scientific reports

OPEN

A five-year examination into the occurrence of herbicide-resistant barnyardgrass populations in paddy from Jiangsu Province, China

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To assess resistance situation and evolutionary risks, 510 *Echinochloa* populations from 13 rice-growing regions in Jiangsu Province (2018–2022) were tested against seven herbicides (penoxsulam, quinclorac, cyhalofop-butyl, bispyribac-sodium, pretilachlor, metamifop, and florpyrauxifen-benzyl), with cross- and multiple-resistance patterns analyzed. Penoxsulam resistance increased ninefold over five years, while quinclorac resistance consistently exceeded 40% annually for four years. Cyhalofop-butyl and bispyribac-sodium resistance frequencies also rose annually, with the strongest resistance to penoxsulam and bispyribac-sodium observed in southern Jiangsu, particularly in Suzhou, Wuxi, Changzhou, and Zhenjiang. In northern Jiangsu, Huaian showed the highest resistance to multiple herbicides, while quinclorac resistance was widespread across all regions. Pretilachlor and metamifop resistance remained low, with only sporadic outbreaks, indicating that they continued to be used. However, prolonged use of single-site herbicides, particularly ALS inhibitors and ACCase inhibitors, has led to cross-resistance evolution. Multiple-resistance analysis indicated that quinclorac, penoxsulam, and cyhalofop-butyl should not be used in binary or ternary mixtures to control resistant *Echinochloa*. Notably, 14 populations exhibited florpyrauxifen-benzyl resistance, with 13 also showing quinclorac resistance, suggesting a potential link between prior quinclorac resistance and florpyrauxifen-benzyl resistance evolution, which warrants further investigation. This study clarifies herbicide resistance patterns in *Echinochloa* in Jiangsu Province, offering critical insights for resistance management strategies.

Keywords *Echinochloa*, Herbicide, Resistance frequency, Multiple resistance

Rice (*Oryza sativa* L.) is one of the three major staple crops globally, with more than half of the world's population relying on rice as their main food source[1]. Weed infestation in farmland is a significant biotic factor that restricts high and stable rice yields. It is estimated that weeds cause a reduction of up to 10 billion kilograms of rice annually in China, with an average loss rate of around 15%, leading to substantial economic losses[2]. *Echinochloa* is one of the top ten most pernicious weeds worldwide and is among the most damaging weeds in rice fields. It is widely distributed and poses severe threats in all rice-growing regions of China[3].As a C4 plant, *Echinochloa* exhibits strong ecological tolerance in the field, competing with crops for water, nutrients, space, and light. It continuously invades and harms crops throughout its entire growth period, making it one of the major biological factors limiting efficient agricultural production[4,5]. *Echinochloa* exhibits a Vavilovian mimicry, making its seedlings very similar to rice, which inevitably leads to rice yield losses[6,7]. The seed production of *Echinochloa* shows high plasticity, primarily depending on local growth conditions, nutrient availability, and daylight duration, and this reproductive ability is also influenced by competition with other crops[8,9]. When rice coexists with species such as *Echinochloa colona*, *Echinochloa crus-galli*, and *Echinochloa glabrescens*, the above-ground growth of rice during the heading stage is significantly inhibited, resulting in yield losses ranging from 10.6–46.5%[10]. *Echinochloa* also interferes with the photosynthetic rate and dry matter accumulation during the rice grain-filling period, leading to declines in both yield and quality[11]. An increase in *Echinochloa* control by 10% can result in an increase of 14 panicles per square meter and an additional 750 kg per hectare of rice

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yield[12]. In the context of global warming and water scarcity, the competitive ability of *Echinochloa* may further enhance[13].

Chemical herbicides are the most economical and effective strategy for controlling weeds in farmland[14]. By the late 1990s, herbicides were widely used for weed control in China, and by 2015, the country had emerged as a major global producer and consumer of herbicides[15]. Herbicides such as pretilachlor, penoxsulam, quinclorac, metamifop, cyhalofop-butyl, and bispyribac-sodium are favored for their effectiveness against *Echinochloa* (Table 1). Bispyribac-sodium and penoxsulam, with nearly 20 years history of field use, are herbicides whose chemical structures belong to the pyrimidinylthiobenzoates and triazolopyridines, respectively[16,17]. They both inhibit acetolactate synthase (ALS) enzyme activity in weeds, disrupting the biosynthesis of branched-chain amino acids and impairing protein synthesis, which ultimately suppresses weed growth and leads to their death[18]. Metamifop and cyhalofop-butyl belong to the aryloxy phenoxy propionate structural subgroup, and their herbicidal mechanism involves inhibiting acetyl CoA carboxylase (ACCase) activity in grass weeds, thereby obstructing fatty acid synthesis[19]. Pretilachlor, a soil-applied herbicide used in China for nearly 30 years, is typically applied between crop sowing and germination in direct-seeded rice fields[20], often combined with the safener CCA123407 to inhibit weed protein synthesis. Quinclorac, a synthetic auxin herbicide with nearly 30 years of application history, is believed to achieve selective weed control by inducing cyanide production, a byproduct of ethylene biosynthesis in plants[4]. Florpyrauxifen-benzyl, a novel aryl-pyridine carboxylate herbicide developed by Dow AgroSciences, was first registered globally in China in 2017 and is considered a promising solution for *Echinochloa* resistance[21].

Herbicide resistance refers to the ability of weeds to survive and reproduce despite being exposed to herbicide doses that would normally be lethal. Excessive reliance on and prolonged use of herbicides with a single action site can lead to the development of herbicide resistance in weeds[22]. From an evolutionary biology perspective, human interventions can dramatically influence the evolution of resistance. *Echinochloa*, due to its polyploid nature and genetic diversity, evolves resistance more rapidly. In recent years, the issue of *Echinochloa* resistance has become increasingly prominent, showing a rising trend annually, resulting in severe reductions in crop yields and even crop failure in some regions. Even more problematic is the emergence of cross-resistance and multiple resistance in *Echinochloa* populations, which makes management more challenging. This is because weeds may develop resistance to different chemical subgroups of the same target site or to herbicides with different modes of action. This significantly shortens the effective lifespan of herbicides and increases agricultural production costs. Previous studies have identified the mechanisms of resistance to several common herbicides in *Echinochloa* populations in China's rice-growing regions, often related to target site mutations and metabolic enzyme mediation[23–27]. At the same time, conducting large-scale surveys to determine the frequency of resistance and urgently investigate the resistance status of *Echinochloa* in different regions is crucial for promoting targeted control measures.

