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Fitness of Malaysian Imidazolinone-Resistant Weedy Rice and Their Growth Responses to Various Herbicides

(Kecergasan Padi Angin Rintang Imidazolinone Malaysia dan Tindak Balas Pertumbuhannya terhadap Pelbagai Racun Herbisida)

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ABSTRACT

The Ser-653-Asn mutation has been identified as the primary factor responsible for imidazolinone (IMI)-resistant weedy rice in Malaysia. This has led to inquiries regarding whether this specific mutation might impose a fitness penalty on the weedy rice population. Consequently, this research examines the fitness of IMI-resistant weedy rice and evaluates its growth responses to different herbicide modes of action (MOA). In the first experiment, the IMI-resistant weedy rice, susceptible weedy rice, and IMI-rice were germinated. Subsequently, the weedy rice seedlings were transplanted interspersed with IMI-rice at varying planting densities. The populations were categorised into herbicide-treated and untreated groups. Photosynthesis parameters were measured at 30, 45, 60, and 75 days after sowing (DAS). Morphological parameters such as leaf area, plant height, dry weight, and tiller numbers were collected after 80 DAS. In the second experiment, the IMI-resistant weedy rice was subjected to different herbicide modes of action (MOA) to evaluate their growth responses. The study was carried out in two parts: Pre-emergence and post-emergence pot trial. In pre-emergence pot trial, the study was conducted using pre-germinated seeds in petri dishes and treated with pretilachlor at 1, 3 and 5 DAS. For post-emergence pot trial, pre-germinated seeds were planted in trays, and at 1-2 leaf stage were treated with quinclorac, imazethapyr and clethodim at the recommended rate. The germination rate and plant height were measured in pre-emergence pot trials after 14 DAS, while survivability and dry weight were recorded in post-emergence pot trials after 21 DAS. A slight fitness cost was observed in IMI-resistant weedy rice, in which it has larger leaf areas and shorter plant height compared to IMI-susceptible weedy rice and MR220CL2 rice. There were no significant differences between the populations in the other morphological parameters observed. In the growth response study, IMI-resistant weedy rice has been observed to have different responses to different herbicide's MOA. Pretilachlor was effective when applied at 1 DAS, and its efficacy reducing when applied later. Clethodim was effective in controlling IMI-resistant weedy rice, quinclorac was ineffective and imazethapyr was only effective in controlling IMI-susceptible weedy rice. Further studies are necessary to establish a standardised assessment of fitness costs and to employ and integrate other MOAs in the management of weedy rice.

Keywords: Fitness cost; herbicide resistance; imidazolinone; MOA; weedy rice

ABSTRAK

Mutasi Ser-653-Asn telah dikenal pasti sebagai faktor utama yang menyebabkan padi angin rintang-imidazolinone (IMI) di Malaysia. Penemuan ini telah menimbulkan persoalan sama ada mutasi tersebut memberi kesan penalti kecergasan kepada populasi padi angin berkenaan. Oleh itu, kajian ini dijalankan untuk menilai kecergasan padi angin rintang-IMI dan tindak balas pertumbuhannya terhadap pelbagai mod tindakan (MOA) racun rumpai. Dalam uji kaji pertama, padi angin rintang-IMI, padi angin rentan dan padi IMI telah dicambahkan. Anak benih padi angin kemudiannya dipindahkan dan ditanam secara berselang-seli dengan padi IMI pada kepadatan tanaman yang berbeza. Populasi ini dibahagikan kepada kumpulan dirawat dengan racun rumpai dan kumpulan tidak dirawat. Parameter fotosintesis diukur pada 30, 45, 60 dan 75 hari selepas tabur (HST). Parameter morfologi seperti luas daun, tinggi pokok, berat kering dan bilangan sulur direkodkan selepas 80 HST. Dalam uji kaji kedua, padi angin rintang-IMI turut diuji dengan pelbagai mod tindakan racun rumpai untuk menilai tindak balas pertumbuhan. Kajian ini dibahagikan kepada dua: percubaan dalam pasu secara pracambah dan pascacambah. Dalam percubaan pracambah, biji benih pracambah diletakkan dalam piring petri dan dirawat dengan pretilachlor pada 1, 3 dan 5 HST. Bagi percubaan pascacambah, biji benih pracambah ditanam dalam dulang dan dirawat dengan quinclorac, imazethapyr dan clethodim pada peringkat 1-2 daun, mengikut kadar yang disyorkan. Kadar percambahan dan tinggi pokok diukur dalam ujian pracambah selepas 14 HLT, manakala kadar kelangsungan hidup dan berat kering direkodkan dalam ujian pascacambah selepas 21 HST. Kesan penalti kecergasan yang kecil telah diperhatikan pada padi angin rintang-IMI, yang mana ia mempunyai luas daun yang lebih besar dan ketinggian yang lebih rendah berbanding padi angin rentan dan padi MR220CL2. Tiada perbezaan signifikan diperhatikan dalam parameter morfologi yang lain. Dalam kajian tindak balas pertumbuhan, padi angin rintang IMI menunjukkan tindak balas yang berbeza terhadap pelbagai MOA racun rumpai lain. Pretilachlor berkesan apabila digunakan pada 1 HLT dan keberkesanannya menurun jika dirawat lewat. Clethodim didapati berkesan dalam mengawal padi angin rintang IMI, manakala quinclorac tidak berkesan dan imazethapyr hanya berkesan ke atas padi angin rentan. Kajian lanjutan diperlukan untuk menilai kesan penalti kecergasan secara piawai dan bagi menerapkan serta menggabungkan MOA lain dalam pengurusan padi angin.

