (Tiangen, Beijing, China) from the leaves of each *D. ciliaris* var. *chrysoblephara* plant. The amplification of a portion of the *ACCase* gene was conducted with previously reported primers (F: 5′-ATTAGGTGGATTATTGACTCTGTTG-3′; R: 5′-TCTGGGTCAAGCCTACCCAT-3′) (Yang et al. 2023), which covered all of the eight mutation sites conferring ACCase-inhibitor resistance in weeds. A polymerase chain reaction (PCR) was run using the following conditions: 4 min of denaturation at 95 C, 35 cycles of 95 C for 30 s, 58 C for 30 s, and 72 C for 2 min, and 10-min elongation at 95 C. The PCR product was directly sequenced by the Sanger method, and the *ACCase* gene sequence was visually checked with Chromas v. 2.3 (Technelysium, Helensvale, Australia).

Dose Response to Other Herbicides with Different MOAs

The YC07 (Ile-1781-Leu), YZ09 (Trp-2027-Cys), SQ03 (Trp-2027-Ser), and HA06 (Ile-2041-Asn) populations with different *ACCase* mutations were used for investigating the cross-resistance patterns to other herbicides. Ten herbicides, including six ACCase inhibitors, one acetolactate synthase (ALS) inhibitor, one protoporphyrinogen oxidase (PPO) inhibitor, one synthetic auxin herbicide, and one very-long-chain fatty-acid (VLCFA) inhibitor, were used for cross-resistance measurement by dose–response experiments as described earlier (Table 1). At 21 DAT, the fresh weight of aboveground parts was measured. Each experiment was conducted twice with three replicates.

Table 1. Herbicide information and application rates for dose–response experiments

Mode of action[a]	Herbicide	Formulation[b]	Supplier	Application rates[c]						
				g ai ha^{-1}						
				1/243X	1/81X	1/27X	1/9X	1/3X	X	3X	9X
ACCase inhibitor	Metamifop	10% EC	FMC	—	1.5	4.4	13.3	40	120	360	1,080
	Cyhalofop-butyl	100 g L^{-1} EC	Corteva	—	1.3	3.9	11.7	35	105	315	945
	Fenoxaprop-*P*-ethyl	69 g L^{-1} EW	Bayer	0.3	0.8	2.3	6.9	20.7	62.1	186.3	—
	Haloxyfop-*P*-methyl	108g L^{-1} EC	Corteva	0.2	0.6	1.8	5.4	16.2	48.6	145.8	437.4
	Clethodim	120 g L^{-1} EC	Qingdao Modern Agrochemical	—	0.9	2.7	8	24	72	—	—
	Sethoxydim	12.5% EC	Shandong Cynda Chemical	—	3.5	10.4	31.3	93.8	281.3	843.9	—
	Pinoxaden	5% EC	Syngenta	—	—	2.2	6.7	20	60	180	540
ALS inhibitor	Bispyribac-sodium	10% SC	Jiangsu Institute of Ecomones	—	—	—	5	15	45	135	405
PPO inhibitor	Pyraclonil	2% GR	Hubei Xianghe Precision Chemistry	—	—	6.67	20	60	180	540	—
Auxin herbicide	Quinclorac	75% WDG	Jiangsu Hormone Research Institute	—	—	16.7	50	150	450	1,350	—
VLCFA inhibitor	Anilofos	30% EC	KingAgroot	—	—	11.7	35	105	315	945	2,835

[a]ACCase, acetyl-CoA carboxylase; ALS, acetolactate synthase; PPO, protoporphyrinogen oxidase; VLCFA, very-long-chain fatty-acid synthesis.
[b]EC, emulsifiable concentrate; EW, emulsion in water; GR, granules; SC, suspension concentrate; WDG, water-dispersible granule.
[c]X, the recommended dose of each herbicide.

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Table 2. Herbicide dose causing 50% growth reduction (GR_{50}) and resistance index (RI) values for metamifop, identification of *ACCase* gene mutations, codon sequence, number of mutants versus number tested in the susceptible (S) and different resistant (R) *Digitaria ciliaris* var. *chrysoblephara* populations

