CHILEAN JOURNAL of AGRICULTURAL RESEARCH

RESEARCH ARTICLE

A new herbicide combination for weed management in dry direct-seeding *indica* rice field

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Received: 1 March 2024; Accepted: 14 May 2024, doi:10.4067/S0718-58392024000400527

ABSTRACT

Dry direct-seeding can get rid of the constraints of high-water demand, and is the simplest and most labor-saving mode in rice (*Oryza sativa* L.) cultivation. However, weeds are more prone to occur in dry direct-seeding rice fields, coupled with increased herbicide resistance, leading to confusion among growers regarding herbicide selection. In this study, our investigation results revealed total grass quantity reached 458.82 plants m^{-2}. Two round of field experiments were conducted to determine suitable post-emergence herbicides for dry direct-seeding *indica* rice. The results showed that among the herbicides safe to *indica* rice, the efficacy of the combination of pyraquinate (a new 4-hydroxyphenylpyruvate dioxygenase inhibitor, 150.0 g ai ha^{-1}) + florpyrauxifen-benzyl (13.5 g ai ha^{-1}) on *Leptochloa chinensis* (L.) Nees, *Eclipta prostrata* (L.) L., *Cyperus iria* L., *Fimbristylis miliacea* auct., and total grasses were 89.1%, 97.8%, 97.2%, 90.1%, and 92.8%, respectively; the efficacy of metamifop (120.0 g ai ha^{-1}) + florpyrauxifen-benzyl (18.0 g ai ha^{-1}) and 22.0% cyhalofop-butyl + pyrazosulfuron-ethyl + bispyribac-sodium OD (148.5 g ai ha^{-1}) on main weeds also exceeds 85.0%, except for cyhalofop-butyl-resistant *L. chinensis*. The combination of metamifop + florpyrauxifen-benzyl and 22.0% cyhalofop-butyl + pyrazosulfuron-ethyl + bispyribac-sodium OD can be selected for fields where *L. chinensis* is not resistant to acetyl-CoA carboxylase (ACCase) inhibitors. The combination of pyraquinate + florpyrauxifen-benzyl is the most promising combination to control multiple weeds, even ACCase inhibitors-resistant weeds. This study could also provide references for herbicides selection and management of ACCase inhibitors-resistant *L. chinensis* in dry direct-seeding *indica* rice field worldwide.

Key words: Herbicide efficacy, selectivity, dry direct-seeding *indica* rice, post-emergence herbicides, weed.

INTRODUCTION

Rice (*Oryza sativa* L.) is grown in more than 95 countries around the world, feeding more people in the world than any other crop (Sanders, 2005). It is estimated that rice can provide the human body with about 27% dietary energy, 20% protein, and 3% fat; and rice also contains Zn, riboflavin, thiamine, and niacin which can only be obtained through dietary intake (Phillip et al., 2018). According to the water management model, rice can be divided into irrigated rice and dry direct-seeding rice. However, compared with other cereal crops such as wheat and corn, transplanting rice consumes two to three times as much water (Liu et al., 2015). Dry direct-seeding rice is always sown directly, usually grown in naturally well-drained soil without surface water, mainly distributed in hilly areas lacking water, such as West Africa, South Asia, and North America (Wopereis et al., 1996; Saito et al., 2010; Ruanpanun and Khunin, 2015). Although dry direct-seeding rice produced by small farmers is a relatively low-yield rice production system, it is expected that the dry direct-seeding rice planting area will further increase in the future due to the global shortage of labor and water resources. The yield of dry direct-seeding rice is limited by temperature, water stress, nitrogen deficiency, weed damage and so on (Saito et al., 2010; Alibu and Mamadou, 2021).

Weeds is one of the most important factors restricting the yield of dry direct-seeding rice (Fofana and Rauber, 2010; Saito, 2010; Saito and Futakuchi, 2014). Because dry direct-seeding rice fields are usually sown directly and the cultivated land was not treated with water sealing or herbicides, the occurrence of weeds is more serious than that of transplanted rice fields. The types of weeds in the dry direct-seeding rice system are different in different places, such as the genus *Cyperus*, *Rottboellia cochinchinensis*, *Digitaria horizontalis*, *Ageratum conyzoides*, *Tridax procumbens*, and *Eleusine indica* in West Africa (Rodenburg et al., 2015; Kraehmer et al., 2016), 26 weeds belonging to 12 families in Shanghai, China, according to our investigation.

Weed control methods in dry direct-seeding rice fields mainly include cultural weed management practices, physical methods, such as deep ploughing, germinating, mechanical weeding, hand-pulling weeding, and chemical methods, that is, the application of herbicides (Singh and Ghosh, 1992; Adigun et al., 2017). The advantages of chemical methods for weed control in dry direct-seeding rice have been reported for decades in terms of higher yields and lower labor costs (Adigun et al., 2017; Kolo et al., 2020). Due to poor soil moisture in dry direct-seeding rice fields, the pre-emergence herbicides are not effective in controlling weeds, which makes the application of post-emergence herbicides extremely important. Currently, the post-emergence commonly used in Chinese rice fields mainly includes penoxsulam, bispyribac-sodium, pyrazosulfuron-ethyl, cyhalofop-butyl, metamifop, quinclorac, MCPA-sodium, bentazone, and so on. In recent years, new 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors have been widely used in rice fields, including benzobicyclon (McKnight et al., 2018), tripyrasulfone (Wang et al., 2020), and pyraquinate (Jiao et al., 2023). The efficacy of the new auxin-type herbicide, florpyrauxifen-benzyl, in paddy fields has also been increasingly recognized by farmers (Duy et al., 2018; Teló et al., 2019).

Choosing appropriate post-emergence herbicides is crucial for the management of weeds in dry direct-seeding rice fields. On the one hand, it depends on the rice varieties. For example, HPPD inhibitor herbicides have been reported cannot be used in *indica* rice fields previously (Kim et al., 2012). On the other hand, herbicide resistance in weed must be taken into consideration. Although *Echinochloa* P. Beauv. (barnyard grass) is very resistant to penoxsulam and quinclorac in China (Xu et al., 2013; Fang et al., 2019), it is currently possible to use florpyrauxifen-benzyl or metamifop as an alternative. The resistance of *Leptochloa chinensis* (L.) Nees (Chinese sprangletop) in rice fields to cyhalofop-butyl has also been reported (Yuan et al., 2021), but there is no herbicide that can effectively replace cyhalofop-butyl. Benzobicyclon treatment requires the coordination of surface water in fields (Brabham et al., 2019), so it is not suitable to be used in dry direct-seeding rice systems. The efficacy of post-emergence herbicides commonly used in paddy fields in dry *indica* rice have not been systematically studied yet.

