Resistance of Three Species of Barnyard Grasses to Quinclorac

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Abstract

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. *Echinochloa spp*. is one of the recognized malignant weeds in rice fields in China and even around the world, causing serious impacts on rice production. Long term use of a single herbicide has led to the development of herbicide resistance in many weeds. This study focuses on the resistance of three common types of *Echinochloa spp* in the middle and lower reaches of the Yangtze River: *E. crusgalli var. zelayensis*, *E. glabrescens*, and *E. cruspavonis* to quinolinic acid. Sensitive biotypes with consistent genetic backgrounds were isolated from them. Using the whole plant method, the relative resistance multiples of each resistant biotype were determined to be XLB-R: 672.38, B-R: 647.75, and KQB-R: 957.29, all showing extremely high resistance to dichloroquinoline acid. This laid the foundation for subsequent research on fitness cost and control of resistant *Echinochloa spp*.

Keywords

***Echinochloa spp*; Rice; Quinclorac; Resistance.**

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops in the world, with about half of the world's population relying on rice as their staple food. Since the beginning of the new century, machine broadcasting and live broadcasting technology have gradually become an important direction for the development of rice production, especially in the middle and lower reaches of the Yangtze River in China. The new model saves labor and costs, but the problem of grass damage is even more serious, which has seriously affected the yield and quality of rice. Weeds in rice fields have the characteristics of large population, multiple species, early occurrence, and rapid growth. They compete with rice for environmental resources, affecting its growth, yield, and quality. According to statistics, weeds occur in all rice growing areas in China, with a harmful area of 15 million hectares, accounting for about 45% of the rice planting area. About 10 million tons of rice are lost each year, with an average loss rate of about 15%. In severe stages, the yield reduction can even reach over 50%. Qiangsheng et al. analyzed the numerical characteristics of weed communities under different rice cultivation modes and found that the dominant weeds in direct seeding rice fields include *Echinochloa spp.* weeds, *Ammania baccifera* L., *Monochoria vaginalis,* and so on; The dominant weeds in dry direct seeding rice fields include weeds in the genus *Echinochloa spp, Ammania baccifera* L, *Euphorbia latiris* L. *Echinochloa spp* and *Ammania baccifera* L are common dominant weeds in various rice cultivation models. Among these weeds, barnyard grass is the most aggressive and harmful to rice, seriously affecting its growth and development, as well as its yield and quality[1-3].

Echinochloa spp, also known as barnyard grass, is native to Europe and India and widely distributed in warm regions of China and the world. It is one of the 18 types of malignant weeds worldwide and is also the most widespread and harmful malignant weed in rice paddies in

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China [4]. According to the classification basis provided by Flora of China published in 2013, Chinese barnyard grass can be divided into 8 species and 6 varieties, including *E. caudata*, *E. colonum*, *E. cruspavonis*, *E. esculenta*, *E. frumentacea*, *E. glabrescen*, *E. oryzoides*, *E. crusgalli var. austro japonensis*, *E. crusgalli var. Breviseta*, *E. crusgalli var. Mitis*, *E. crusgalli var. Praticola*, and *E. crusgalli var. zelayensis.* Barnyard grass is waterlogging resistant, hygroscopic, salt alkali resistant, widely distributed, and has strong environmental adaptability. It mainly enters rice fields through rice seeds, soil, and agricultural operations . Barnyard grass is closely related to rice in terms of morphological characteristics, growth and development cycle, physiological and biochemical metabolism, ecological environment requirements, etc.. Moreover, as a C_4 plant, barnyard grass is much stronger in growth situation, stress resistance, and competition for water, light, and soil nutrients than rice, and has a strong competitive advantage which severely inhibits the growth, development, and yield of rice[5-8]. At the same time, barnyard grass is also the host of rice planthoppers, giant borers, iron beetles, rice stink bugs, rice leafhoppers, rice sheath blight, rice blast, rice yellow dwarf disease and other pests and diseases [9-11], significantly increasing the frequency of rice field diseases and pests.