Population	GR_{50} ± SE	RI	*ACCase* mutation	Codon at mutation site	Numbers of mutant/tested plants
	—g ai ha^{-1}—				
S	4.65 ± 0.23	1.0	No mutations	—	0/10
YC07	127.19 ± 17.34	27.4	Ile-1781-Leu	ATA-CTA	10/10
YZ06	103.82 ± 27.39	22.3	Ile-2041-Asn	ATT-AAT	10/10
YZ07	77.78 ± 11.83	16.7	Trp-2027-Cys	TGG-TGT/C	6/4/10
YZ08	83.99 ± 3.06	13.8	Ile-2041-Asn	ATT-AAT	10/10
YZ09	96.03 ± 1.03	20.7	Trp-2027-Cys	TGG-TGT	10/10
YZ11	20.47 ± 2.71	4.4	Trp-2027-Cys	TGG-TGT	6/6
YZ12	20.20 ± 3.28	4.3	Ile-2041-Asn	ATT-AAT	5/5
YZ14	12.62 ± 0.71	2.7	Ile-2041-Asn	ATT-AAT	5/5
YZ15	149.06 ± 15.46	32.1	Trp-2027-Cys	TGG-TGT	10/10
YZ17	14.46 ± 2.11	3.1	Ile-2041-Asn	ATT-AAT	6/6
YZ19	112.49 ± 8.01	24.2	Trp-2027-Cys	TGG-TGT	10/10
HA06	127.09 ± 12.13	27.3	Ile-2041-Asn	ATT-AAT	10/10
HA10	34.80 ± 3.07	7.5	Ile-2041-Asn	ATT-AAT	6/6
HA11	14.14 ± 3.87	3.0	Ile-2041-Asn	ATT-AAT	6/6
SQ03	141.08 ± 15.10	30.3	Trp-2027-Ser	TGG-TGT	10/10
SQ07	123.34 ± 14.88	26.5	Trp-2027-Cys	TGG-TGT	8/10
SQ08	109.89 ± 27.54	23.6	Trp-2027-Cys	TGG-TGC	10/10

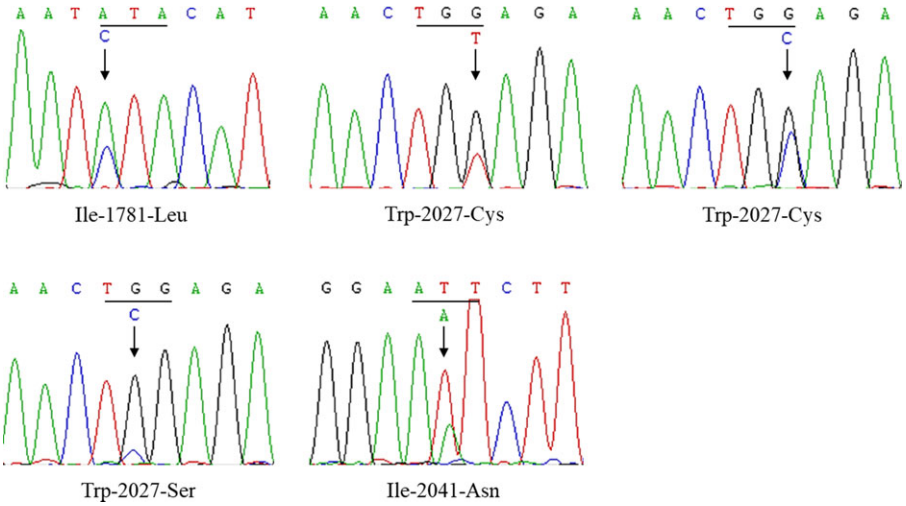


Figure 2. The chromatograms of different *ACCase* mutations involving Ile-1781-Leu, Trp-2027-Cys, Trp-2027-Ser, and Ile-2041-Asn.

Data Analysis

Dose–response curves were constructed using SigmaPlot v. 12.0 (Systat Software), and the herbicide dose causing 50% growth reduction (GR_{50}) was calculated with a log-logistic model function (Equation 1) (Seefeldt et al. 1995). In Equation 1, y is the percentage reduction of aboveground fresh weight, x is variable dose of each herbicide, and C and D are the lower and upper limits, respectively. The resistance index (RI) is expressed by GR_{50} values (R) versus GR_{50} values (S).

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

Results and Discussion

Resistance Status to Metamifop in Digitaria ciliaris var. chrysoblephara

Digitaria ciliaris var. *chrysoblephara* has been widely infesting rice fields, especially dry-seeded rice, across Jiangsu Province in China in recent years. Weed control of this species primarily relied on ACCase-inhibiting herbicides, and continuous metamifop selection pressure caused the rapid spread of herbicide resistance within rice fields (Cao et al. 2023). For the current study, the metamifop sensitivity of 53 *D. ciliaris* var. *chrysoblephara* populations was first evaluated with a single-dose spraying. The screening results showed that 17 populations had evolved resistance (R) to metamifop, with a resistance frequency of 32.1%, and 5 populations

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were in the process of developing resistant (DR) to metamifop, accounting for 9.4% (Figure 1; Supplementary Table S1). Plants of the S population completely died at one-half of the recommended dose (60 g ai ha^{-1}), with a GR_{50} value of 4.65 g ai ha^{-1} (Table 2; Supplementary Table S1). The GR_{50} values of the R populations ranged from 12.62 to 149.06 g ai ha^{-1}, producing 2.7- to 32.1-fold resistance to metamifop (Table 2).