In this study, we compared the selectivity and efficacy of multiple post-emergence herbicides in dry direct-seeding rice fields. The aim was to find a selective and effective herbicide combinations for dry direct-seeding *indica* rice. Furthermore, to screen herbicide combinations that can control the acetyl-CoA carboxylase (ACCase) inhibitor-resistant Chinese sprangletop in *indica* rice fields is also an important goal. We hope to provide a reference for the selection of post-emergence herbicides used for dry *indica* rice fields worldwide.

MATERIALS AND METHODS

The dry direct-seeding rice variety and herbicides

Indica rice (*Oryza sativa* L.), provided by Shanghai Agrobiological Gene Center, Shanghai, China, was planted at the end of April in China. The herbicides used for herbicide screening experiments in this study are shown in Table 1.

Investigation of weed flora and the efficacy of common herbicides used

Random sampling was used to select more than 10 points in the dry direct-seeding *indica* rice field in the lower reaches of the Yangtze River, China. The sampling frame made by polyvinylchloride (PVC) tubes was a square sampling frame of 0.25 m^2 (0.5 m × 0.5 m). The number of each weed was recorded and finally converted into the number of weeds per square meter. A field for continuous dry direct-seeding rice cultivation has been selected, located in Jinshan District, Shanghai (30°49’2.9’’N;121°11’5.0’’E), China, for continuous monitoring (2020, 2021, and 2023) of its efficacy of conventional herbicides. The five-point sampling method was used to investigate the weed species and quantity in a 0.25 m^2 (0.5 m × 0.5 m) sample point.

Table 1. Herbicides tested in this study. IUPAC: International Union of Pure and Applied Chemistry.

IUPAC name	Permissible dosage range (g ai ha^{-1})	Manufacturer
Ethyl (2*R*)-2-[4-[(6-chloro-1,3-benzoxazol-2-yl)oxy]phenoxy]propanoate	31.05 ~ 62.1	BAYER
Benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate	18.0 ~ 36.0	Corteva Agriscience
(2*R*)-2-[4-[(6-Chloro-1,3-benzoxazol-2-yl)oxy]phenoxy]-N-(2-fluorophenyl)-N-methylpropanamide	90.0 ~ 120.0	FMC
[4-[2-Chloro-3-[(3,5-dimethylpyrazol-1-yl)methyl]-4-methylsulfonylbenzoyl]-2,5-dimethylpyrazol-3-yl]1,3-dimethylpyrazole-4-carboxylate	135.0 ~ 225.0	KingAgroot
N-(3,4-Dichlorophenyl) propenamide + Tripyrasulfone	840.0 ~ 1050	KingAgroot
[4-[3-(3-Chlorophenyl)-1,5-dimethyl-2,4-dioxoquinazoline-6-carbonyl]2,5-dimethylpyrazol-3-yl] N,N-diethylcarbamate	112.5 ~ 225.0	Shandong Cynda (Chemical)
Butyl (2*R*)-2-[4-(4-cyano-2-fluorophenoxy)phenoxy]propanoate + Ethyl 5-[(4,6-dimethoxypyrimidin-2-yl)carbamoylsulfamoyl]-1-methylpyrazole-4-carboxylate + 2,6-bis[(4,6-Dimethoxypyrimidin-2-yl)oxy]benzoate	99.0 ~ 148.5	Anhui Shanghe Voda Biotechnology Co., Ltd., China
2-Chloro-4-fluoro-5-[3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl]-N-[methyl(propan-2-yl)sulfamoyl] benzamide	26.3 ~ 52.5	BASF SE

Field experiment design

The experimental site located in Chongming District, Shanghai (31°37'7.2" N; 121°40'31.4" E), China. We have previously reported that the Chinese sprangletop (*Leptochloa chinensis* (L.) Nees) in this area is resistant to cyhalofop-butyl (Yuan et al., 2021). There was a total of 21 herbicide treatments groups set in the first ground of test (2021), including 19 herbicide treatment groups, one hand-pulling group, and one control group, each treatment group contains four biological replicates. The dosages setting of each herbicide and combination are shown in Table 2. The area of each biological replicate was set to 20 m^2 (5 m × 4 m), called test plot. A total of 84 test plots were completely randomized design. In the 4-5 leaf stage of dry *indica* rice (28 d after sowing), each test plot was sprayed with herbicides to stem and leaf of rice and weed. The herbicide of each test plot was mixed with water and dissolved into 900 mL solution, and sprayed evenly into the plot with a sprayer produced by an HD 900 electric sprayer (Agrolex, Singapore) with fan-shaped nozzle until the herbicide solution was completely sprayed. The spray pressure was set to 230.0 kPa. The weather during herbicide application was cloudy, with a temperature of 28.2 °C, humidity of 60.1%, and wind speed of 1.2 m s^{-1}. The second round of the experiment was a validation test for the herbicide combinations that were safe to rice in the first round. The herbicide combination treatment entering the second round has been marked in Table 2. Other experimental details were consistent with the first round of experiments. The weather during herbicide application in the second round of experiment was sunny, with a temperature of 27.2 °C, humidity of 55.4%, and wind speed of 1.4 m s^{-1}.

Table 2. Dosage setting of each herbicide treatment.