Jiangsu Province (E 116°18' – 121°57', N 30°45' – 35°20') is one of China's top rice-producing regions, with yields consistently exceeding 35 billion kilograms for ten consecutive years. Administratively, Jiangsu province is divided into 13 cities and geographically into Southern Jiangsu, Northern Jiangsu, and Central Jiangsu (**Figure S1**). Given the diversity of *Echinochloa* species in Jiangsu's rice fields and the long history of herbicide use, 510 *Echinochloa* populations were collected from 13 cities of Jiangsu from 2018 to 2022. Resistance screening was conducted using several commonly used herbicides, and an investigation was carried out on the frequency of resistance and the occurrence of multiple and cross-resistance. This research aims to elucidate the herbicide resistance evolution of *Echinochloa* in Jiangsu's rice fields *Echinochloa*, provide insights into regional resistance status, predict resistance trends, and offer references for managing resistant *Echinochloa* in rice fields, thereby promoting the green and sustainable development of rice cultivation.

Materials and methods

Seed collection

Echinochloa seeds in paddy were sampled from August to October during 2018–2022. From 13 prefecture-level cities in Jiangsu Province, 99, 115, 100, 106, and 93 *Echinochloa* populations have been collected in the five consecutive years 2018 to 2022 accordingly (**Table S1**).

The collections were conducted by driving to all 13 cities in Jiangsu Province, with 3–5 sampling points collected from each village, which were then combined to form a single sample. The design of the sampling locations aimed to distribute the *Echinochloa* collection sites and provide representative *Echinochloa* populations from rice fields where farmers had failed to control *Echinochloa* weeds. After selecting the collection sites, seeds

Herbicide	Mode of action	Chemical subgroup	Application method	First registration in China
Bispyribac-sodium	ALS	Pyrimidinylthiobenzoates	Stem and leaf	2004
Penoxsulam	ALS	Triazolopyrimidines	Stem and leaf	2004
Metamifop	ACCase	Aryloxy phenoxy propionate	Stem and leaf	2010
Cyhalofop-butyl	ACCase	Aryloxy phenoxy propionate	Stem and leaf	1998
Quinclorac	Synthetic hormones	Quinoline carboxylic acids	Stem and leaf	1993
Florpyrauxifen-benzyl	Synthetic hormones	Pyridine carboxylic acids	Stem and leaf	2017
Pretilachlor	Cell division inhibitor	Chloramides	Soil	1992

Table 1. Several herbicides used in paddy in China.

were harvested from 10 to 30 individuals *Echinochloa* to create a composite sample for that site, referred to as a population. Each location's longitude and latitude were recorded using a handheld GPS device. The seeds were labeled with collection site information and stored in brown paper bags. After collection, the seeds were dried using an electric hot air oven and then stored in a temperature and humidity-controlled seed storage cabinet for future use.

Seedlings culture and resistance screening

The *Echinochloa* seeds were cleaned using sieves and a blower to ensure that mature seeds from each collection site had an equal probability of being collected. After overcoming seed dormancy, resistance screening was conducted at the agricultural experiment station of the Jiangsu academy of agricultural sciences. About 10–15 seeds from each population were sown in a plastic pot, with each pot serving as one replication. Each population was sown in four separate pots to ensure four biological replicates. A mixture of loamy soil and substrate ($V:V=1:1$) was used, and the soil was sterilized with high-pressure steam (121 °C, 20 min) before use to prevent the growth of any other weeds during the seedling stage. *Echinochloa* seed germination and seedling cultivation are conducted outdoors in June. Because the average temperature in Nanjing, Jiangsu Province, has ranged from 21 °C to 29 °C during the last five years, and the minimum temperature exceeds 16.5 °C (from http://data.cma.cn/dataService/cdcindex/datacode/A.0012.0001/show_value/normal.html), the conditions are suitable for *Echinochloa* growing. A transparent plastic film was placed over the trays to maintain warmth and moisture until the seeds germinated, and seedlings were grown outdoors until they reached the 3–5 leaf stage.

When the seedlings reached the 3–5 leaf stage, herbicides were applied using a laboratory sprayer with a cone nozzle (Model: 3 WPSH-500D, Nanjing Agricultural Mechanization Research Institute, China, with a delivery rate of 280 L ha^{-1} at 0.3 MPa). The survival rates were observed 28 days after treatment, and the number of resistant and susceptible populations was recorded. The herbicide information utilized in the experiments is shown in Table 2, and all pesticides used in this experiment are recommended.

Cross resistance and multiple resistance validation

If a population can survive treatment with herbicides that have the same target site but different chemical structures, it is considered to exhibit cross resistance. For example, a population resistant to both bispyribac-sodium and penoxsulam is considered to have cross-resistance, or a population resistant to both metamifop and cyhalofop-butyl is also considered to have cross-resistance. Populations resistant to herbicides with different modes of action are considered to exhibit multiple resistance.

Data analysis and ploting

After screening with single herbicide doses, the number of surviving populations was recorded. Susceptibility was assessed based on plant survival rates following herbicide application. *Echinochloa* spp. populations were classified as 'susceptible' (S) if the survival rate was less than 20%, and 'resistant' (R) if the survival rate was 20% or higher, based on the resistance classification system introduced by Busi et al.[28]. The annual resistance frequency is calculated as Xn/Pn, where Xn represents the number of populations resistant to the herbicide in year *n*, and Pn denotes the total number of populations tested for resistance to the herbicide in the same year. Similarly, the regional annual resistance frequency is calculated as X1n/P1n, where X1n indicates the number of populations resistant to the herbicide in a specific region in year *n*, and P1n refers to the total number of populations tested for resistance to the herbicide in that region during the same year. Graphs were created using Origin 2019b.

Results

The annual resistance frequency of *Echinochloa* to several herbicides in paddy of Jiangsu Province

The six herbicides used in this study—penoxsulam, quinclorac, metamifop, pretilachlor, cyhalofop-butyl, and bispyribac-sodium—have been applied in rice fields for at least 15 years. It is essential to evaluate the annual resistance frequency of *Echinochloa* to these herbicides over consecutive years. The annual resistance frequency

Herbicide	Manufacturer	Pesticide preparation	Recommended dose
Bispyribac-sodium	Kumiai Chemical Industry Co., Ltd.	100 g/L SC	23 g a.i. ha^{-1}
Penoxsulam	Corteva Agriscience Corporation	25 g/L OD	30 g a.i. ha^{-1}
Metamifop	FMC Corporation	10% EC	120 g a.i. ha^{-1}
Cyhalofop-butyl	Qingdao KingAgroot CropScience Co., Ltd.	40% OD	90 g a.i. ha^{-1}
Pretilachlor	Syngenta Group Co., Ltd.	300 g/L EC	450 g a.i. ha^{-1}
Quinclorac	Jiangsu Institute of Ecomones Co., Ltd.	50% WP	375 g a.i. ha^{-1}
Florpyrauxifen-benzyl (Rinskor™)	Corteva Agriscience Corporation	3% EC	30 g a.i. ha^{-1}

Table 2. Herbicide information used in resistance determination. Notes: SC (Suspension Concentrate); OD (Oil Dispersion); EC (Emulsifiable Concentrate); WP (Wettable Powder).