However, the widespread use of single herbicides throughout the year has led to an increasing number of weeds developing resistance under the powerful selection of herbicides, making the problem of weed resistance even more severe[12-13]. There are currently 521 unique cases (species x site of action) of herbicide resistant weeds globally, with 268 species (154 dicots and 114 monocots). Weeds have evolved resistance to 21 of the 31 known herbicide sites of action and to 165 different herbicides. Herbicide resistant weeds have been reported in 98 crops in 72 countries[14]. Quinclorac is the most commonly used herbicide for removing barnyard grass in rice paddies in China. It is a selective auxiliary herbicide that is mainly absorbed by the leaves and roots, transported through the xylem and phloem, leading to leaf atrophy and necrosis of new leaves. In 1997, the world's first case of quinclorac resistant barnyard grass was discovered and reported in a single season rice field in Spain [15]. Subsequently, quinclorac resistant barnyard grass was found in rice fields in Louisiana, Brazil, the Philippines, and Colombia in the United States [16-18]. In recent years, barnyard grass that has developed resistance to quinclorac has also been discovered in China.People discovered barnyard grass resistant to quinclorac in major rice producing areas such as Heilongjiang, Liaoning, Hubei, Zhejiang, and Guangdong [19-24]. The types of barnyard grass that develop resistance to quinclorac include *E. crus-galli var. crus-galli*, *E. crus-galli var. zelayensis*, *E. cruspavonis*., and *E. colonum* (L.) Link.

At present, the types and quantities of resistant barnyard grass to dichloroquinoline acid are increasing year by year, and the research on the resistance of barnyard grass to dichloroquinoline acid has become particularly important. Therefore, this study selected three common types of barnyard grass resistant to quinolinic acid in the middle and lower reaches of the Yangtze River basin as the research object. Through two generations of screening and cultivation, sensitive biotypes with consistent genetic backgrounds were isolated, and their resistance levels and multi resistance were studied. To provide theoretical support for the subsequent scientific management of resistant barnyard grass and to curb the frequency of its occurrence.

2. MATERIALS AND METHODS

2.1. Test materials

The seeds of barnyard grass used in the experiment were selected by our research group in the early stage to be resistant to quinclorac. The collection locations and historical background of the medication are shown in Table 1.

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Table 1. Seed collection information for testing

Types	Locality	Latitude and longitude	Background of herbicide application	Effect of prevention
E. crus-galli var. zelayensis	Jintan District, Jiangsu Province	119° 28' E, 31° 39' N	quinclorac	ordinary
E. crus-galli var. crus-galli	Liuhe District, Jiangsu Province	118° 49' E, 32° 90' N	quinclorac	ordinary
E. cruspavonis	Jurong County, Jiangsu Province	119° 8' E, 31° 48' N	quinclorac	ordinary

2.2. Test site

The screening work was conducted at the experimental site of the Old Plant Protection Institute of Jiangsu Academy of Agricultural Sciences (118 ° 52'E, 32 ° 01'N), and the resistance level analysis experiment was conducted in the greenhouse of the Plant Protection Institute of Jiangsu Academy of Agricultural Sciences.

2.3. Test reagent

50% quinclorac wettable powder (recommended dosage: 750 g/hm^2, Jiangsu Hormone Research Institute Co., Ltd.)

2.4. Test method

1) Separation of sensitive materials with consistent genetic background

In order to isolate sensitive biotype materials that are consistent with the genetic background of the resistant biotype, appropriate amounts of plump and mature seeds of three types of resistant barnyard grass were selected and placed in a refrigerator at 4 ℃ for 24 hours to release dormancy. Subsequently, the seeds were cultured and germinated in a light incubator (conditions: light cycle of 10D/14L, light intensity of 10000 lux, daytime temperature of 30 ℃, evening temperature of 25 ℃, relative humidity of 60%~85%). After the seeds sprout until exposed to white, they are transplanted, with 3 seeds per pot and 50 pots for each type of barnyard grass. After the seedlings produce 3-4 tillers, remove the tillers from the main stem and plant them separately. Divide the extracted tillers into two parts for cultivation. After the tillers survive, one part is treated with the recommended dose (750 g. ha-1) of quinclorac, and the other part is treated with four times the recommended dose of quinclorac. The main stem is not treated and used for seed retention. If the tillering plant dies during the recommended dose of quinclorac treatment, the seed bearing on the main stem is recorded as a sensitive material; If the tillering plant survives treatment with 4 times the recommended dose of quinclorac, the seeds bearing on the main stem are considered as resistant materials. Separate and cultivate the two biotype materials of each type of barnyard grass under the same environmental conditions, and harvest the first generation of seeds after maturity. Dry and store it at room temperature (25 ℃) for subsequent testing purposes.