Group number	Dosage (g ai ha^{-1})	Treatments
1	31.05 + 13.5	69.0 g L^{-1} Fenoxaprop-*P*-ethyl + 3% Florpyrauxifen-benzyl
2-1	120.0 + 9.0	10% Metamifop + 3% Florpyrauxifen-benzyl
2-2	120.0 + 18.0	10% Metamifop + 3% Florpyrauxifen-benzyl
3-1	180.0 + 13.5	6% Tripyrasulfone + 3% Florpyrauxifen-benzyl
3-2	270.0 + 13.5	6% Tripyrasulfone + 3% Florpyrauxifen-benzyl
4-1	1050.0	28% Propanil + Tripyrasulfone
4-2	2100.0	28% Propanil + Tripyrasulfone
5-1	225.0 + 9.0	5% Pyraquinate + 3% Florpyrauxifen-benzyl
5-2	225.0 + 13.5	5% Pyraquinate + 3% Florpyrauxifen-benzyl
5-3	225.0 + 18.0	5% Pyraquinate + 3% Florpyrauxifen-benzyl
5-4	225.0 + 22.5	5% Pyraquinate + 3% Florpyrauxifen-benzyl
5-5	300.0 + 13.5	5% Pyraquinate + 3% Florpyrauxifen-benzyl
5-6	150.0 + 13.5	5% Pyraquinate + 3% Florpyrauxifen-benzyl
6-1	99.0	22% Cyhalofop-butyl + Pyrazosulfuron-ethyl + Bispyribac-sodium
6-2	148.5	22% Cyhalofop-butyl + Pyrazosulfuron-ethyl + Bispyribac-sodium
7-1	120.0 + 21.0	10% Metamifop + 70% Saflufenacil
7-2	120.0 + 31.5	10% Metamifop + 70% Saflufenacil
8-1	31.05 + 21.0	69.0 g L^{-1} Fenoxaprop-*P*-ethyl + 70% Saflufenacil
8-2	31.05 + 31.5	69.0 g L^{-1} Fenoxaprop-*P*-ethyl + 70% Saflufenacil
9	/	Hand-weeding
10	/	Control

Investigation of selectivity of herbicide treatments on rice

On the 7th day after herbicide application, the symptoms of rice poisoning were visually investigated and recorded; on the 14th day after herbicide application, the poisoning symptoms or recovery from poisoning of rice were visually evaluated and recorded; at harvest (at the end of August that year), number of effective ears, number of grains per ear, and weight of 1000 grains per unit area of each treatment group were investigated. When collecting rice ears for yield measurement, a sampling frame (0.5 m × 0.5 m) was randomly placed in the experimental plot. It should be noted that the sampling frame cannot be tightly placed against the edge of the plot due to edge effects. Each plot was randomly selected with four frames, and there was no intersection between the four frames. The complete ears of all rice in each frame collected were marked and dried to a constant weight. A precision balance (ME2002E, Mettler-Toledo, Barcelona, Spain; max = 2200 g, d = 0.01 g) was used to measure 1000 grains weight.

Investigation of efficacy of herbicide treatments on weed

On the 14th day after herbicide application, the number of each kind of weed in each test plot was investigated and recorded; on the 56th day after herbicide application, the persistence of control effect on total grass was visually estimated and recorded. The indicator we use to evaluate the efficacy of herbicide combinations on weed was the inhibition rate of the number of weeds. The details of weed sampling method were the same as the description of rice sampling in the above section. The species and numbers of each weed in the sampling frame were investigated and recorded. At the same time, the control group and the hand-weeding treatment group were also investigated. Visual estimation needs to be evaluated for the entire test plot and was jointly completed by four experienced researchers (who have carried out field herbicide-related experiments for more than 5 yr). The hand-weeding treatment group was carried out only once on the 14th day after herbicide treatment in other herbicide treatment groups.

Data analysis
All data of control effects on weeds and rice yield indicators were subjected to significance analysis respectively using SPSS Statistics (for Windows, Version 20.0.; IBM, Armonk, New York, USA) by Duncan's multiple range test ($p < 0.05$).

RESULTS

Weed flora in experimental fields and efficacy of common herbicides used
We investigated weed flora in dry direct-seeding *indica* rice field in the lower reaches of the Yangtze River, China (Figure 1a). The results showed that the total grass quantity reached 458.82 plants m^{-2}. Chinese sprangletop, with 153.11 plants m^{-2}, was the main grass weed, accounting for 33.37%; *Eclipta prostrata* (false daisy), with 49.78 plants m^{-2}, was the main broad-leaved weed, accounting for 10.85%; *Cyperus iria* (rice flat sedge) and *Fimbristylis littoralis* (globe fringerush), with 79.26 and 93.04 plants m^{-2}, respectively, were the main *Cyperaceae* weeds, accounting for 20.28% and 12.27%, respectively. Other weeds included large weeds, such as *Aeschynomene indica* (Indian joint-vetch) and *Polygonum lapathifolium* (Figure 1b). We investigated the efficacy of conventional herbicides used by farmers in the dry direct-seeding rice fields. They usually used pendimethalin for the first time, cyhalofop-butyl + penoxsulam + bentazone for the second time, and the herbicide for the third time depends on the specific weed flora. The monitoring results for 3 yr showed that after the second herbicides treatment, Chinese sprangletop, barnyard grass, false daisy, Indian joint-vetch, rice flat sedge, and globe fringerush were difficult to control, and the number of total weeds reached 216.80 plants m^{-2} in 2023 (Table 3).