Herbicide	2018	2019	2020	2021	2022
Penoxsulam	2.02%	9.57%	26.00%	29.13%	19.35%
Quinclorac	26.26%	56.52%	41.00%	47.57%	50.54%
Metamifop	1.01%	3.48%	2.00%	5.83%	0.00%
Pretilachlor	6.06%	7.83%	1.00%	-	-
Cyhalofop-butyl	-	-	19.00%	41.75%	22.58%
Bispyribac-sodium	-	-	-	4.85%	29.03%
Florpyrauxifen-benzyl -methyl	-	-	-	-	16.13%

Table 3. The annual resistance frequency of *Echinochloa* populations to herbicides in 2018–2022.

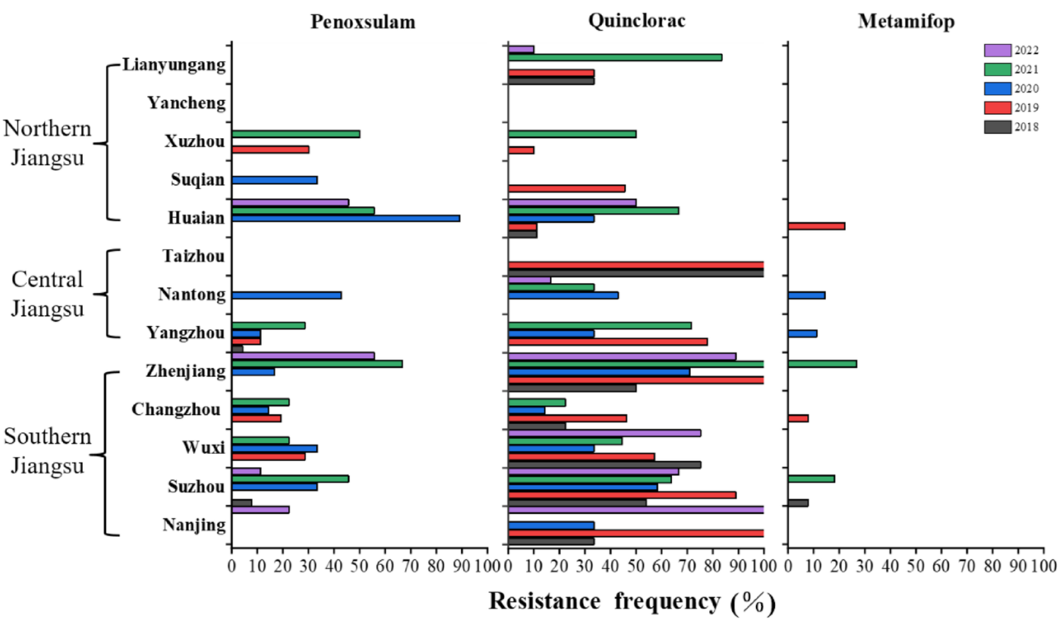

Fig. 1. The regional annual resistance frequency of *Echinochloa* in paddy fields to penoxsulam, quinclorac, and metamifop in 13 cities of Jiangsu province from 2018 to 2022. No populations were collected at the following sampling points: Nantong (2018), Suqian (2018, 2022), Taizhou (2020–2022), Xuzhou (2020, 2022), Changzhou(2022), and Yangzhou (2022), so no resistance frequency data was available.

of 510 *Echinochloa* populations to the paddy herbicides in 2018–2022 was investigated (Table 3). The resistance frequency of *Echinochloa* in Jiangsu to penoxsulam showed a cumulative increase from 2018 to 2021, with a slight decrease in 2022, but still reached 19.35%. The resistance frequency to quinclorac showed fluctuations within a certain range, but remained above 40.0% from 2019 to 2022. The resistance frequency to metamifop over 5 years never exceeded 6.0%. Data from 3 years of trials showed that the resistance frequency to pretilachlor never exceeded 8.0%. The resistance frequency of *Echinochloa* to cyhalofop-butyl ranged from 19.00 to 41.75% during 2020–2022. The resistance frequency to bispyribac-sodium was 4.85% in 2021 and 29.03% in 2022.

Frequency difference of herbicide resistance in 13 cities of Jiangsu Province

From 2018 to 2022, resistance surveys were conducted on *Echinochloa* populations collected five years using the herbicides penoxsulam, quinclorac, and metamifop (Fig. 1). In the annual resistance screening for penoxsulam, resistant populations were detected in Suzhou, Wuxi, Changzhou, Zhenjiang, Yangzhou, and Huaian for at least three years. The annual average resistance frequencies for penoxsulam were 19.5%, 16.8%, 13.9%, 27.8%, 13.7%, and 40.0% in above regions (excluding years with no available samples). Huaian and Zhenjiang exhibited notably high annual average resistance frequencies to penoxsulam, both exceeding 25.0%. Over the course of five years, no populations in Lianyungang were discovered to be resistant to penoxsulam. With smaller sample sizes, Taizhou and Yancheng were less representative. Across the broader region, Southern Jiangsu reported 14 counts of penoxsulam-resistant populations, with detections in five agricultural zones, suggesting that resistance to penoxsulam is more prevalent in this area. In contrast, Northern Jiangsu recorded only 6 counts, but the annual resistance frequencies in its three agricultural zones ranged from 30.0 to 88.9%.

Except for Suqian and Yancheng, the detection of quinclorac-resistant populations was extremely common in other regions (Fig. 1). The highest regional annual average quinclorac resistance frequencies were observed in Zhenjiang (81.9%), while the resistance frequencies in Nanjing (53.3%), Suzhou (66.3%), and Wuxi (57.0%) all exceeded 50%. In some places, the regional annual resistance frequency of *Echinochloa* to quinclorac was an incredible 100.0%. Notably, it was 100% in Taizhou during 2018 and 2019, in Zhenjiang during 2019 and 2021, and in Nanjing during 2019 and 2022. Across the broader region, Southern Jiangsu reported 23 counts of quinclorac-resistant populations, with annual resistance frequencies ranging from 14.3 to 100.0% across its five agricultural zones. Northern Jiangsu recorded 12 counts, with resistance frequencies ranging from 10.0 to 83.3% across its five agricultural zones. Central Jiangsu recorded 8 counts in three agricultural zones, with resistance frequencies ranging from 16.7 to 100.0%.