Kata kunci: Imidazolinone; kerintangan racun rumpai; kos kecergasan; MOA; padi angin

INTRODUCTION

The introduction of rice cultivars carrying imidazolinone (IMI)-resistance genes has facilitated the management of weedy rice proliferation (Dilipkumar et al. 2021; Sang et al. 2024). However, the fitness cost related to herbicide resistance can constrain the efficacy of these cultivars (Hassanpour-Bourkheili et al. 2020b; Li et al. 2023). The fitness of herbicide-resistant weeds generally exhibits variability, with some experiencing deleterious effects, others showing beneficial outcomes, and yet some demonstrating no discernible differences (Gherekhloo et al. 2021). Some studies have suggested that IMI-resistant weedy rice may display increased growth and competitive capabilities under specific conditions, however, the long-term ecological implications of these characteristics remain insufficiently understood (Piveta et al. 2020).

The application of chemical weed management through herbicides that are selective for rice proves largely ineffective against their weedy counterparts (Wang et al. 2023). An exception to this limitation is the transgenic varieties that have been suitably engineered to withstand herbicides selective for cultivated rice, exhibiting a wide range of efficacy (Fang et al. 2020). Nonetheless, the effectiveness of herbicides in managing infestations of weedy rice is minimal (Roma-Burgos et al. 2021). Comprehending the mode of action (MOA) of herbicides is crucial for the formulation of an effective weed control programme. The MOA of a herbicide encompasses all processes that render plants sensitive to the active ingredient of the herbicide, subsequently affecting normal plant development and growth (Székács 2021). The development of a control programme for IMI-resistant weedy rice should be feasible, provided an appropriate MOA distinct from IMI is identified, with a concomitantly low likelihood of inducing herbicide resistance.

Research concerning the fitness cost of weedy rice remains scarce in Malaysia. Comprehending the fitness cost of weedy rice in relation to IMI herbicides is crucial to formulate effective weed management strategies in rice cultivation (Ruzmi et al. 2021). The excessive application of IMI herbicides has substantially diminished their effectiveness, thereby necessitating the investigation of alternative herbicides with varying MOA to efficiently control resistant weed populations (Patterson et al. 2022; Ruzmi et al. 2021). Moreover, the lack of new herbicides with innovative MOA entering the market during the past thirty years intensifies the difficulty of managing herbicide-resistant weed populations (da Costa et al. 2021). Therefore, this study aims to evaluate the fitness of weedy rice in competition with cultivated rice across various planting densities and to investigate the growth responses of IMI-resistant and IMI-susceptible weedy rice populations to alternative herbicide modes of action.