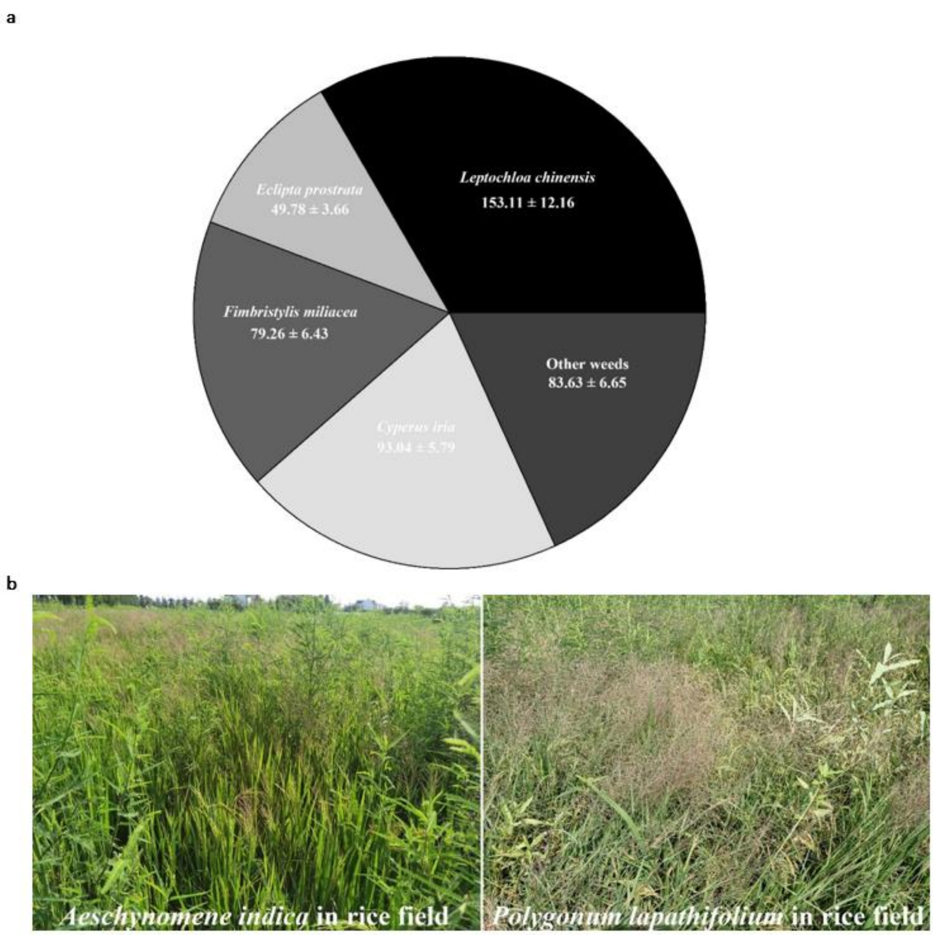

Figure 1. (a) Weed community in the experimental field. (b) Damage of *Aeschynomene indica* and *Polygonum lapathifolium* to dry direct-seeding rice.

Table 3. Residual weeds in dry direct-seeding rice field after application of herbicides commonly used by growers.

Year	Chinese sprangletop	Barnyard grass	False daisy	Indian joint-vetch	Rice flat sedge	Globe fringerush	Total grass
	Nr m^{-2}						
2020	44.80 ± 6.97	10.40 ± 4.83	21.60 ± 5.46	11.20 ± 3.44	24.00 ± 3.58	29.60 ± 7.44	146.40 ± 19.21
2021	52.80 ± 16.85	7.20 ± 2.33	8.80 ± 1.50	12.00 ± 5.22	28.80 ± 11.41	21.60 ± 4.66	133.60 ± 20.92
2023	77.60 ± 16.90	3.20 ± 1.50	19.20 ± 4.45	16.00 ± 4.38	33.60 ± 9.93	65.60 ± 15.16	216.80 ± 13.05

Selectivity of each herbicide combination on dry direct-seeding *indica* rice

Main symptoms of phytotoxicity. We investigated the symptoms of *indica* rice in each test plot on the seventh day and 14th day after herbicide treatment. The results of all herbicide treatment groups are shown in Table 4 and Figure 2. In summary, treatment groups containing fenoxaprop-*P*-ethyl and saflufenacil (treatment groups 1, 7, and 8) were likely to cause dead seedlings of rice; treatment groups containing HPPD inhibitors, including tripyrasulfone and pyraquinate (treatment groups 3, 4, and 5) were likely to cause bleaching of rice seedlings. Pyraquinate at 150.0 g ai ha^{-1} caused slight leaf bleaching in rice on the seventh day after treatment, but returned to normal on the 14th day. After treatment with traditional herbicides such as metamifop, cyhalofop-butyl, and pyrazosulfuron-ethyl, *indica* rice could grow normally during the investigation period. Florpyrauxifen-benzyl was also safe for dry direct-seeding *indica* rice at the dosage of 18.0 g ai ha^{-1} base on treatment group 2-2. The safe herbicide combinations for *indica* rice were groups 2-1, 2-2, 5-6, 6-1, and 6-2.

Table 4. Symptoms of phytotoxicity on direct-seeding *indica* rice on the 7th and 14th day after herbicide treatments. DAT: Days after herbicide treatment.

Group	Treatments	Dosage (g ai ha^{-1})	Symptoms (7 DAT)	Symptoms (14 DAT)
1	Fenoxaprop-*P*-ethyl + Florpyrauxifen-benzyl	31.05 + 13.5	Dead seedlings	Dead seedlings
2-1	Metamifop + Florpyrauxifen-benzyl	120.0 + 9.0	Normal growth	Normal growth
2-2	Metamifop + Florpyrauxifen-benzyl	120.0 + 18.0	Normal growth	Normal growth
3-1	Tripyrasulfone + Florpyrauxifen-benzyl	180.0 + 13.5	Leaf bleaching	Leaf bleaching
3-2	Tripyrasulfone + Florpyrauxifen-benzyl	270.0 + 13.5	Leaf bleaching	Leaf bleaching
4-1	Propanil + Tripyrasulfone	1050.0	Leaf bleaching and dead seedlings	Leaf bleaching and dead seedlings
4-2	Propanil + Tripyrasulfone	2100.0	Leaf bleaching and dead seedlings	Leaf bleaching and dead seedlings
5-1	Pyraquinate + Florpyrauxifen-benzyl	225.0 + 9.0	Leaf bleaching	Leaf bleaching
5-2	Pyraquinate + Florpyrauxifen-benzyl	225.0 + 13.5	Leaf bleaching	Leaf bleaching
5-3	Pyraquinate + Florpyrauxifen-benzyl	225.0 + 18.0	Leaf bleaching	Leaf bleaching
5-4	Pyraquinate + Florpyrauxifen-benzyl	225.0 + 22.5	Leaf bleaching and dead seedlings	Leaf bleaching and dead seedlings
5-5	Pyraquinate + Florpyrauxifen-benzyl	300.0 + 13.5	Leaf bleaching and dead seedlings	Leaf bleaching and dead seedlings
5-6	Pyraquinate + Florpyrauxifen-benzyl	150.0 + 13.5	Slight leaves bleaching	Normal growth
6-1	Cyhalofop-butyl + Pyrazosulfuron-ethyl + Bispyribac-sodium	99.0	Normal growth	Normal growth
6-2	Cyhalofop-butyl + Pyrazosulfuron-ethyl + Bispyribac-sodium	148.5	Normal growth	Normal growth
7-1	Metamifop + Saflufenacil	120.0 + 21.0	Dead seedlings	Dead seedlings
7-2	Metamifop + Saflufenacil	120.0 + 31.5	Dead seedlings	Dead seedlings
8-1	Fenoxaprop-*P*-ethyl + Saflufenacil	31.05 + 21.0	Dead seedlings	Dead seedlings
8-2	Fenoxaprop-*P*-ethyl + Saflufenacil	31.05 + 31.5	Dead seedlings	Dead seedlings