Since the first case of reported metamifop-resistance of *Digitaria* spp. in rice fields in 2017 (Jiang et al. 2017), the frequency of resistance has continuously increased in China, especially in areas with extensive application of herbicides. The rapid development of resistance to metamifop in *D. ciliaris* var. *chrysoblephara* may be related to the following two factors. First of all, the risk of resistance is quite high for ACCase-inhibiting herbicides, suggesting a marked decrease in herbicide sensitivity will be observed in a relatively short period of time (Bagavathiannan et al. 2014; Kaundun 2014). Second, the *Digitaria* spp. had been reported resistant to diverse herbicides with different MOAs worldwide, including ACCase inhibitors, ALS inhibitors, and glyphosate, indicating these species were prone to develop resistance to herbicides (de Carvalho et al. 2012; Laforest et al. 2017; Li et al. 2017; Takano et al. 2020; Yang et al. 2023; Yanniccari et al. 2022; Zhao et al. 2023). Due to a dilution effect by susceptible homoeologous gene copies, ACCase herbicide resistance evolves faster in grass weeds that contain only one or two *ACCase* gene copies, such as *E. indica* (Deng et al. 2023a) and rigid ryegrass (*Lolium rigidum* Gaudin) (Yu et al. 2007), than in hexaploid wild oat (*Avena fatua* L.) (Yu et al. 2013) and *E. crus-galli* (Yang et al. 2021), which contain of three and six *ACCase* copies, respectively. This helps to explain the rapid spread of metamifop resistance in *D. ciliaris* var. *chrysoblephara* in recent years.

ACCase Mutations in Resistant Digitaria ciliaris var. chrysoblephara

The sequence of a 1,088-bp fragment covering part of the *ACCase* gene was amplified from each *D. ciliaris* var. *chrysoblephara* plant using a PCR-based approach, and it exhibited more than 98% nucleotide identity with sequences deposited in GenBank (accession nos. OQ354339 and OQ354340). Because there were two *ACCase* genes in *D. ciliaris* var. *chrysoblephara* (Yang et al. 2023), double peaks were observed in specific single-nucleotide polymorphism sites between two gene copies. The sequencing results showed that all 10 plants from the S population have no mutations in *ACCase* genes. Four amino acid substitutions, Ile-1781-Leu, Trp-2027-Cys/Ser, and Ile-2041-Asn, were found in a single *ACCase* gene of different resistant populations (Table 2; Figure 2). Among these mutations, the Trp-2027-Cys was the most common *ACCase* mutation identified in resistant *D. ciliaris* var. *chrysoblephara*. A total of 78 plants in 7 R populations and 5 DR populations survived metamifop treatment, and these populations contained a Trp-2027-Cys mutation in the *ACCase* gene (Supplementary Table S1). Two nucleotide mutations, TGT and TGC, were detected in codon position 2027, and conferred the same amino acid substitution of Trp-2027-Cys (Figure 2). Second, the Ile-2041-Asn substitution occurred in plants survived from 8 R populations. Conversely, both Ile-1781-Leu and Trp-2027-Ser were detected in only one R population, YC07 and SQ03, respectively. Notably, *ACCase* gene mutations were detected in all of the R and DR populations, indicating that the target-based mechanism plays a key role in the resistance evolution to metamifop in *D. ciliaris* var. *chrysoblephara*.

Table 3. Sensitivity to different herbicides for the susceptible (S) and resistant (YC07, YZ09, SQ03, and HA06) *Digitaria ciliaris* var. *chrysoblephara* populations[a]