After breaking the dormancy of the first generation seeds of three different biotypes of barnyard grass, they were placed on a seedling tray with a size of 0.54 meters × 0.28 m, 4 per tray × 8 holes) are filled with mixed nutrient soil (vermiculite: nutrient soil: quartz sand=1: 2: 1; pH value 5.6, organic matter content 1.4%). After watering with water, select full and mature seeds for sowing, 20~30 seeds per hole, repeat 4 holes, and cover them with fine sand. When the seedlings reach the 3-leaf stage, the recommended concentration of dichloroquinoline acid is sprayed on individuals with antagonistic biotypes to ensure that the resistant materials do not contain sensitive biotype individuals. Subsequently, the two biotypes of each type of barnyard grass were transplanted into flower pots and separated and cultured under the same

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environmental conditions. Before flowering, pollen cover was used to prevent cross pollination. After maturity, the second generation seeds were harvested, dried and stored at room temperature (25 ℃). At this point, the two biotypes have been separated, and resistant and sensitive biotype materials with consistent genetic backgrounds have been obtained. The seeds used in subsequent experiments are all the second generation.

2) Analysis of resistancelevels of three different biotypes of barnyard grass to quinclorac

Three different biotypes of barnyard grass seeds were subjected to dormancy breaking treatment, and after the treatment, the barnyard grass seeds were placed in a light incubator for cultivation and germination. Fill a plastic cup with a hole at the bottom with mixed nutrient soil, absorb water at the bottom until the soil moisture is saturated, wait for the barnyard grass seeds to sprout and become white, then transplant them into a plastic cup, with 12 seeds per cup, sowing at a depth of 0.5 cm, and covering the surface with a layer of fine sand. After transplanting, place it in a light incubator for cultivation, manage it with normal water and fertilizer, and wait for the barnyard grass to grow to the 3-leaf stage before preparing for spraying treatment.

Before spraying, perform seedling fixation treatment, remove the seedlings with abnormal growth, and leave 10 identical barnyard grass seedlings in each cup. After the completion of seeding, the stem and leaf of barnyardgrass were treated with spray of quinclorac from low to high dosage, and a blank control (CK) without medicine was set. Each treatment was repeated three times. The dosage of quinclorac for each biotype was shown in Table 2. The application dosage is set according to the commonly used dosage in the field. The recommended dosage for dichloroquinoline acid in the field is 750 g/hm_2. After applying the medicine, cultivate it normally. After 21 days, observe and record the death of barnyard grass, cut off the aboveground part of the plant, weigh the fresh weight, and calculate its fresh weight inhibition rate and growth inhibition medium GR_{50} value.

Table 2. Application dosage of resistant and sensitive biotypes of three types barnyard grass

Types of barnyard grass	Dose of herbicide application (g a.i.ha^{-1})						
XLB-S	0	6.25	12.5	25	50	100	200
B-S							
XQB-S							
XLB-R	0	200	400	800	1 600	3200	6400
B-R							
XQB-R							

3) Data processing

The collected experimental data was statistically analyzed using Excel 365, GraphPad Prism, and Sigmaplot 12.0. After ANOVA analysis, it was found that there were no significant differences between the repeated experiments. The data was processed using a dual logistic nonlinear regression model described by Seefeldt et al. to obtain the GR_{50} values of dichloroquinoline acid for these three different biotypes of barnyard grass, and the relative resistance index RI was calculated. The specific formula is as follows:

$$Y = C + \frac{D - C}{1 + \left(\frac{X}{GR_{50}}\right)^{b}}$$

In the formula, “Y” is the percentage of fresh weight on the mixed grassland measured under a specific herbicide dosage relative to the non spraying control; “X” is the dosage of herbicide; “C” is the lower limit of dose response; “D” is the upper limit of dose response; “B” is the

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slopeGR_{50} is the moderate amount of growth inhibition (the herbicide dosage when the aboveground biomass is inhibited by 50%).