Figure 2. Morphology of rice in each treatment group on the 14th day after herbicide treatments. CK: control; 1: Fenoxaprop-*P*-ethyl + Florpyrauxifen-benzyl (31.05 + 13.5 g ai ha^{-1}); 2-1: Metamifop + Florpyrauxifen-benzyl (120.0 + 9.0 g ai ha^{-1}); 2-2: Metamifop + Florpyrauxifen-benzyl (120.0 + 18.0 g ai ha^{-1}); 3-1: Tripyrasulfone + Florpyrauxifen-benzyl (180.0 + 13.5 g ai ha^{-1}); 3-2: Tripyrasulfone + Florpyrauxifen-benzyl (270.0 + 13.5 g ai ha^{-1}); 4-1: Propanil + Tripyrasulfone (1050.0 g ai ha^{-1}); 4-2: Propanil + Tripyrasulfone (2100.0 g ai ha^{-1}); 5-1: Pyraquinate + Florpyrauxifen-benzyl (225.0 + 9.0 g ai ha^{-1}); 5-2: Pyraquinate + Florpyrauxifen-benzyl (225.0 + 13.5 g ai ha^{-1}); 5-3: Pyraquinate + Florpyrauxifen-benzyl (225.0 + 18.0 g ai ha^{-1}); 5-4: Pyraquinate + Florpyrauxifen-benzyl (225.0 + 22.5 g ai ha^{-1}); 5-5: Pyraquinate + Florpyrauxifen-benzyl (300.0 + 13.5 g ai ha^{-1}); 5-6: Pyraquinate + Florpyrauxifen-benzyl (150.0 + 13.5 g ai ha^{-1}); 6-1: Cyhalofop-butyl + Pyraquinate + Bispyribac-sodium (99.0 g ai ha^{-1}); 6-2: Cyhalofop-butyl + Pyraquinate + Bispyribac-sodium (148.5 g ai ha^{-1}); 7-1: Metamifop + Saflufenacil (120.0 + 21.0 g ai ha^{-1}); 7-2: Metamifop + Saflufenacil (120.0 + 31.5 g ai ha^{-1}); 8-1: Fenoxaprop-*P*-ethyl + Saflufenacil (31.05 + 21.0 g ai ha^{-1}); 8-2: Fenoxaprop-*P*-ethyl + Saflufenacil (31.05 + 31.5 g ai ha^{-1}).

Yield of rice in safe herbicide treatment group. To further verify the impact of treatment groups 2-1, 2-2, 5-6, 6-1, and 6-2 on rice yield, the effective number of ears, grains per ear, and 1000 grain weight of rice in these treatment groups were tested continuously for two round of field experiment conducted in 2021 and 2022 (Figure 3). The number of effective ears of rice in all herbicide treatment groups was significantly higher than that in the control group ($p < 0.05$), and there was nonsignificant difference compared to the hand-weeding group. Among them, the treatment group 5-6 (150.0 g ai ha^{-1} 5% pyraquinate OD + 13.5 g ai ha^{-1} 3% florpyrauxifen-benzyl EC) had the most effective ears, reaching 442.50, which was more than 4.4 million when converted to the number of ears per hectare. The number of effective ears in this treatment group was significantly higher than that in any other groups ($p < 0.05$). The number of grains per ear of rice in all herbicide treatment groups and the hand-weeding treatment group ranged from 73.00 to 80.50, and there was nonsignificant difference between them. Except for the control group, dry weight of 1000 grains in each treatment group exceeded 20 g. Among them, hand-weeding group had the highest 1000 grains weight, reaching 21.06 g. There was nonsignificant difference in the 1000 grains weight between all herbicide treatment groups and the hand-weeding group. Additionally, the 1000 grains weight of all treatment groups was significantly higher than that of the control group ($p < 0.05$).

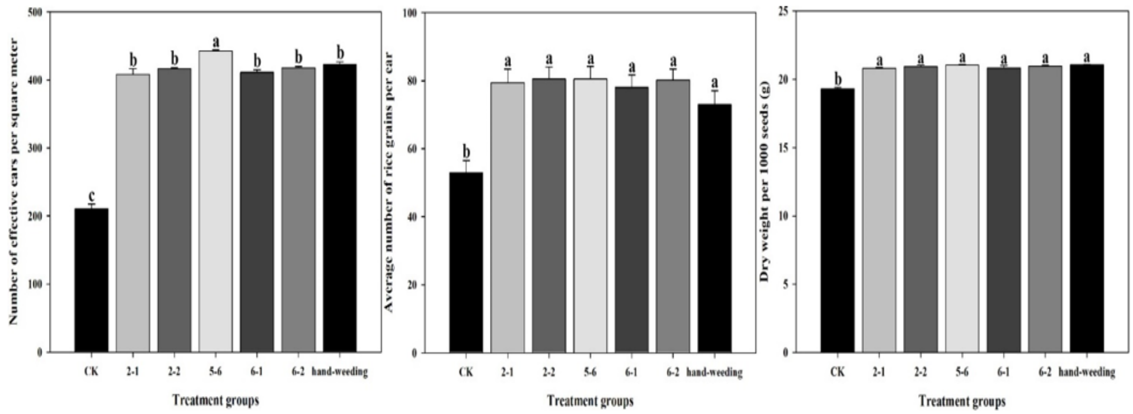

Figure 3. Differences in rice yields of some treatment groups. Different letters represent significant difference in different treatments ($p < 0.05$). CK: Control; 2-1: Metamifop + Florpyrauxifen-benzyl (120.0 + 9.0 g ai ha^{-1}); 2-2: Metamifop + Florpyrauxifen-benzyl (120.0 + 18.0 g ai ha^{-1}); 5-6: Pyraquinate + Florpyrauxifen-benzyl (150.0 + 13.5 g ai ha^{-1}); 6-1: Cyhalofop-butyl + Pyrazosulfuron-ethyl + Bispyribac-sodium (99.0 g ai ha^{-1}); 6-2: Cyhalofop-butyl + Pyrazosulfuron-ethyl + Bispyribac-sodium (148.5 g ai ha^{-1}).