Herbicide	Population	GR_{50} ± SE	RI
		—g ai ha^{-1}—	
Cyhalofop-butyl	S	12.07 ± 1.03	1.0
	YC07	214.39 ± 20.62	17.8
	YZ09	120.33 ± 7.48	10.0
	SQ03	143.58 ± 6.48	11.9
	HA06	239.85 ± 24.80	19.9
Fenoxaprop-*P*-ethyl	S	0.42 ± 0.03	1.0
	YC07	55.78 ± 4.44	132.8
	YZ09	32.39 ± 3.98	77.1
	SQ03	34.34 ± 3.04	81.8
	HA06	22.57 ± 2.34	53.7
Haloxyfop-*P*-methyl	S	0.77 ± 0.14	1.0
	YC07	19.23 ± 0.89	25.0
	YZ09	5.21 ± 0.60	6.8
	SQ03	4.74 ± 0.67	6.2
	HA06	48.19 ± 5.02	62.6
Clethodim	S	6.55 ± 0.43	1.0
	YC07	17.57 ± 1.88	2.7
	YZ09	9.77 ± 1.17	1.5
	SQ03	6.13 ± 0.49	0.9
	HA06	10.11 ± 1.17	1.5
Sethoxydim	S	8.05 ± 0.45	1.0
	YC07	106.64 ± 7.78	13.2
	YZ09	11.33 ± 0.49	1.4
	SQ03	10.00 ± 0.34	1.2
	HA06	46.05 ± 3.51	5.7
Pinoxaden	S	15.18 ± 1.67	1.0
	YC07	81.58 ± 6.56	5.4
	YZ09	42.80 ± 6.75	2.8
	SQ03	35.53 ± 4.38	2.3
	HA06	62.54 ± 6.15	4.1
Bispyribac-sodium	S	66.62 ± 10.46	1.0
	YC07	67.93 ± 15.23	1.0
	YZ09	90.62 ± 17.92	1.4
	SQ03	82.49 ± 18.26	1.2
	HA06	97.13 ± 18.63	1.5
Pyraclonil	S	31.82 ± 5.54	1.0
	YC07	23.19 ± 4.92	0.7
	YZ09	28.63 ± 6.41	0.9
	SQ03	29.32 ± 6.85	0.9
	HA06	24.50 ± 3.24	0.8
Quinclorac	S	154.64 ± 21.18	1.0
	YC07	188.21 ± 19.67	1.2
	YZ09	163.11 ± 17.94	1.1
	SQ03	150.32 ± 23.81	1.0
	HA06	135.38 ± 21.68	0.9
Anilofos	S	1,131.29 ± 251.61	1.0
	YC07	1,210.77 ± 282.42	1.1
	YZ09	1,375.44 ± 158.23	1.2
	SQ03	1,296.36 ± 235.65	1.1
	HA06	1,086.58 ± 217.78	1.0

[a]GR_{50}, herbicide dose causing 50% growth reduction; RI, resistance index.

Allelic variants in *ACCase* genes are the most commonly reported TSR mechanisms and have been characterized as yielding resistance in different kinds of grass weeds, including *Alopecurus* spp. (Petit et al. 2010), American sloughgrass [*Beckmannia syzigachne* (Steud.) Fernald] (Tang et al. 2015), *Echinochloa* spp. (Iwakami et al. 2024), *E. indica* (Deng et al. 2023a), *L. chinensis* (Deng et al. 2023b), and *L. rigidum* (Yu et al. 2007). For *Digitaria* spp., the Ile-1781-Leu and Trp-2027-Cys mutations have been reported to confer high-level resistance to ACCase-inhibiting herbicides in previous studies (Basak et al. 2020; Cao et al. 2023; Yang et al. 2023). The other two mutations, Trp-2027-Ser and Ile-2041-Asn, were first reported in the *ACCase* of *Digitaria* spp. Although NTSR mechanisms were not investigated in the current

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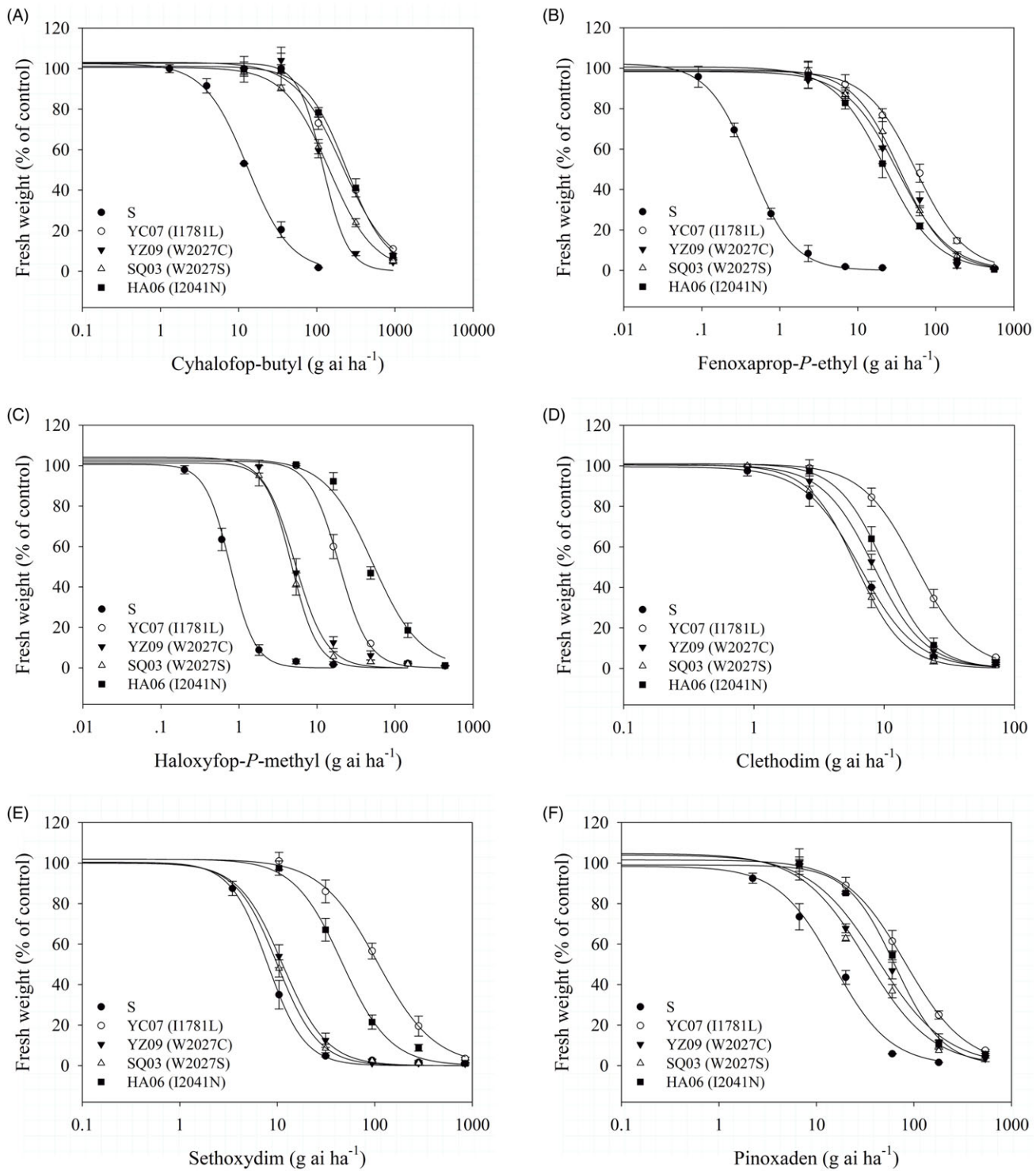