Resistance to metamifop remained generally low, with sporadic detections in specific regions in certain years (Fig. 1). In the five-year monitoring across 13 regions, a total of 7 detections of resistant populations were recorded, involving 6 agricultural zones, with the highest annual resistance frequency observed in Zhenjiang in 2021 at 26.7%, and the lowest in Changzhou in 2019 at 7.7%. In the remaining seven regions, no resistant populations were found. These results suggest that metamifop still maintains good efficacy against *Echinochloa*.

As shown in Fig. 2, resistance screening of *Echinochloa* populations collected from 2018 to 2020 revealed that resistance to pretilachlor was detected in only a few regions and years. The highest annual resistance frequency was observed in Suzhou in 2019 (33.3%), while the lowest was in Zhenjiang in 2020 (4.2%). High resistance frequencies appeared as sporadic outbreaks. Overall, the herbicide still shows significant potential for use.

Screening for resistance to cyhalofop-butyl was conducted on *Echinochloa* populations collected from 2020 to 2022 (Fig. 3). The results reveal that resistance was detected in Suzhou, Wuxi, and Zhenjiang over the three-year investigation, with regional average annual resistance frequencies of 36.6%, 35.2%, and 34.6%, respectively. When *Echinochloa* collected by Southern Jiangsu was tested once a year, a total of 13 batches tests found resistant populations, with annual resistance frequencies across the five cultivation zones ranging from 8.3 to 73.3%. During the annual testing of *Echinochloa* populations from Northern Jiangsu, no cyhalofop-butyl-resistant populations were detected in Suqian or Yancheng. However, four batches resistant populations were identified in Lianyungang, Xuzhou, and Huaian, with the highest resistance frequency of 50% observed in the Huai'an population in 2022. In the annual testing of *Echinochloa* populations from Central Jiangsu, resistance was detected in four batches, with the highest frequency reaching 57.1% in the Nantong population in 2020.

In 2021 and 2022, resistance testing of *Echinochloa* to bispyribac-sodium was carried out across 13 cities in Jiangsu Province. varying levels of resistance (Fig. 4). Except for Changzhou, Taizhou, and Suqian, resistant populations were detected in all other 10 cultivation zones. The highest annual resistance frequency was observed in Xuzhou in 2021, with 50%. Overall, the number of resistance population detection batches was the lowest in Central Jiangsu, with a lower resistance frequency. The resistance detection batches and annual resistance frequencies in Southern and Northern Jiangsu were similar.

Cross resistance

As shown in Fig. 5, cross-resistance analysis was conducted for the ACCase-targeted herbicides (cyhalofop-butyl and metamifop), and the ALS-targeted herbicides (penoxsulam and bispyribac-sodium). In 2020, 2021, and 2022, cross-resistance to ACCase inhibitor herbicides was assessed in 100, 103, and 93 *Echinochloa* populations, respectively. In 2020, two populations demonstrated cross-resistance to both cyhalofop-butyl and metamifop, for a cross-resistance frequency of 2.0%. In 2021, six populations demonstrated cross-resistance, at a frequency

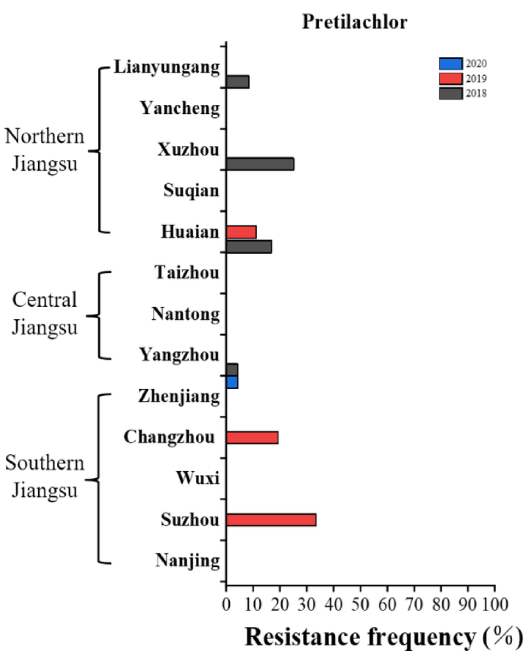

Fig. 2. Resistance status of *Echinochloa* in paddy fields to pretilachlor in 13 cities of Jiangsu province from 2018 to 2020. No populations were collected at the following sampling points: Nantong (2018), Suqian (2018), Taizhou (2020), and Xuzhou (2020), so no resistance frequency data was available.

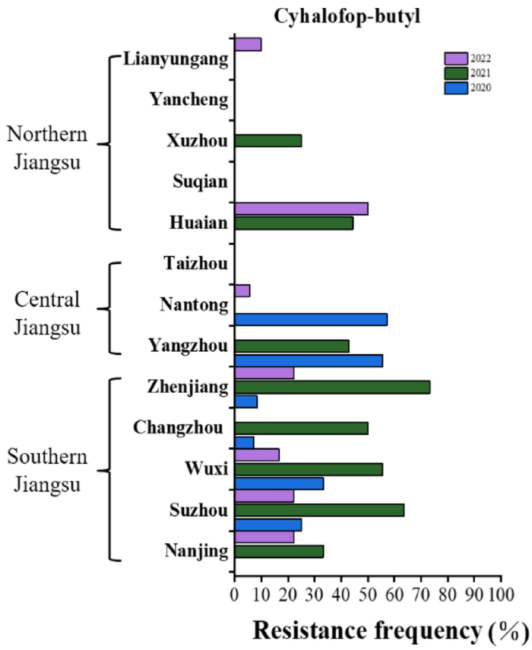

Fig. 3. Resistance status of *Echinochloa* in paddy fields to cyhalofop-butyl in 13 cities of Jiangsu province from 2020 to 2022. No populations were collected at the following sampling points: Suqian (2022), Taizhou (2020–2022), Xuzhou (2020, 2022), Changzhou(2022), and Yangzhou (2022), so no resistance frequency data was available.