Efficacy of herbicide combinations on weeds

Inhibition rate of herbicides on weeds. On the 21th day after herbicide treatment, the control effect on weeds number of all herbicide treatment groups on Chinese sprangletop, false daisy, rice flat sedge, globe fringerush, and total grass (including other non-main weeds) were investigated (Table 5). There were 13 combinations that have a control effect over 80% on the total grass, and five combinations that have a control effect over 90% on the total grass. Among them, the control effect of 10% metamifop EC + 3% florpyrauxifen-benzyl EC at the dosage of 120.0 g ai ha^{-1} + 9.0 g ai ha^{-1} on Chinese sprangletop, false daisy, rice flat sedge, globe fringerush, and total grass were 56.7%, 99.0%, 96.1%, 92.1%, and 81.6%, and the control effects were 69.8%, 99.3%, 97.1%, 87.9%, and 85.1% at 120.0 g ai ha^{-1} + 18.0 g ai ha^{-1}, respectively. The control effects of 22% cyhalofop-butyl + pyrazosulfuron-ethyl + bispyribac-sodium OD at 148.5 g ai ha^{-1} on Chinese sprangletop, false daisy, rice flat sedge, globe fringerush, and total grass were 49.7%, 98.3%, 99.7%, 99.4%, and 80.3%, respectively. The control effect of 5% pyraquinate OD + 3% florpyrauxifen-benzyl EC at a dose of 150.0 + 13.5 g ai ha^{-1} on Chinese sprangletop, false daisy, rice flat sedge, globe fringerush, and total grass were 89.1%, 97.8%, 97.2%, 90.1%, and 92.8%, respectively. Among all combinations, only the combination containing 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors had a control effect on Chinese sprangletop exceeding 80.0%. Fenoxaprop-*P*-ethyl, metamifop, and cyhalofop-butyl have poor inhibitory effects on Chinese sprangletop in our experimental site (< 75.0%).

Persistence of herbicides efficacy on weed. After 56 d of herbicides treatment, the control effect of treatments groups 2-1, 2-2, 5-6, 6-1, 6-2, and 9 were investigated again. We visually estimated the control effect of each herbicide treatment on total grass and recorded the main remaining weeds (Table 6, Figure 4). The results showed that among all the treatment groups that were safe for dry direct-seeding *indica* rice, only treatment group 5-6 (150.0 g ai ha^{-1} 5% pyraquinate OD + 13.5 g ai ha^{-1} 3% florpyrauxifen-benzyl EC) still had a control effect on total grass higher than 90.0%, which was significantly higher than other herbicide treatment groups ($p < 0.05$). The control effect of other herbicide treatment groups on weeds decreased to less than 60.0%. Compared to the control group, the broad-leaved weeds and sedge weeds in the herbicide treatment group were effectively inhibited. Except for treatment group 5-6, the main remaining weed in the other herbicide treatment groups was the gramineous Chinese sprangletop.

Table 5. Control effect (%) (21 d after herbicide treatment) of each herbicide treatment on main weeds in the experimental field. The bold group numbers require a second round of experimental verification. Different letters represent the significant difference in different treatment groups ($p < 0.05$).

Group	Treatments	Dosages (g ai ha^{-1})	Chinese sprangletop	False daisy	Rice flat sedge	*Fimbristylis miliacea*	Total grass
1	Fenoxaprop-P-ethyl + Florpyrauxifen-benzyl	31.05 + 13.5	42.3^{ij}	100.0^{a}	91.4^{ef}	89.4^{abcd}	76.3^{de}
2-1	**Metamifop + Florpyrauxifen-benzyl**	**120.0 + 9.0**	**56.7^{fghi}**	**99.0^{a}**	**96.1^{abcde}**	**92.1^{abcd}**	**81.6^{cd}**
2-2	**Metamifop + Florpyrauxifen-benzyl**	**120.0 + 18.0**	**69.8^{defg}**	**99.3^{a}**	**97.1^{abcd}**	**87.9^{bcde}**	**85.1^{bc}**
3-1	Tripyrasulfone + Florpyrauxifen-benzyl	180.0 + 13.5	93.5^{ab}	98.6^{a}	93.9^{bcdef}	87.8^{bcde}	93.2^{ab}
3-2	Tripyrasulfone + Florpyrauxifen-benzyl	270.0 + 13.5	97.9^{a}	99.5^{a}	94.6^{abcdef}	89.2^{abcd}	94.0^{a}
4-1	Propanil + Tripyrasulfone	1050.0	70.4^{cdefg}	83.6^{b}	58.2^{j}	89.9^{abcd}	75.2^{de}
4-2	Propanil + Tripyrasulfone	2100.0	80.4^{abcde}	71.0^{c}	71.0^{g}	95.5^{abc}	82.5^{cd}
5-1	Pyraquinate + Florpyrauxifen-benzyl	225.0 + 9.0	87.1^{abcd}	98.1^{a}	92.2^{def}	77.8^{e}	86.5^{abc}
5-2	Pyraquinate + Florpyrauxifen-benzyl	225.0 + 13.5	89.5^{abcd}	95.2^{a}	92.7^{cdef}	82.0^{de}	88.3^{abc}
5-3	Pyraquinate + Florpyrauxifen-benzyl	225.0 + 18.0	90.2^{abc}	100.0^{a}	93.5^{cdef}	85.2^{cde}	87.7^{abc}
5-4	Pyraquinate + Florpyrauxifen-benzyl	225.0 + 22.5	89.6^{abcd}	100.0^{a}	99.2^{ab}	86.2^{bcde}	91.4^{ab}
5-5	Pyraquinate + Florpyrauxifen-benzyl	300.0 + 13.5	96.3^{a}	100.0^{a}	89.8^{f}	83.6^{de}	91.1^{ab}
5-6	**Pyraquinate + Florpyrauxifen-benzyl**	**150.0 + 13.5**	**89.1^{abcd}**	**97.8^{a}**	**97.2^{abcd}**	**90.1^{abcd}**	**92.8^{ab}**
6-1	**Cyhalofop-butyl + Pyraquinate + Bispyribac-sodium**	**99.0**	**15.4^{k}**	**97.8^{a}**	**98.4^{abc}**	**97.2^{ab}**	**62.2^{f}**
6-2	**Cyhalofop-butyl + Pyraquinate + Bispyribac-sodium**	**148.5**	**49.7^{hi}**	**98.3^{a}**	**99.7^{a}**	**99.4^{a}**	**80.3^{cd}**
7-1	Metamifop + Saflufenacil	120.0 + 21.0	27.8^{jk}	97.1^{a}	65.4^{hi}	82.8^{de}	59.8^{f}
7-2	Metamifop + Saflufenacil	120.0 + 31.5	52.9^{ghi}	100.0^{a}	67.3^{gh}	88.1^{bcde}	71.5^{e}
8-1	Fenoxaprop-*P*-ethyl + Saflufenacil	31.05 + 21.0	66.4^{efgh}	96.6^{a}	57.0^{j}	100.0^{a}	75.9^{de}
8-2	Fenoxaprop-*P*-ethyl + Saflufenacil	31.05 + 31.5	74.8^{bcdef}	99.5^{a}	61.9^{j}	99.5^{a}	81.1^{cd}