Figure 3. Dose–response curves of the susceptible (S) and four resistant (R) (YC07, YZ09, SQ03, and HA06) *Digitaria ciliaris* var. *chrysoblephara* populations to different acetyl-CoA carboxylase (ACCase)-inhibiting herbicides: (A) cyhalofop-butyl, (B) fenoxaprop-*P*-ethyl, (C) haloxyfop-*P*-methyl, (D) clethodim, (E) sethoxydim, and (F) pinoxaden.

study, P450-involved metabolism was determined to mediate ACCase resistance in certain populations in our previous paper (Yang et al. 2023). In addition, both TSR and NTSR mechanisms simultaneously participating in ACCase-inhibiting herbicide resistance have been identified in some other grass weed species (Golmohammadzadeh et al. 2020; Zhao et al. 2019, 2022). Hence, although we focused on the target-site *ACCase* gene mutations in *D. ciliaris* var. *chrysoblephara* in this paper, non–target site enhanced metabolism resistance was not ruled out in these resistant populations.

Cross-Resistance Patterns of Specific Mutations to Different Herbicides

Four R populations, YC07, YZ09, SQ03, and HA06, which carried the target *ACCase* Ile-1781-Leu, Trp-2027-Cys, Trp-2027-Ser, and

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Ile-2041-Asn mutations, respectively, were used for determining cross-resistance profiles to other herbicides with different MOAs. The results showed that all four R populations exhibited cross-resistance to several specific ACCase inhibitors, but no resistance to the ALS inhibitor bispyribac-sodium, the PPO inhibitor pyraclonil, the synthetic auxin herbicide quinclorac, and the VLCFA inhibitor anilofos, indicating that *D. ciliaris* var. *chrysoblephara* developed exclusive resistance to ACCase herbicides in rice-cropping systems (Table 3). The whole-plant experiment indicated that the S plants died completely at the recommended dose of each ACCase inhibitor (Figure 3). All four R populations exhibited different levels of cross-resistance to cyhalofop-butyl, fenoxaprop-*P*-ethyl, haloxyfop-*P*-methyl, and pinoxaden, producing 10.0- to 19.9-fold, 53.7- to 132.8-fold, 6.2 to 62.6-fold, and 2.3- to 5.4-fold decreases in sensitivity compared with the S population, respectively (Table 3; Figure 3). In addition, the YC07 (Ile-1781-Leu) and HA06 (Ile-2041-Asn) populations were resistant to sethoxydim (RI = 13.2 and 5.7, respectively), and the YC07 population was resistant to clethodim (RI = 2.7) as well (Table 3; Figure 3). It should also be noted that none of the four herbicides with other MOAs could kill the S population if they were applied at rates below the recommended field dosage recommended (data not shown), suggesting these herbicides were unable to control this weed due to poor effectiveness.