Resistance index (RI)=resistant population GR50/sensitive population GR50. The calculation method for the inhibition rate of herbicides on weed bud growth is as follows:

$$\text{Fresh weight inhibition rate} = \frac{\text{Control fresh weight} - \text{Processing fresh weight}}{\text{Control fresh weight}} \times 100\%$$

3. RESULT

3.1. Separation of sensitive materials with consistent genetic background

In order to obtain resistant and sensitive biotype materials with consistent genetic backgrounds, each type of barnyard grass was divided into three groups by cloning and tillering. The screening of dichloroquinoline acid was performed on the three groups of barnyard grass, and the results are shown in Table 3. 38 resistant materials (XLB-R) with consistent genetic background and 11 sensitive materials (XLB-S) were selected from the original resistant Echinochloa crusgalli. 39 resistant materials (B-R) and 8 sensitive materials (B-S) were selected from the original resistant Echinochloa crusgalli. 40 resistant materials (XQB-R) and 9 sensitive materials (KQB-S) were selected from the original resistant Echinochloa crusgalli. Cultivate the resistant and sensitive biotype materials of each selected barnyard grass in the same environment separately, and collect the first generation seeds for subsequent experiments after maturity.

Cultivate the first generation seeds obtained, and treat the three resistant biotypes of barnyard grass with the recommended dosage of dichloroquinoline acid. After treatment, all of them can survive, and it is believed that there are no sensitive biotype individuals in them, which can be used for subsequent experiments. After the maturity of the barnyard grass, the second generation resistant and sensitive biotype materials were harvested and numbered. The sensitive and resistant biotypes of the Xilai barnyard grass were XLB-S and XLB-R; The sensitive and resistant biotypes of barnyard grass are B-S and B-R; The sensitive and resistant biotypes of peacock barnyard grass are KQB-S and KQB-R. At this point, the two biotypes have been separated, and resistant and sensitive biotype materials with consistent genetic backgrounds have been obtained. The seeds used in subsequent experiments are all second-generation seeds with this number.

3.2. Analysis of resistance levels of Three different biotypes of barnyard grass to Quinclorac

To clarify the resistance levels of three different biotypes of barnyard grass to dichloroquinoline acid, the whole plant method was used to determine them. This method is simple, feasible, and can be used in large batches for detection. It is commonly used for detecting the resistance of barnyard grass to dichloroquinoline acid. By comparing and analyzing biological indicators such as mortality rate or fresh dry weight inhibition rate of resistant and sensitive biotype materials, the level of resistance can be determined.

By using the whole plant method, the fresh weight changes of XLB-S and XLB-R, B-S and B-R, as well as KQB-S and KQB-R, three different biotypes of barnyard grass, were determined under the gradient concentration treatment of dichloroquinoline acid (see Figure 1), and their resistance index was calculated (see Table 4). The results showed that the sensitive biotype plants of three types of barnyard grass (XLB-S, B-S, KQB-S) showed slight growth inhibition after spraying 6.25 g a.i.ha-1 of dichloroquinoline acid. Compared with the sensitive biotype plants without spraying, the fresh weight decreased by 15.18% to 18.77%; After spraying 12.5 g a.i.ha-1 dose of dichloroquinoline acid, the plants showed significant growth inhibition. Compared with the sensitive plants without spraying, the fresh weight decreased by 41.45%~56.47%;

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After spraying a dose of 25 g a.i.ha-1 of dichloroquinoline acid, the growth inhibition of the plant worsened again, with a fresh weight reduction of 73.73%~87.71% and frequent death; After spraying a dose of 50 g a.i.ha-1 of dichloroquinoline acid, the fresh weight of the plants was only 3.81% to 7.90% of that of the untreated plants, and almost all of them died; After spraying doses of 100 and 200 g a.i.ha-1, all plants died, with a fresh weight of 0.