Table 6. Persistence of control effect (56 d after herbicide treatment) of safe herbicide treatment groups. Different letters represent significant difference in different treatment groups ($p < 0.05$).

Group	Treatments	Dosages	Control effect	Major weeds present
		g ai ha^{-1}	%	
2-1	Metamifop + Florpyrauxifen-benzyl	120.0 + 9.0	35.63^{c}	Chinese sprangletop
2-2	Metamifop + Florpyrauxifen-benzyl	120.0 + 18.0	65.63^{b}	Chinese sprangletop
5-6	Pyraquinate + Florpyrauxifen-benzyl	150.0 + 13.5	92.50^{a}	-
6-1	Cyhalofop-butyl + Pyrazosulfuron-ethyl + Bispyribac-sodium	99.0	28.75^{c}	Chinese sprangletop
6-2	Cyhalofop-butyl + Pyrazosulfuron-ethyl + Bispyribac-sodium	148.5	60.63^{b}	Chinese sprangletop
9	Hand-weeding	/	86.25^{a}	Chinese sprangletop

Figure 4. Persistence of herbicides in each treatment group. The investigation was conducted on the 56th day after herbicide treatment. CK: Control; 2-1: Metamifop + Florpyrauxifen-benzyl (120.0 + 9.0 g ai ha^{-1}); 2-2: Metamifop EC + Florpyrauxifen-benzyl (120.0 + 18.0 g ai ha^{-1}); 5-6: Pyraquinate + Florpyrauxifen-benzyl (150.0 + 13.5 g ai ha^{-1}); 6-1: Cyhalofop-butyl + Pyrazosulfuron-ethyl + Bispyribac-sodium (99.0 g ai ha^{-1}); 6-2: Cyhalofop-butyl + Pyrazosulfuron-ethyl + Bispyribac-sodium (148.5 g ai ha^{-1}).

DISCUSSION

The planting area of dry direct-seeding rice is increasing owing to the global shortage of agricultural labor force and water resources. Ensuring high and stable yields of rice is an important guarantee for food security. At present, weeds are already a key factor that impairs the yield and quality of dry direct-seeding rice (Fofana and Rauber, 2010; Saito, 2010; Saito and Futakuchi, 2014). The damage of weed in dry direct-seeding rice fields has been reported frequently (Rodenburg et al., 2015; Kraehmer et al., 2016). The rough management mode of this farming pattern of direct-seeding rice field system further aggravates the ravages of weeds. This study found that the amount of weeds per square meter in the investigation site reached 458.82 (Figure 1a), which also included large weeds such as *Aeschynomene indica* and *Polygonum lapathifolium* (Figure 1b). It is undoubtedly disastrous for the growth of rice. Studies have shown that when the density of weeds reaches 15 plants m^{-2}, the yield of rice will decrease or even reach 50%; as the density of weeds increases, plant height, number of tillers, leaf area, and DM of rice showed a linear decline (Khairuddin, 2012). In fact, the yield of rice in the control group (without herbicide or other weeding methods treatments) in this study was significantly much lower than the safe herbicide treatment groups ($p < 0.05$) (Figure 3). Because the soil moisture is usually poor in dry direct-seeding rice cultivation areas, the effect of pre-emergence herbicides is limited. The most used herbicides in dry direct-seeding rice fields in China do not actually meet the needs of weed control (Table 3). Therefore, the selection of post-emergence herbicides is particularly critical.

Among the herbicides tested in this study, there were two herbicides that were not registered in paddy fields, saflufenacil and fenoxaprop-*P*-ethyl. The reason for testing these two herbicides is that some Chinese farmers prefer to use them in rice fields. Saflufenacil is a protoporphyrinogen IX oxidase (PPO) inhibitor, which was commercially introduced in 2010 for crop desiccation, mainly used for broadleaf weed control (Grossmann et al., 2011; Roskamp and Johnson, 2013). Saflufenacil is currently mainly registered for use in orchards, non-arable land, and dry rice field (for pre-emergence treatment to control broadleaf weeds) in China. Saflufenacil is also often used to control weeds that are resistant to glyphosate. Fenoxaprop-*P*-ethyl is an ACCase inhibitor, widely used in wheat fields for controlling Gramineae weeds, like *Alopecurus japonicus* and *Beckmannia syzigachne* (Pan et al., 2016; Xu et al., 2017). Although the above two herbicides have excellent performance in their respective application fields, they are not advisable to be applied to *indica* rice fields for post-emergence treatment; because *indica* rice showed severe sensitivity to saflufenacil and fenoxaprop-*P*-ethyl, its growth was seriously inhibited (Figure 2, Table 4). Therefore, the application of herbicides from other crop fields to *indica* rice fields should be banned, because herbicides are prescribed to be used on certain crop fields based on rigorous previous tests.