Based on the results, we concluded that the Ile-1781-Leu substitution conferred wide-spectrum resistance to all of the APP, CHD, and DEN herbicides tested, which is in line with previous research in other weed species (Basak et al. 2020; Deng et al. 2019; Petit et al. 2010; Yu et al. 2007). Ile-1781 is within a domain close to the binding site for ACCase herbicides across three chemical groups, explaining the resistance spectrum to all three families (Beckie and Tardif 2012; Gaines et al. 2020). In contrast, two substitutions at codon position 2027, Trp-2027-Cys and Trp-2027-Ser, endowed resistance to the APP and DEN herbicides, but susceptibility to the CHD herbicides. Similar results were obtained in Trp-2027-Cys and Trp-2027-Ser mutant *L. chinensis*, which showed resistance to metamifop, cyhalofop-butyl, and fenoxaprop-*P*-ethyl (APPs), but no resistance to clethodim (CHD) (Jiang et al. 2022). Moreover, the HA06 population carrying an Ile-2041-Asn mutation was resistant to the APP herbicides, the DEN herbicide, and the CHD herbicide sethoxydim, but susceptible to the CHD herbicide clethodim. Accordingly, the *ACCase* mutations at amino acid positions 1781, 2027, and 2041 exhibited varied cross-resistance patterns to ACCase inhibitors.

In addition, the whole-plant bioassays showed poor control of *D. ciliaris* var. *chrysoblephara* with other postemergence herbicides registered for rice cropping, including bispyribac-sodium, pyraclonil, quinclorac, and anilofos. Thus far, in rice fields, few alternative herbicides can be used for controlling *D. ciliaris* var. *chrysoblephara*, except ACCase inhibitors. Our previous study reported that three preemergence herbicides, including pretilachlor, pendimethalin, and oxadiazon, could greatly inhibit the seed germination of this weed (Yang et al. 2023). On the basis of this analysis, reasonable use of preemergence herbicides can greatly reduce the selection pressure of postemergence herbicides and thus mitigate the risk of ACCase-inhibiting herbicide resistance in *D. ciliaris* var. *chrysoblephara*. In combination with chemical control, agricultural and ecological measures, such as crop rotation, straw mulching, and depleting the weed seedbank, can effectively improve the biodiversity of agroecosystems and reduce the number of viable seeds in the soil (Zhang et al. 2021; Zhu et al. 2020). Therefore, integrated approaches including chemical and nonchemical control can be employed to develop diverse and sustainable weed management strategies in cropping systems.

In conclusion, it is evident that the *D. ciliaris* var. *chrysoblephara* in direct-seeded rice fields has evolved resistance to metamifop in Jiangsu Province, China. Four *ACCase* mutations, Ile-1781-Leu, Trp-2027-Cys/Ser, and Ile-2041-Asn, were found in resistant *D. ciliaris* var. *chrysoblephara* plants, resulting in various cross-resistance profiles to ACCase herbicides. Few postemergence herbicides can be selected for controlling *D. ciliaris* var. *chrysoblephara* plants in rice cultivation except ACCase inhibitors, and this poses a huge challenge to weed management for farmers. Integrated weed management approaches need to be adopted to delay the resistance evolution in *D. ciliaris* var. *chrysoblephara* and promote sustainable development of agroecosystems.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wsc.2024.30

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References

Bagavathiannan MV, Norsworthy JK, Smith KL, Neve P (2014) Modeling the simultaneous evolution of resistance to ALS- and ACCase-inhibiting herbicides in barnyardgrass (*Echinochloa crus-galli*) in Clearfield® rice. Weed Technol 28:89–103

Basak S, McElroy JS, Brown AM, Gonçalves CG, Patel JD, McCullough PE (2020) Plastidic *ACCase* Ile-1781-Leu is present in pinoxaden-resistant southern crabgrass (*Digitaria ciliaris*). Weed Sci 68:41–50

Beckie HJ, Tardif FJ (2012) Herbicide cross resistance in weeds. Crop Prot 35:15–28

Cao J, Tao Y, Zhang Z, Gu T, Li G, Lou Y, Wang H (2023) Mechanism of metamifop resistance in *Digitaria ciliaris* var. *chrysoblephara* from Jiangsu, China. Front Plant Sci 14:1133798

de Carvalho LB, Alves PL, González-Torralva F, Cruz-Hipolito HE, Rojano-Delgado AM, De Prado R, Gil-Humanes J, Barro F, de Castro MD (2012) Pool of resistance mechanisms to glyphosate in *Digitaria insularis*. J Agric Food Chem 60:615–622

Délye C, Jasieniuk M, Le Corre V (2013) Deciphering the evolution of herbicide resistance in weeds. Trends Genet 29:649–658

Deng W, Cai J, Zhang J, Chen Y, Chen Y, Di Y, Yuan S (2019) Molecular basis of resistance to ACCase-inhibiting herbicide cyhalofop-butyl in Chinese sprangletop (*Leptochloa chinensis* (L.) Nees) from China. Pestic Biochem Physiol 158:143–148