Table 3. Isolation and screening of sensitive biotypes of three barnyard grass

XLB	Quinclorac			B	Quinclorac			KQB	Quinclorac		
	CK	1×	4×		CK	1×	4×		CK	1×	4×
XLB-1	√	√	√	B-1	√	√	√	KQB-1	√	√	√
XLB-2	√	√	√	B-2	√	√	√	KQB-2	√	×	×
XLB-3	√	√	×	B-3	√	√	√	KQB-3	√	√	√
XLB-4	√	√	√	B-4	√	√	√	KQB-4	√	√	√
XLB-5	√	√	√	B-5	√	√	√	KQB-5	√	×	×
XLB-6	√	√	√	B-6	√	×	×	KQB-6	√	√	√
XLB-7	√	×	×	B-7	√	×	×	KQB-7	√	√	√
XLB-8	√	√	√	B-8	√	√	√	KQB-8	√	√	×
XLB-9	√	√	√	B-9	—	—	—	KQB-9	√	√	√
XLB-10	√	√	√	B-10	√	√	√	KQB-10	√	√	√
XLB-11	√	×	×	B-11	√	√	√	KQB-11	√	√	√
XLB-12	√	√	√	B-12	√	√	√	KQB-12	√	√	√
XLB-13	—	—	—	B-13	√	√	×	KQB-13	√	×	×
XLB-14	√	√	√	B-14	√	√	√	KQB-14	√	√	×
XLB-15	√	√	√	B-15	√	√	√	KQB-15	√	√	√
XLB-16	√	√	√	B-16	√	√	√	KQB-16	√	√	√
XLB-17	√	√	√	B-17	√	√	√	KQB-17	√	√	×
XLB-18	√	×	×	B-18	√	√	√	KQB-18	—	—	—
XLB-19	√	√	√	B-19	√	√	√	KQB-19	√	×	×
XLB-20	√	√	√	B-20	√	√	√	KQB-20	√	√	√
XLB-21	√	×	×	B-21	√	√	√	KQB-21	√	√	√
XLB-22	√	×	×	B-22	√	√	√	KQB-22	√	√	√
XLB-23	√	√	√	B-23	√	√	√	KQB-23	√	×	×
XLB-24	√	√	√	B-24	√	×	×	KQB-24	√	√	√
XLB-25	√	√	√	B-25	√	√	√	KQB-25	√	√	√
XLB-26	√	√	√	B-26	—	—	—	KQB-26	√	√	√
XLB-27	√	√	√	B-27	√	√	√	KQB-27	√	√	√
XLB-28	√	×	×	B-28	√	×	×	KQB-28	√	√	√
XLB-29	√	√	√	B-29	√	√	√	KQB-29	√	√	√
XLB-30	√	√	√	B-30	√	√	√	KQB-30	√	√	√
XLB-31	√	√	√	B-31	√	√	√	KQB-31	√	√	√
XLB-32	√	√	√	B-32	—	—	—	KQB-32	√	√	√
XLB-33	√	×	×	B-33	√	√	√	KQB-33	√	√	√
XLB-34	√	√	√	B-34	√	√	√	KQB-34	√	×	×
XLB-35	√	√	√	B-35	√	√	√	KQB-35	√	√	√
XLB-36	√	×	×	B-36	√	×	×	KQB-36	√	√	√
XLB-37	√	√	√	B-37	√	√	√	KQB-37	√	√	√
XLB-38	√	√	√	B-38	√	×	×	KQB-38	√	√	√
XLB-39	√	√	√	B-39	√	√	√	KQB-39	√	×	×
XLB-40	√	×	×	B-40	√	√	√	KQB-40	√	√	√
XLB-41	√	√	√	B-41	√	√	√	KQB-41	√	√	√
XLB-42	√	√	√	B-42	√	×	×	KQB-42	√	×	×
XLB-43	√	×	×	B-43	√	√	√	KQB-43	√	√	√
XLB-44	√	√	√	B-44	√	√	√	KQB-44	√	×	×
XLB-45	√	√	√	B-45	√	√	√	KQB-45	√	√	√
XLB-46	√	√	√	B-46	√	√	√	KQB-46	√	√	√
XLB-47	√	√	√	B-47	√	√	√	KQB-47	√	√	√
XLB-48	√	√	√	B-48	√	×	×	KQB-48	√	√	×
XLB-49	√	√	√	B-49	√	√	√	KQB-49	√	√	√
XLB-50	√	×	×	B-50	√	√	√	KQB-50	√	√	√

Note: (1) Mark "√" as a surviving plant, "×" as a dead plant, "—" as invalid plants that died before using herbicides;(2) "CK" is recorded as the survival of the main stem without the application of herbicide(Quinclorac), "1 ×" is recorded as the survival status of plants under the

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recommended dosage, "4 ×" is recorded as the survival status of the plant at 4 times the recommended dosage.