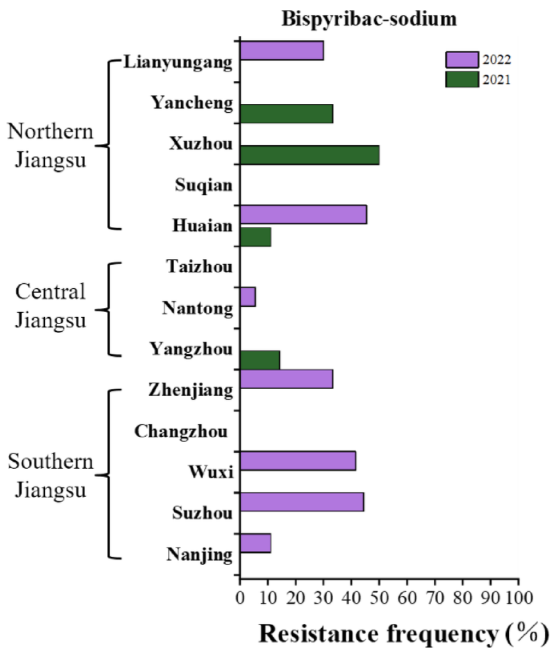

Fig. 4. Resistance frequency of *Echinochloa* to bispyribac-sodium in 13 cities of Jiangsu province from 2021 to 2022. No populations were collected at the following sampling points: Suqian (2022), Taizhou (2021, 2022), Xuzhou (2022), Changzhou(2022), and Yangzhou (2022), so no resistance frequency data was available.

of 5.8%. In 2022, no cross-resistant populations were discovered. A total of 8 *Echinochloa* populations exhibiting cross-resistance to ACCase inhibitors cyhalofop-butyl and metamifop were found, distributed across Zhenjiang (6 populations), Changzhou (1 population), and Nantong (1 population). Overall, the proportion of cross-resistance to the two ACCase inhibitors is relatively low, and the number of cross-resistant populations is almost entirely concentrated in Southern Jiangsu.

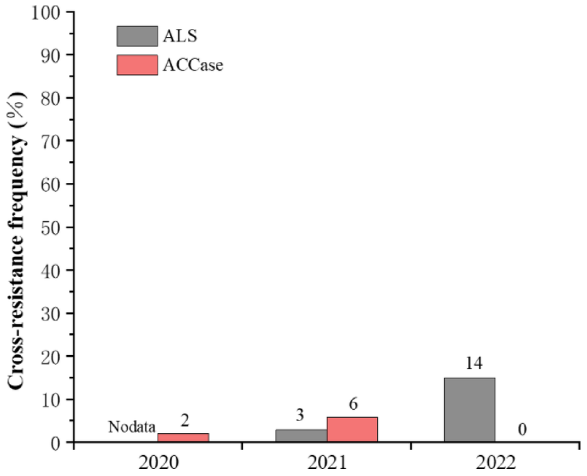

Fig. 5. The cross-resistance to ALS or ACCase inhibitor herbicides in 2020–2022.

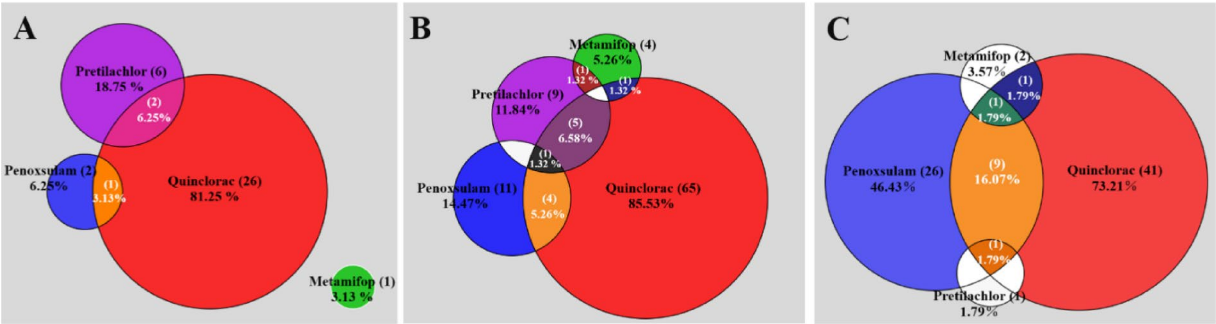

Fig. 6. Multi-resistance frequency of *Echinochloa* populations to penoxsulam, quinclorac, metamifop, and pretilachlor in (**A**) 2018, (**B**) 2019, and (**C**) 2020.

In 2021 and 2022, cross-resistance to ALS inhibitor herbicides was measured in 103 and 93 *Echinochloa* populations, respectively (Fig. 5). In 2021, three populations showed cross-resistance to both penoxsulam and bispyribac-sodium, resulting in a cross-resistance frequency of 2.9%. In 2022, 14 cross-resistant populations were found, with a cross-resistance frequency of 15.1%. A total of 17 populations exhibiting cross-resistance to penoxsulam and bispyribac-sodium were identified, distributed across Zhenjiang (3 populations), Nanjing (2 populations), Suzhou (2 populations), Yangzhou (1 population), and Huaian (9 populations). It is worth noting that these ALS inhibitor cross-resistant populations are almost equally distributed between Southern and Northern Jiangsu, with a very low proportion in Central Jiangsu.

Multiple resistance

Multiple resistance in *Echinochloa* spp., defined as resistance to two or more herbicides with different modes of action, has emerged as a growing concern in rice fields. Monitoring its frequency is crucial for informed resistance management. Figure 6 shows the frequency of multiple resistance in *Echinochloa* populations to four herbicides—penoxsulam, quinclorac, metamifop, and pretilachlor—during the period from 2018 to 2020. In 2018, multiple resistance was detected in *Echinochloa* populations, with 32 populations found to be resistant to at least one of the four herbicides: penoxsulam, quinclorac, metamifop, and pretilachlor. Among these, one population exhibited dual resistance to penoxsulam and quinclorac, while two populations were resistant to both quinclorac and pretilachlor. In 2019, a total of 76 populations were resistant to at least one of the same four herbicides. Among them, four populations exhibited dual resistance to quinclorac and penoxsulam, five to quinclorac and pretilachlor, one to quinclorac and metamifop, and one to pretilachlor and metamifop. Additionally, one population showed triple resistance to quinclorac, penoxsulam, and pretilachlor. In 2020, 56 populations were found to be resistant to at least one of the four herbicides. Among them, nine populations exhibited dual resistance to quinclorac and penoxsulam, and one to quinclorac and metamifop. Moreover, one population displayed triple resistance to quinclorac, penoxsulam, and metamifop, and another to quinclorac, penoxsulam, and pretilachlor. These findings indicate a rising trend in multiple resistance over the three years, highlighting the urgent need for integrated weed management strategies to mitigate the further spread of resistant *Echinochloa* populations.