Deng W, Li Y, Yao S, Duan Z, Yang Q, Yuan S (2023a) *ACCase* gene mutations and P450-mediated metabolism contribute to cyhalofop-butyl resistance in *Eleusine indica* biotypes from direct-seeding paddy fields. Pestic Biochem Physiol 194:105530

Deng W, Li Y, Yao S, Wu J, Zhu A, Yang Q, Yuan S (2023b) Current status of cyhalofop-butyl and metamifop resistance and diversity of the *ACCase* gene mutations in Chinese sprangletop (*Leptochloa chinensis*) from China. Pestic Biochem Physiol 197:105648

Deng W, Yang M, Li Y, Xia Z, Chen Y, Yuan S, Yang Q (2021) Enhanced metabolism confers a high level of cyhalofop-butyl resistance in a Chinese sprangletop (*Leptochloa chinensis* (L.) Nees) population. Pest Manag Sci 77:2576–2583

Gaines TA, Duke SO, Morran S, Rigon CAG, Tranel PJ, Küpper A, Dayan FE (2020) Mechanisms of evolved herbicide resistance. J Biol Chem 295:10307–10330

Golmohammadzadeh S, Rojano-Delgado AM, Vázquez-García JG, Romano Y, Osuna MD, Gherekhloo J, De Prado R (2020) Cross-resistance mechanisms to ACCase-inhibiting herbicides in short-spike canarygrass (*Phalaris brachystachys*). Plant Physiol Biochem 151:681–688

Guo W, Zhang TJ, Zhang C, Tian XS (2022) Response of seed germination and seedling establishment of *Digitaria sanguinalis* to different environmental factors. Plant Prot 48:85–93. Chinese

Iwakami S, Ishizawa H, Sugiura K, Kashiwagi K, Oga T, Niwayama S, Uchino A (2024) Syntenic analysis of *ACCase* loci and target-site-resistance mutations in cyhalofop-butyl resistant *Echinochloa crus-galli* var. *crus-galli* in Japan. Pest Manag Sci 80:627–636

Jiang M, Wang Y, Li Wei, Li Q, Zhang J, Liao M, Zhao N, Cao H (2022) Investigating resistance levels to cyhalofop-butyl and mechanisms involved in Chinese sprangletop (*Leptochloa chinensis* L.) from Anhui Province, China. Pestic Biochem Physiol 186:105165

Jiang Y, Chen G, Dong L (2017) Resistance level of *Digitaria sanguinalis* to post-emergence herbicides frequently used in rice fields. J Weed Sci 35:67–72. Chinese

Jin M, Chen L, Deng XW, Tang X (2022) Development of herbicide resistance genes and their application in rice. Crop J 10:26–35

Kaundun SS (2014) Resistance to acetyl-CoA carboxylase-inhibiting herbicides. Pest Manag Sci 70:1405–1417

Khammassi M, Hajri H, Menchari Y, Chaabane H, Souissi T (2019) Current status in resistance to ACCase and ALS-inhibiting herbicides in rigid ryegrass populations from cereal crops in North of Tunisia. J Agric Sci 157: 676–683

Laforest M, Soufiane B, Simard MJ, Obeid K, Page E, Nurse RE (2017) Acetyl-CoA carboxylase overexpression in herbicide-resistant large crabgrass (*Digitaria sanguinalis*). Pest Manag Sci 73:2227–2235

Li J, Li M, Gao X, Fang F (2017) A novel amino acid substitution Trp574Arg in acetolactate synthase (ALS) confers broad resistance to ALS-inhibiting herbicides in crabgrass (*Digitaria sanguinalis*). Pest Manag Sci 73:2538–2543

Li Q, Zhao N, Jiang M, Wang M, Zhang J, Cao H, Liao M (2023) Metamifop resistance in *Echinochloa glabrescens* via glutathione *S*-transferase-involved enhanced metabolism. Pest Manag Sci 79:2725–2736

Liu X, Hou Z, Zhang Y, Merchant A, Zhong M, Ma G, Zeng Q, Wu L, Zhou X, Luo K, Ding C (2023) Cloning and functional characterization of a tau class glutathione transferase associated with haloxyfop-*P*-methyl resistance in *Digitaria sanguinalis*. Pest Manag Sci 79:3950–3958

Petit C, Bay G, Pernin F, Délye C (2010) Prevalence of cross or multiple resistance to the acetylcoenzyme A carboxylase inhibitors fenoxaprop, clodinafop and pinoxaden in black-grass (*Alopecurus myosuroides* Huds.) in France. Pest Manag Sci 66:168–177

Powles SB, Yu Q (2010) Evolution in action: plants resistant to herbicides. Annu Rev Plant Biol 61:317–347

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-logistic analysis of herbicide dose-response relationships. Weed Technol 9:218–227

Sun P, Niu L, Lan X, Yu H, Cui H, Chen J, Li X (2023) Enhanced metabolic resistance mechanism endows resistance to metamifop in *Echinochloa crus-galli* (L.) P. Beauv. Pestic Biochem Physiol 197:105656