The growth inhibition of the three resistant biotypes of barnyard grass (XLB-R, B-R, KQB-R) was relatively mild. After spraying 200 g a.i.ha-1 of dichloroquinoline acid, compared with the non sprayed plants, the fresh weight decreased by 4.28% to 6.37%; After spraying 400 g a.i.ha-1 dose of dichloroquinoline acid, the plants showed a slight growth inhibition state, and compared with the non sprayed plants, the fresh weight decreased by 8.46% to 1.19%; After spraying 800 g a.i.ha-1 dose of dichloroquinoline acid, the fresh weight of the control plant decreased by 15.84% to 20.67%; After spraying a dose of 1600 g a.i.ha-1 of dichloroquinoline acid, the growth inhibition of the plants slightly increased, and compared to the non sprayed plants, the fresh weight decreased by 22.54% to 25.79%; After spraying a dose of 3200 g a.i.ha-1 of dichloroquinoline acid, the growth inhibition state of the plants was more obvious, which was about 28.82%~36.80% lower than the fresh weight of the plants without spraying; After spraying a dose of 6400 g a.i.ha-1 of dichloroquinoline acid, the growth inhibition status of the plant worsened again, and the fresh weight decreased by 40.14% to 49.33%.

The GR_{50} values of each biotype were obtained through regression equation fitting, and their resistance indices were determined to be XLB-R: 672.38, B-R: 647.75, and KQB-R: 957.29, respectively. This indicates that XLB-R, B-R, and KQB-R have strong resistance to dichloroquinoline acid, all reaching extremely high levels of resistance.