MATERIALS AND METHODS

PLANTING MATERIALS AND DESIGN

Progeny of the imidazolinone (IMI)-resistant weedy rice (R), susceptible (S) weedy rice, and IMI-rice (MR220CL2) populations were used in this study. The seeds were pre-germinated and transplanted in 30 × 40 cm planting trays in the glasshouse. There are two main experiments in this study namely: 1) Fitness of weedy rice and 2) Growth response of IMI-resistant weedy rice towards other herbicide mode of action (MOA).

FITNESS OF WEEDY RICE

The design of the experiment follows Anthimidou et al. (2020) with some modifications. Weedy rice seedlings were grown in a tray according to planting densities listed in Table 1. Two weedy rice populations were planted, resistant (R) and susceptible (S), in separate trays. Each population and density have four replications each. The treatments were divided into two: the absence and presence of the herbicide. The assigned treatments for the populations are IMI-resistant weedy rice in the presence of herbicide (HR), IMI-resistant weedy rice in the absence of herbicide (NR), susceptible weedy rice in the presence of herbicide (HS) and susceptible weedy rice in the absence of herbicide (NS). For the fitness study with the presence of IMI-herbicide, a recommended rate of IMI-herbicide imazapic + imazapyr (On Duty®) was applied as treatment at the 1-2 leaf stage. In the absence of IMI-herbicide treatment, the plants were left as it is. The plants were managed following the standard of rice cultivation practices (Azmi et al. 2008) and were left to grow up to 85 days after sowing (DAS). Photosynthesis rates were taken using the LI-6400XT portable photosynthesis system (LI-COR Biosciences, Atlanta, Georgia, USA) at 30, 45, 60, and 75 DAS. After 85 DAS, the leaf area of each treatment and densities were measured using LI-3100C leaf area meter (LI-COR Biosciences, Atlanta, Georgia, USA). Weedy rice plant height and the number of productive tillers were also taken. The plants were weighed after being harvested 1 cm aboveground and oven-dried at 65 °C for 72 h.

GROWTH RESPONSE OF IMI-RESISTANT WEEDY RICE TOWARDS OTHER HERBICIDE MODE OF ACTION (MOA)

Pre-Emergence Pot Trial

The pre-emergence pot trial follows the method of Ruzmi et al. (2020) with some modifications. The R, S, and MR220CL2 population seeds were soaked in water for 24 h. Twenty seeds from each population were transferred to a Petri dish lined with filter paper and replicated four times. The seeds were then treated with the pre-emergence herbicide pretilachlor at the recommended rate at 1, 3, and 5 days after sowing (DAS). The concentration of pretilachlor is listed in Table 2. The samples were left on the laboratory bench at ambient room temperature (24-27 °C) and light for 14 DAS. Subsequently, the germination rate, plant height, and leaf count were quantified.

Post-Emergence Pot Trial

The experiment was conducted in the glasshouse. This post-emergence pot trial follows the method of Ruzmi et al. (2020) with some modification. Twenty pre-germinated seeds of R, S, and MR220CL2 populations were transplanted into 30 × 40 cm trays with four replicates for each population. The trays contained soil saturated with water to emulate rice-growing conditions. The recommended rate of post-emergence herbicides imazethapyr, quinclorac, and clethodim were applied at the 1-2 leaf stage (Table 2). The samples were left to grow for 21 DAS, and the growing conditions and water level followed those of the rice planting method (Azmi et al. 2008). After 21 DAS, the survival rate was recorded, and the plants were harvested 1 cm aboveground. Their dry weight was determined after drying the harvested plants at 65 °C for 72 h in an oven.

STATISTICAL ANALYSIS

A randomised complete block design (RCBD) was used to arrange and design the planting trays. Two-way analysis of variance (ANOVA) and Fischer's Least Significant Differences (LSD) were done for all treatment and planting densities, utilising R Studio (R Core Team 2025) to calculate the significance of the data. The two factors analysed in the two-way ANOVA were IMI-herbicide treatment (absence and presence) and planting density. A one-way ANOVA was conducted for the growth response of weedy rice

TABLE 1. Planting densities (total number of plants per tray), number of weedy rice plants and number of MR220CL2 rice plants in each treatment and population

Planting densities (total number of plants per tray)	Number of weedy rice plants	Number of MR220CL2 rice plants
20	20	0
25	20	5
30	20	10
35	20	15
40	20	20

TABLE 2. Concentrations (g ai L^{-1}) of herbicides used in the pot trials

Pot trial	Herbicide	Concentration
Pre-emergence	Pretilachlor	1.44
Post-emergence	Imazethapyr	0.54
	Quinclorac	0.66
	Clethodim	0.31

towards other modes of herbicide experiment with the significance of the data set at $P<0.001$. A polynomial linear regression plot was done for the plant growth parameters using SigmaPlot version 14.0 (Systat Software Inc., GmbH, Germany) using the following equation:

$$y=b_0+b_1x$$

where y is the dependent variable; b_0 and b_1 are the coefficients; x is the dependent variable.