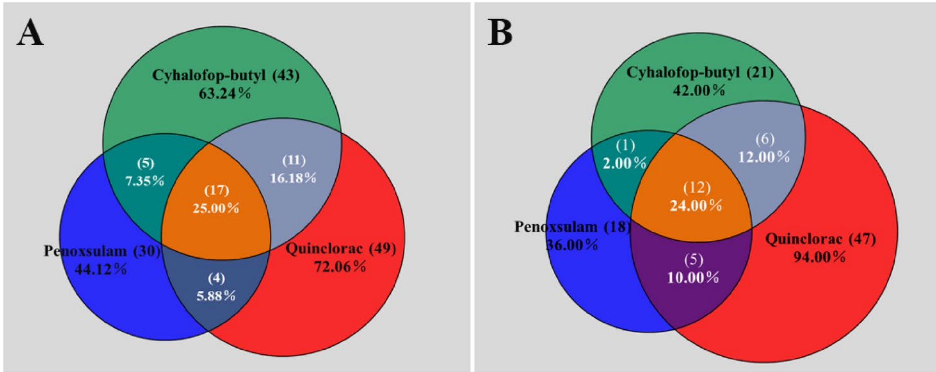

Fig. 7. Multi-resistance frequency of *Echinochloa* populations to penoxsulam, quinclorac, and cyhalofop-butyl in (**A**) 2021 and (**B**) 2022.

Statistics of multiple resistance		2018	2019	2020	2021	2022
R-quinclorac & penoxsulam	Populations	1	5	11	21	17
	Ratio (%)	1.0	4.4	11.0	20.4	18.3
R-quinclorac & pretilachlor	Populations	2	6	1	-	-
	Ratio (%)	2.0	5.2	1.0	-	-
R-pretilachlor & penoxsulam	Populations	0	1	0	-	-
	Ratio (%)	0.0	0.9	0.0	-	-
R-quinclorac &penoxsulam & metamifop or pretilachlor	Populations	0	1	2	-	-
	Ratio (%)	0.0	0.9	2.0	-	-
R-quinclorac & cyhalofop-butyl	Populations	-	-	-	28	18
	Ratio (%)	-	-	-	27.2	19.4
R-penoxsulam & cyhalofop-butyl	Populations	-	-	-	23	13
	Ratio (%)	-	-	-	22.3	14.0
R-quinclorac & penoxsulam & cyhalofop-butyl	Populations	-	-	-	17	12
	Ratio (%)	-	-	-	16.5	12.9

Table 4. Number and proportion of *Echinochloa* populations with multiple resistance to several herbicides (2018–2022). Notes: The ratio was calculated as the number of populations with multiple resistance divided by the total number of populations tested each year, expressed as a percentage and rounded to one decimal place.

The multi-resistance frequency of *Echinochloa* populations to penoxsulam, quinclorac, and cyhalofop-butyl from 2021 to 2022 is described in Fig. 7. In 2021, a total of 68 populations were found to have resistance to at least one of the three herbicides: penoxsulam, quinclorac, and cyhalofop-butyl. Among these, four populations showed dual resistance to quinclorac and penoxsulam, eleven populations showed dual resistance to quinclorac and cyhalofop-butyl, and five populations showed dual resistance to cyhalofop-butyl and penoxsulam. Additionally, seventeen populations showed triple resistance to quinclorac, penoxsulam, and cyhalofop-butyl.

In 2022, a total of 50 populations were found to have resistance to at least one of the three herbicides. Among these, five populations exhibited dual resistance to quinclorac and penoxsulam, six populations showed dual resistance to quinclorac and cyhalofop-butyl, and one population demonstrated dual resistance to cyhalofop-butyl and penoxsulam. Furthermore, twelve populations exhibited triple resistance to quinclorac, penoxsulam, and cyhalofop-butyl.

The number of multi-herbicide-resistant *Echinochloa* populations and their corresponding annual proportions from 2018 to 2022 were summarized (Table 4). From 2018 to 2022, "R-quinclorac & penoxsulam" populations exhibited an upward trend in both population count and proportion over the years, reaching 18.3% in 2022 despite a slight decline that year. From 2018 to 2020, "R-quinclorac & pretilachlor" and "R-pretilachlor & penoxsulam" had relatively low population counts and proportions. Over a three-year period, only three populations of "R-quinclorac, penoxsulam, metamifop or pretilachlor" were discovered. "R-quinclorac & cyhalofop-butyl" was detected in 28 and 18 populations in 2021 and 2022, with proportions of 27.2% and 19.4%, respectively. Similarly, "R-penoxsulam & cyhalofop-butyl" was detected in 23 and 13 populations in 2021 and 2022, respectively, with proportions of 22.3% and 14.0%. Notably, "R-quinclorac & penoxsulam & cyhalofop-butyl" was discovered in 17 and 12 populations in 2021 and 2022, accounting for 16.5% and 12.9%, respectively.

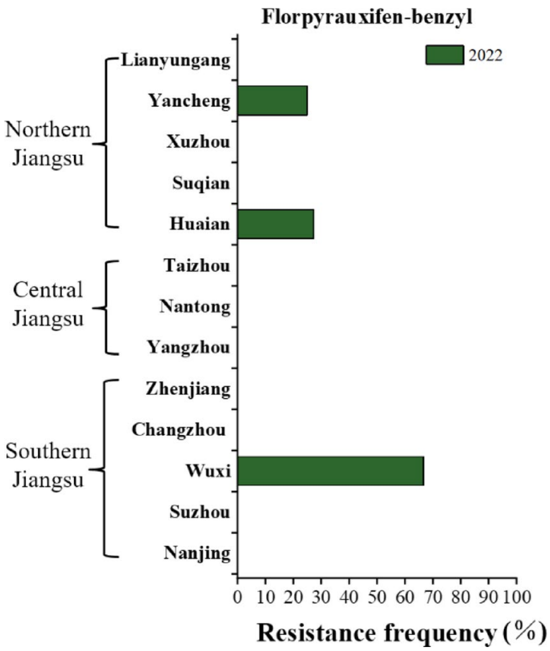

Fig. 8. Resistance status of *Echinochloa* in paddy fields to florpyrauxifen-benzyl in 13 cities of Jiangsu province in 2022. No populations from 2022 were collected at the following sampling points: Suqian, Taizhou, Xuzhou, Changzhou, and Yangzhou, so no resistance frequency data was available.