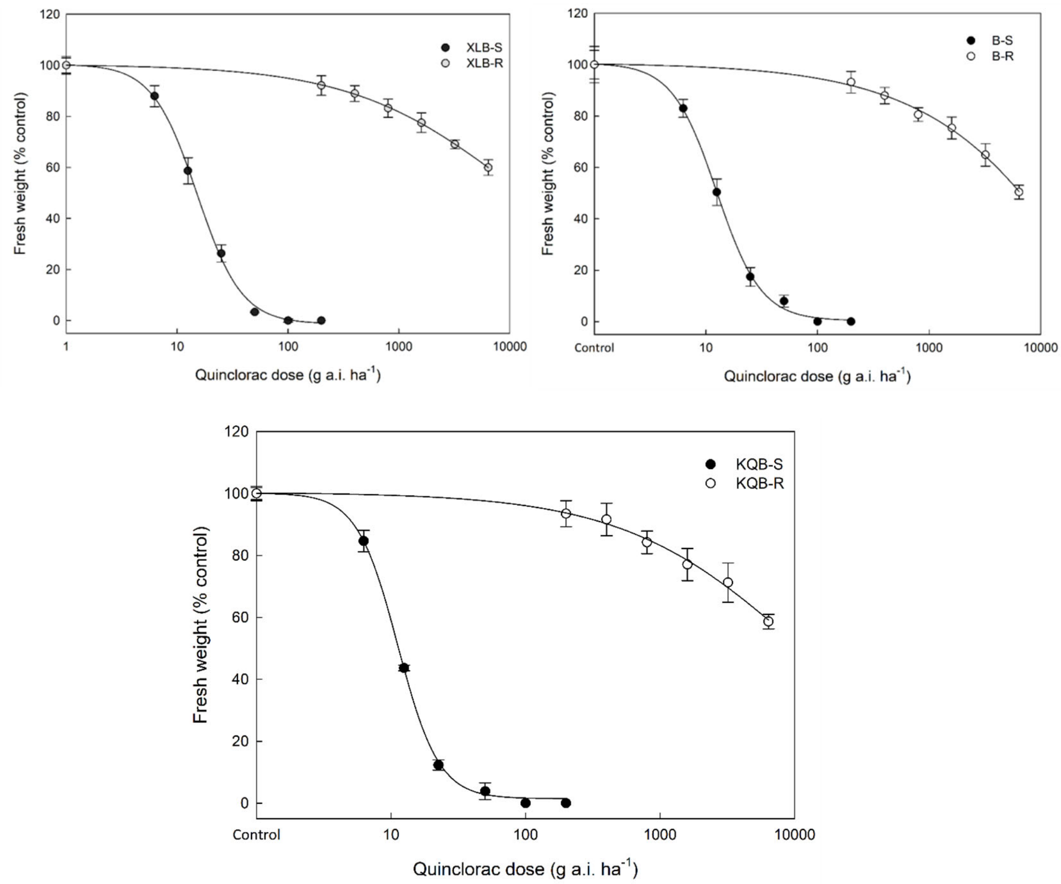

Figure 1. Dose-response curves of resistant and sensitive biotypes of three types barnyard grass to quinclorac

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Table 4. Resistance level of three types of barnyard grass to quinclorac

Biotype	GR50(g a.i.ha^{-1})	RI
XLB-S	14.11±0.84	—
XLB-R	9487.35±403.77	672.38
B-S	12.26±1.84	—
B-R	7941.48±662.36	647.75
KQB-S	11.22±0.35	—
KQB-R	10740.89±930.15	957.29

4. DISCUSSION

The unified genetic background is a prerequisite for conducting all fitness cost studies, and plays a very important role in the study of the fitness of resistant weeds. Both resistant and sensitive biotypes must have the same genetic background except for mutated resistance alleles [83]. In previous studies, the control of genetic background was often overlooked, resulting in inaccurate observations of the fitness cost of herbicide resistance. This chapter studies the method of separating heterozygous individuals to isolate the first generation of sensitive biotype materials from the original resistant populations of Xilai Echinochloa, Echinochloa crusgalli, and Peacock Echinochloa. Through cultivation and screening of two generations, resistant (R) and sensitive (S) biotype materials with the same genetic background were ultimately obtained. This method is generally applicable to single gene mutations [97]. Barnyard grass is a self pollinating species, so it is suitable to adopt this method to unify the genetic background.

This article measured the resistance levels of different biotypes of Echinochloa crusgalli, Echinochloa crusgalli, and Peacock Echinochloa. The results showed that the resistance index of XLB-R was 672.38, the resistance index of B-R was 647.75, and the resistance index of KQB-R was 957.29. These three types of barnyard grass have developed extremely high resistance to dichloroquinoline acid, with peacock barnyard grass having the highest resistance. Echinochloa crusgalli is a polyploid plant that is theoretically less resistant to herbicides due to the "dilution effect" [98]. However, a large number of literature reports indicate that some barnyard grass has also developed a high level of resistance to dichloroquinoline acid, which is consistent with the results obtained in this experiment [99]. The reason for this may be related to the medication history of the sampling site and the biological characteristics of barnyard grass. The samples of barnyard grass in this experiment were collected from areas with severe weed occurrence in rice fields such as Nanjing, Changzhou, and Zhenjiang. During the sampling, detailed records were also kept of the drug use history in these areas. The sampling sites of all three types of barnyard grass had a history of long-term use of dichloroquinoline acid, and long-term external selection pressure further evolved the resistance of barnyard grass to drugs [84]. In addition, with the emergence of resistance, farmers often use much higher doses of dichloroquinoline acid than recommended to achieve the effect of controlling barnyard grass, which will undoubtedly further exacerbate the resistance level of barnyard grass [60]. In addition, barnyard grass itself has the characteristics of short growth cycle, active metabolism, high seed yield, and ripening with falling [17], which helps to accumulate mutated genes or resistance factors, thus making barnyard grass resistant to drugs in rapid iterative reproduction.

At present, the research on the resistance of barnyard grass to pesticides is a hot topic, but there is less research from the perspective of resistance cost. Studying the resistance cost of barnyard grass can further understand the biological characteristics differences between resistant and sensitive barnyard grass, and can be applied to the management of resistant barnyard grass. This study lays the foundation for systematically exploring the resistance costs of three types of barnyard grass in the future.

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