Takano HK, Melo MSC, Ovejero RFL, Ovejero RFL, Westra PH, Todd A, Gaines TA, Dayan FE (2020) Trp2027Cys mutation evolves in *Digitaria insularis* with cross-resistance to ACCase inhibitors. Pestic Biochem Physiol 164:1–6

Tang W, Zhou F, Zhang Y, Chen J (2015) Resistance of American sloughgrass (*Bechmannia syzigachne*) populations to ACCase-inhibiting herbicides involves three different target site mutations from China. Pestic Biochem Physiol 124: 93–96

Yang Q, Yang X, Zhang Z, Wang J, Fu W, Li Y (2021) Investigating the resistance levels and mechanisms to penoxsulam and cyhalofop-butyl in barnyardgrass (*Echinochloa crus-galli*) from Ningxia Province, China. Weed Sci 69:422–429

Yang Q, Yang X, Zhu J, Wei T, Lv M, Li Y (2022) Metabolic resistance to acetyl-CoA carboxylase-inhibiting herbicide cyhalofop-butyl in a Chinese *Echinochloa crus-galli* population. Agronomy 12:2724

Yang Q, Zhu J, Yang X, Wei T, Lv M, Li Y (2023) Ile-1781-Leu target mutation and non-target-site mechanism confer resistance to acetyl-CoA carboxylase-inhibiting herbicides in *Digitaria ciliaris* var. *chrysoblephara*. J Agric Food Chem 71:7988–7995

Yanniccari M, Vázquez-García JG, Gigón R, Palma-Bautista C, Vila-Aiub M, De Prado D (2022) A novel EPSPS Pro-106-His mutation confers the first case of glyphosate resistance in *Digitaria sanguinalis*. Pest Manag Sci 78:3135–3143

Yu J, McCullough PE, Czarnota MA (2017) First report of acetyl-CoA carboxylase–resistant southern crabgrass (*Digitaria ciliaris*) in the United States. Weed Technol 31:252–259

Yu Q, Ahmad-Hamdani MS, Han H, Christoffers MJ, Powles SB (2013) Herbicide resistance-endowing ACCase gene mutations in hexaploidy wild oat (*Avena fatua*): insights into resistance evolution in a hexaploid species. Heredity 110:220–231

Yu Q, Collavo A, Zheng MQ, Owen M, Sattin M, Powles SB (2007) Diversity of acetyl-coenzyme A carboxylase mutations in resistant *Lolium* populations: evaluation using clethodim. Plant Physiol 145:547–558

Yuan S, Di Y, Chen Y, Chen Y, Cai J, Deng W (2019) Target-site resistance to cyhalofop-butyl in bearded sprangletop (*Diplachne fusca*) from China. Weed Sci 67:534–538

Zhang L, Wang W, Du Y, Deng Y, Bai T, Ji M (2023) Multiple resistance of *Echinochloa phyllopogon* to synthetic auxin, ALS-, and ACCase-inhibiting herbicides in northeast China. Pestic Biochem Physiol 193:105450

Zhang Y, Chen L, Song W, Cang T, Xu M, Wu C (2022) Diverse mechanisms associated with cyhalofop-butyl resistance in Chinese sprangletop (*Leptochloa chinensis* (L.) Nees): characterization of target-site mutations and metabolic resistance-related genes in two resistant populations. Front Plant Sci 13:990085

Zhang Z, Li R, Zhao C, Qiang S (2021) Reduction in weed infestation through integrated depletion of the weed seed bank in a rice-wheat cropping system. Agron Sustain Dev 41:10

Zhao B, Xu X, Li B, Qi Z, Huang J, Hu A, Wang G, Liu X (2023) Target-site mutation and enhanced metabolism endow resistance to nicosulfuron in a *Digitaria sanguinalis* population. Pestic Biochem Physiol 194:105488

Zhao N, Ge L, Yan Y, Bai S, Wang D, Liu W, Wang J (2019) Trp-1999-Ser mutation of acetyl-CoA carboxylase and cytochrome P450s-involved metabolism confer resistance to fenoxaprop-*P*-ethyl in *Polypogon fugax*. Pest Manag Sci 75:3175–3183

Zhao N, Jiang M, Li Q, Gao Q, Zhang J, Liao M, Cao H (2022) Cyhalofop-butyl resistance conferred by a novel Trp-2027-Leu mutation of acetyl-CoA carboxylase and enhanced metabolism in *Leptochloa chinensis*. Pest Manag Sci 78:1176–1186

Zhu J, Wang J, DiTommaso A, Zhang C, Zheng G, Liang W, Islam F, Yang C, Chen X, Zhou W (2020) Weed research status, challenges, and opportunities in China. Crop Prot 134:104449

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