Detection of suspected resistant *Echinochloa* populations to florpyrauxifen-benzyl

Florpyrauxifen-benzyl, marketed as Rinksor, is a herbicide with a novel mechanism of action and is considered promising for addressing herbicide-resistant weeds in rice fields. Resistance screening of *Echinochloa* populations collected in 2022 was conducted with florpyrauxifen-benzyl (Fig. 8). Fifteen resistant populations were found in Wuxi (8), Huaian (6), and Yancheng (1), which survived 21 days after a single-dose treatment. The remaining 10 sampling sites showed no survival of barnyard grass populations after treatment. In 2022, 16.13% of the 93 barnyard grass populations tested showed resistance to florpyrauxifen-benzyl (Table 3). The further analysis showed that 14 of the 15 populations were resistant to quinclorac. Overall, most *Echinochloa* populations remain highly sensitive to florpyrauxifen-benzyl, with resistance only sporadically occurring in specific areas. Apart from Wuxi, Huaian, and Yancheng, no florpyrauxifen-benzyl-resistant populations were detected in other regions of Jiangsu Province. This indicates that *Echinochloa* remains highly sensitive to this herbicide in most areas, suggesting broad potential for its application. Although *Echinochloa* seedlings were able to survive after treatment, the impact on their later-stage reproductive capacity and growth restrictions to crops is still unclear and requires further evaluation.

Discussion

Trend of annual resistance frequency from 2018 to 2022

Long-term use of herbicides with a single mode of action can exert significant selection pressure on weeds, resulting in an increase in the prevalence, geographic spread, and resistance levels of resistant weed populations[29]. According to data from the International Herbicide-Resistant Weed Database, the majority of global cases of herbicide-resistant *Echinochloa* are found in ALS inhibitors and ACCase inhibitors, accounting for two-thirds of all cases[30]. In this study, a significant upward trend in annual resistance frequency to the ALS inhibitor penoxsulam was observed over a short span of five years, with the 2022 value being more than nine times that of 2018. Studies have shown that repeated applications of ALS and ACCase inhibitor herbicides can accelerate resistance selection in *Echinochloa*, and even herbicide rotation, sequencing, or mixing may not be effective if the time intervals between applications are too short[31]. The declining agricultural labor force in China has driven the widespread promotion and adoption of lightweight wet direct-seeded rice cultivation systems, resulting in shifts in weed community composition and an overall increase in weed incidence[32]. However, whether the large-scale adoption of direct-seeded rice will accelerate the evolution of herbicide resistance remains unreported.

The occurrence of quinclorac-resistant weeds has been frequently reported in China[33]. This study revealed a spiraling upward trend in quinclorac resistance frequency, exceeding 40.0% from 2019 to 2022. Such widespread resistance urgently calls for an in-depth analysis of its underlying mechanisms. The resistance frequency of *Echinochloa* to cyhalofop-butyl ranged from 19.00 to 41.75% between 2020 and 2022, indicating a probable continuous increase in the coming years. The resistance frequency to metamifop and pretilachlor has remained relatively low, indicating a slower spread of resistant populations and highlighting their continued potential for effective application. Metamifop and cyhalofop-butyl, both ACCase inhibitors, exhibit a marked differential in resistance frequency, probably attributable to the considerably shorter usage history of metamifop—approximately ten years less than that of cyhalofop-butyl[34,35]. Although pretilachlor has been applied in rice fields for several decades, the frequency of resistance development remains relatively low[36]. This could potentially be attributed to its application as a soil treatment, which may slow down the evolution of weed resistance by targeting seeds and emerging seedlings at an early stage, reducing selection pressure on established weed populations[37,38].

In addition to conducting regular annual surveys, tracking year-by-year changes in resistance levels would offer a more comprehensive understanding. *Echinochloa* species are diverse, and there are significant differences in biological chara cteristics among species[39], as well as different sensitivities to herbicides. Therefore, performing classification surveys to pinpoint the specific *Echinochloa* species driving the surge in resistance frequency would be particularly meaningful.

The disparity in the annual resistance frequency of *Echinochloa* across several regions

In the five-year survey on penoxsulam resistance, the resistance frequency to penoxsulam was highest in southern Jiangsu, particularly in Suzhou, Wuxi, Changzhou, and Zhenjiang, for at least three consecutive years. Northern and central Jiangsu also showed higher resistance frequencies in certain cultivation areas, with Yangzhou and Huaian being particularly notable. The regional differences in resistance frequencies may be attributed to variations in field application practices and rice cultivation patterns.

Except for Yancheng, resistance to quinclorac has been widely detected across various regions, warranting significant attention from agricultural producers. Even at low resistance levels, the rapid accumulation of resistance genes could pose a substantial challenge in the coming years. When the use of quinclorac is unavoidable, applying it at the maximum recommended rate is essential to delay the accelerated development of resistance[40–42].

The resistance frequency to metamifop was relatively low, not exceeding 6.0% in any region. Resistance to pretilachlor was only found in a few regions during certain years, with the highest annual resistance frequency in Suzhou (33.3%) and the lowest in Zhenjiang and Yangzhou (4.2%). As a pre-emergent herbicide, pretilachlor's effectiveness varies among *Echinochloa* populations in different regions. Although high resistance frequencies to metamifop and pretilachlor occasionally occur in isolated outbreaks, these herbicides still retain significant potential for effective use.

A three-year investigation into cyhalofop-butyl resistance revealed that the highest and most widespread resistance frequencies were concentrated in southern Jiangsu, including five cultivation areas: Nanjing, Suzhou, Wuxi, Changzhou, and Zhenjiang. In central Jiangsu, resistance was most notable in Yangzhou and Nantong, while in northern Jiangsu, severe outbreaks were limited to specific areas, such as Huaian.

Resistance frequency surveys for bispyribac-sodium conducted in 2021 and 2022 revealed that central Jiangsu had the fewest detected resistant populations and the lowest resistance frequency. In contrast, southern and northern Jiangsu exhibited more severe resistance, with similar levels of annual resistance frequencies.