The polynomial linear regression is used to assess the relationship between different growth parameters in the absence and presence of IMI-herbicide treatments and planting density. Photosynthesis parameters and growth response to various herbicides results were presented in tables.

RESULTS AND DISCUSSION

FITNESS OF WEEDY RICE: MORPHOLOGICAL PARAMETERS

Significant reductions in physiological parameters were recorded among herbicide-resistant (HR) and non-treated resistant (NR) weedy rice across various planting densities (Table 3). All parameters measured in all treatments across the populations were significantly different at $P<0.001$. Dry weight decreased relative to the control are 75-19% in HR and by 73-15% in NR, while susceptible weedy rice (HS) was completely controlled by herbicide. Both HR and NR had 22-29% lower dry weight compared to susceptible (NS) rice, indicating reduced growth potentially linked to the imidazolinone (IMI)-resistance trait. The leaf area of HR relative to the control was measured at 91-0%, NR were 81-24%, and NS were 87-12%. The flag leaf area relative to control for HR was 90-17%, NR were 96-20%, and NS exhibited 91-14% reduction. The most productive tillers were observed in NR, followed sequentially by HR, NS, and HS across all planting densities. The percentage of productive tillers in NR, relative to the control, was observed to be 96-45%. Conversely, NS recorded a 74-35% reduction. In HR, the percentage of productive tillers was at 89-53%, whereas HS exhibited a value of 0% because of plant mortality following herbicide application.

Plant height reductions ranged from 79-20% in HR and 84-63% in NR, compared to 84-31% in NS. Leaf

TABLE 3. Dry weight, plant height, leaf area, flag leaf area, and productive tillers of resistant and susceptible weedy rice population in different planting densities with and without IMI-herbicide application

IMI-Herbicide application	Weedy rice population	Planting density (plants per pot)	Dry weight (g)	Plant height (cm)	Leaf area (cm^2)	Flag leaf area (cm^2)	Productive tillers (number)
Untreated	Resistant (NR)	20	51.93±3.21a	39.40±1.80a	16.38±0.41a	14.50±0.59a	30.00±1.10a
		25	37.85±1.43b	32.80±1.31b	13.16±0.63b	13.89±0.76a	29.00±0.63a
		30	27.70±0.66c	30.80±1.34b	9.61±0.47c	9.01±0.32b	18.40±2.49b
		35	16.20±0.30d	25.60±0.46c	8.44±0.71c	8.57±1.06b	17.20±1.99bc
		40	7.85±0.55e	24.40±0.83c	3.90±0.62d	3.00±0.46c	13.60±0.83c
	Susceptible (NS)	20	66.48±2.78a	52.20±0.59a	15.72±0.98a	15.88±1.08a	29.00±0.80a
		25	56.00±1.94b	43.80±1.21b	13.50±0.29b	14.42±0.70ab	21.60±0.67b
		30	38.03±0.81c	41.60±1.37b	12.29±0.54b	12.89±0.66b	18.00±0.75c
		35	22.30±1.82d	20.20±1.04c	3.83±0.65c	5.90±0.26c	16.40±1.19c
		40	8.50±0.98e	16.40±0.73d	1.82±0.09d	2.26±0.80d	10.40±0.83d
Treated	Resistant (HR)	20	47.23±1.43a	49.00±0.63a	20.23±0.72a	23.07±2.17a	23.80±0.77a
		25	35.45±1.76b	39.00±1.17b	18.35±0.70b	20.58±2.00a	21.40±0.83a
		30	28.28±0.63c	27.40±1.22c	17.29±0.72b	15.66±2.04b	20.60±1.56ab
		35	21.70±1.67d	20.80±1.04d	6.69±0.44c	7.51±0.32c	17.60±1.15b
		40	9.28±1.15e	9.80±0.59e	5.97±0.65c	3.90±0.25c	12.80±0.77c
	Susceptible (HS)	20	n.a.	n.a.	n.a.	n.a.	n.a.
		25	n.a.	n.a.	n.a.	n.a.	n.a.
		30	n.a.	n.a.	n.a.	n.a.	n.a.
		35	n.a.	n.a.	n.a.	n.a.	n.a.
		40	n.a.	n.a.	n.a.	n.a.	n.a.