The 4-hydroxyphenylpyruvate dioxygenase (HPPD) is an important enzyme in plants, that can catabolize tyrosine and anabolism of plastoquinones, tocopherols, and subsequently carotenoid biosynthesis (Beaudegnies et al., 2009). The HPPD inhibitors are a relatively new class of herbicides discovered about three decades ago and are widely used in agriculture for weed management currently (Nakka et al., 2017). The HPPD inhibitors that have been registered for use in Chinese rice fields, mainly include tefuryltrione, benzobicyclon, tripyrasulfone, and pyraquinate (recently registered). Among them, tripyrasulfone and pyraquinate have more potential in dry direct-seeding rice fields. The significant advantage of the HPPD inhibitors is that it has a new mode of action to manage acetolactate synthase (ALS) inhibitors resistant-, ACCase inhibitors resistant-, or auxin-type herbicides resistant-weeds (Nakka et al., 2017). However, we found that HPPD inhibitors can easily cause chlorosis and bleaching of *indica* rice leaves in several previous experiments (data not shown). Pyraquinate ($C_{27}H_{28}ClN_5O_5$) is a recently developed HPPD inhibitor and has higher safety for *indica* rice, which is different from other HPPD inhibitors. This study confirmed the above view, because treatments groups containing HPPD inhibitors showed serious phytotoxicity to *indica* rice (Figures 2 and 3, Table 4). However, pyraquinate is safe for *indica* rice at a dose of 150 g ai ha^{-1}, despite slight poisoning symptoms in the early stages (Figure 2). In general, pyraquinate should be used with caution in dry direct-seeding *indica* rice fields. Detailed crop selectivity experiments should be conducted for different *indica* rice varieties.

Florpyrauxifen-benzyl (CAS: 1390661-72-9) belongs to the pyridine carboxylic acids class of auxin mimics and is a novel post-emergence herbicide exploited by Dow AgroSciences (Indianapolis, Indiana, USA); it was registered to use in paddy fields in China in 2017 (Wang et al., 2021). Florpyrauxifen-benzyl is a good choice for weed management in both irrigated and dry direct-seeding paddy fields. The recommended dosage of florpyrauxifen-benzyl in Chinese paddy fields is 18-36 g ai ha^{-1}. It exhibited a control effect in at least 10 weed species that infest rice fields, especially in barnyard grass and broadleaf weeds (Wang et al., 2021). However, the price of the commercial product florpyrauxifen-benzyl, Rinskor, is relatively high (80-160 USD ha^{-1}), which could increase the cost of agricultural production and hard to be accepted by farmers. Therefore, selecting a suitable herbicide to mix with florpyrauxifen-benzyl is the key to solving the cost problem. In this study, metamifop and pyraquinate which are used at a reduced dose (less than 150.0 g ai ha^{-1}) were mixed with florpyrauxifen-benzyl, respectively, which achieved satisfactory results (Figures 2-4, Tables 4-6). The dosage of florpyrauxifen-benzyl can be reduced to 13.5 g ai ha^{-1}.

The choice of herbicide application is very important in farmland production, because improper herbicide application can result in crop poisoning and yield losses (Pereira et al., 2015; Langaro et al., 2017). In this study, through the screening test of 19 herbicide treatments, three herbicide combinations that were safer for dry direct-seeding *indica* rice were initially selected (Figure 3). This experimental system is typical because the Chinese sprangletop is resistant to ACCase inhibitors (Yuan et al., 2021). In fact, the severe resistance of Chinese sprangletop to ACCase inhibitors in Chinese rice fields was very common (Yuan et al., 2021). It is very difficult to control cyhalofop-butyl-resistant Chinese sprangletop that exceeds the 5-leaf stage, according to current chemical methods. This study provides a new approach to address this challenge. If there exist gramineous weeds, broad-leaved weeds, and sedges in dry direct-seeding *indica* rice fields, and the weeds are not herbicide-resistant, metamifop + florpyrauxifen-benzyl (120.0 + 9.0 g ai ha^{-1}) or 22% cyhalofop-butyl + pyrazosulfuron-ethyl + bispyribac-sodium OD (99.0 g ai ha^{-1}) is recommended; if there exist weeds that are herbicide-resistant, pyraquinate + florpyrauxifen-benzyl (150.0 +13.5 g ai ha^{-1}) is recommended.

CONCLUSIONS

This study innovatively proposes the application of 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors in *indica* rice fields, under the premise of screening for selective dosage. It also provides a potential herbicide combination (150.0 g ai ha^{-1} pyraquinate + 13.5 g ai ha^{-1} florpyrauxifen-benzyl) for weed management in dry direct-seeding *indica* rice fields, worldwide. This new combination has significant advantages in efficacy and selectivity, especially in the control of acetyl-CoA carboxylase (ACCase) inhibitors-resistant weeds. However, this study also has limitations. Firstly, we only used the main *indica* rice varieties from the lower reaches of the Yangtze River in China in our experiment, which do not have extensive representativeness. Before using this combination, strict testing is required for the safety of other rice varieties. Secondly, this study only compared the methods of chemical control, while physical and biological control methods are the future research and development focus.

Author contribution

Conceptualization: Y.G., G-H.S. Methodology: Y.G., Z-H.T., G-H.S. Software: J-P.F., G-H.Y. Validation: Y.G., G-H.S. Formal analysis: Y.G., J-P.F. Investigation: Y.G., J-P.F., G-H.Y., Y.L., Z-H.T. Resources: G-H.Y., Z-H.T., Y.L. Data curation: G-H.S. Writing-original draft: Y.G. Writing-review & editing: Z-H.T., G-H.S. Visualization: Y.G. Supervision: Z-H.T., G-H.S. Project administration: Y.G., G-H.S. Funding acquisition: Y.G., J-P.F. All co-authors reviewed the final version and approved the manuscript before submission

Acknowledgements

This research was funded by Shanghai Agriculture Applied Technology Development Program, China (Grant number 2022-02-08-00-12-F01166) and Special research project in the field of agricultural technology innovation support of Eco-Environmental Protection Research Institute, Shanghai Academy of Agricultural Sciences, China (Grant number Shengkechuang-XJ-2024-6).

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