Cross resistance

Cross-resistance, where resistance to one herbicide also confers resistance to another, can significantly reduce the effectiveness of herbicide rotations, mixtures, and multiple-HR crop technologies, typically in weeds with non-target site resistance mechanisms[43]. Cross-resistance to three ACCase-inhibiting herbicides, cyhalofop-butyl (CyB), fenoxaprop-ethyl (FeE), and quizalofop-ethyl (QuE), has been reported in resistant biotypes of *Echinochloa crus-galli* (L.) P. Beauv[44]. The overall incidence rate remained low, with ACCase cross-resistance in *Echinochloa* occurring at frequencies of 2.0% in 2020, 5.8% in 2021, and 0% in 2022. Six of the eight *Echinochloa* populations with ACCase cross-resistance identified over the three years were located in Zhenjiang. Riar et al. reported that barnyardgrass (*Echinochloa crus-galli*) biotypes AR1 and AR2 from Arkansas exhibited cross-resistance to imazamox, imazethapyr, and penoxsulam, while AR1 and the MS1 biotype from Mississippi also showed resistance to bispyribac-sodium[45]. Among 14 *Echinochloa crus-galli* populations resistant to at least one ALS-inhibiting herbicide, seven exhibited high cross-resistance to all ALS inhibitors tested, including azimsulfuron (SU), penoxsulam (PTB), imazamox (IMI), and bispyribac-sodium (PTB), as reported in Italian rice fields[46]. This study identified 17 *Echinochloa* populations with cross-resistance to ALS-inhibiting herbicides penoxsulam and bispyribac-sodium, 9 of which are located in Huaian. Interestingly, cross-resistance to ALS and ACCase inhibitors in *Echinochloa* was predominantly found in Huaian and Zhenjiang, respectively, rather than being evenly distributed across all regions. To prevent the spread of cross-resistance in these specific regions, it is essential to conduct regular monitoring of its occurrence. Additionally, the rotation and alternation of herbicides with different modes of action within these regions should be emphasized to extend the effective lifespan of herbicides.

Multiple resistance

Herbicide mixtures are favored by pesticide manufacturers for their synergistic effects, broader spectrum of weed control, and increased commercial benefits[47]. However, with the rise of multi-resistant weed populations, these mixtures may lose their synergistic effectiveness, leading to suboptimal control results. Multiple resistance in *Echinochloa* has surged—R-quinclorac & penoxsulam increased from 1% in 2018 to 18.3% in 2022. Double-resistant populations (R-quinclorac & cyhalofop-butyl and R-penoxsulam & cyhalofop-butyl) are now the most common, and over 10% of populations are triple-resistant to quinclorac, penoxsulam, and cyhalofop-butyl. This reflects the intensive use of these tank mixes in Jiangsu's rice fields and highlights the strong adaptive potential of *Echinochloa* under repeated selection pressure. Key resistance mechanisms include ALS and ACCase target-site mutations conferring resistance to penoxsulam and cyhalofop-butyl, along with enhanced detoxification of quinclorac through the ethylene biosynthesis pathway involving ACS and ACO enzymes[4,23,46]. To slow resistance development, implement integrated weed management—employ agronomic practices and ecological controls, alternate with nonchemical methods, and maximize the use of preemergence herbicides[48–50]. Future work should prioritize long-term monitoring of multiple resistance to improve assessment and management.

The sensitivity status of *Echinochloa* populations to florpyrauxifen-benzyl

In 2022, 15 resistant populations were identified among 93 tested populations, 14 of which also exhibited resistance to quinclorac. Both quinclorac and florpyrauxifen-benzyl control weeds by disrupting endogenous hormonal balance through complex mechanisms[51,52]. This complexity stems from the fact that most functional cells in plants can act as hormone receptors, and the pathways involved may vary significantly across different weed species. Research indicates that two resistant populations share a common resistance mechanism to florpyrauxifen-benzyl and penoxsulam—hydrolysis of a methoxy group followed by glucose conjugation, suggesting that some *E. crus-galli* populations may have evolved resistance prior to the commercialization of florpyrauxifen-benzyl[53]. After 24 h post florpyrauxifen-benzyl treatment, the susceptible (S) biotype of *Echinochloa crus-galli*(L.) exhibited an enhanced auxin response and elevated expression of genes linked to ethylene and abscisic acid production and signaling, in contrast to the resistant (R) biotype[54]. Zhao et al. analyzed the expression of seven IAA-related genes in R and S *Echinochloa crusgalli var. zelayensis*, suggesting that changes in IAA synthesis, conjugation, and oxidation led to reduced IAA induction in the resistant biotype, decreasing ethylene burst and facilitating quinclorac resistance[55]. Given the widespread quinclorac resistance and its longer use history, we hypothesize that the emergence of florpyrauxifen-benzyl resistance may be primarily driven by cross-resistance developed under prolonged quinclorac selection, rather than long-term evolutionary processes. Further studies should focus on determining the resistance levels of populations resistant to both quinclorac and florpyrauxifen-benzyl, as well as analyzing the underlying mechanisms. When promoting florpyrauxifen-benzyl, particular caution should be exercised in areas where *Echinochloa* has developed high resistance to quinclorac.

Conclusion

In this work, the annual resistance frequency to penoxsulam increased sharply, multiplying ninefold over five years. Quinclorac exhibited a steadily escalating resistance trend, with annual resistance frequencies exceeding 40%. From 2020 to 2022, the resistance frequency to cyhalofop-butyl ranged between 19.00% and 41.75%. Metamifop and pretilachlor showed relatively low resistance frequencies, with only sporadic outbreaks in certain regions. Although the efficacy of pretilachlor has deteriorated in some areas, its overall resistance levels remain low, indicating substantial potential for *Echinochloa* management in rice fields. Similarly, resistance to metamifop remains relatively low, making it a viable option for controlling *Echinochloa*. Although resistance to florpyrauxifen-benzyl was detected in 14 populations, it remains a promising herbicide. To address the issue of herbicide resistance, the rotation of herbicides based on different modes of action and application methods is essential for mitigating resistance and prolonging herbicide effectiveness. This study provides critical insights into the resistance status of *Echinochloa* in Jiangsu's rice fields, offering valuable guidance for managing resistant populations and supporting sustainable rice cultivation practices.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Received: 22 February 2025; Accepted: 23 April 2025
Published online: 28 April 2025

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Author contributions

Conceived and designed the experiments: Y.L. and X.Y. Collected public datasets and performed experiments: X.H., Y.X., C.L., H.M., Z.L. and Y.X. Analyzed the data: X.H. Wrote the manuscript: X.H. The final version of the manuscript was approved by all authors, who have agreed to take responsibility for the work in its entirety.

Funding
This research was supported by the National Key Research and Development Program of China (Grant No. 2023YFD1401100), the National Natural Science Foundation of China (Grant No. 32272565).

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-99846-4.

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