Data is expressed as mean±standard error. The means are significant at $P<0.001$. n.a. means not available and flag leaf areas were generally larger in HR than NR across densities but decreased under high-density conditions (Figure 1). This trend corresponds with findings that resistant populations may show altered or enhanced morphology depending on environmental pressures and resistance mechanisms (Chen et al. 2021; Jin et al. 2022). Mutations in the enzyme responsible for herbicide resistance might lead to interference with the metabolism of a plant and could redirect their resources from growth to defence, thus, altering their phenotype (Hassanpour-Bourkheili et al. 2020a; Yu et al. 2020). NR populations showed the highest number of productive tillers, followed by HR, NS, and HS. Tillering declined by 96-45% in NR, 89-53% in HR, and 74-35% in NS, while HS produced none due to mortality. The enhanced tillering observed in HR may relate to hormetic responses, although evidence for hormesis under field conditions remains limited (Mollaee et al. 2020).

Morphological changes such as reduced plant height, leaf area, and dry weight are consistent with the presence of fitness penalties or altered resource allocation in resistant populations (Unan et al. 2024; Zakaria & Ahmad Hamdani 2023). However, such traits are not always linked to reduced fitness and may reflect adaptive strategies depending on density and competition. Weedy rice, regardless of resistance status, generally exhibits greater plant height (140-150 cm) and tillering capacity (average 14 panicles/hills) than cultivated rice (115-120 cm height, average 9 panicles/hill), aligning with earlier observations (Juliano et al. 2020). In this study, the plant height of IMI-resistant population was overall shorter than IMI-susceptible population (Table 3). The Ser-653-Asn mutation imposes a fitness penalty towards the height of the IMI-resistant weedy rice as similar finding in IMI-resistant *Euphorbia heterophylla* with similar Ser-653-Asn mutation were observed with shorter plant height (120 cm) compared to its susceptible (137 cm) counterpart (Hassanpour-bourkheili et al. 2020b).

FITNESS OF WEEDY RICE: PHOTOSYNTHESIS PARAMETERS

Photosynthesis rates measured at 30, 45, 60, and 75 days after sowing (DAS) declined significantly compared to controls, following the order HR > NR > NS > HS (Table 4). HS plants were completely controlled by herbicide, recording no photosynthetic activity. HR maintained 80-95% photosynthesis at 25 plants density, decreasing to 23-60% at 40 plants density. NR and NS showed a similar density-dependent decline, with NR ranging from 96% to 17% and NS from 91% to 7%. Net photosynthesis in weedy rice decreased with increasing density, while intercellular CO_2 remained relatively stable. As planting density increase, the competition for resources such as water, light and air increases causing a decline in overall net photosynthesis (Liu et al. 2020). Intercellular CO_2 should be affected similarly; however, the stable reading might be caused by a decrease of soil water availability (Huang et al. 2021) especially nearing plant maturity. HR had higher photosynthesis rates than NR in early stages, possibly due to non-target site resistance (NTSR) mechanisms (Yean et al. 2021). Although R populations initially outperformed S, the latter exhibited higher photosynthesis at 60 and 75 DAS, suggesting reduced fitness in R populations later in growth, likely due to the Ser-653-Asn AHAS mutation from gene flow with IMI-rice (Ruzmi et al. 2021; Unan et al. 2024). Weedy rice's higher photosynthesis rates, especially early in development, may also be linked to larger leaf areas (Figure 1), enhancing light interception (Ayalew et al. 2022; Rahma Harti et al. 2024).

GROWTH RESPONSE OF WEEDY RICE TOWARDS DIFFERENT HERBICIDE MODE OF ACTION (MOA): PRE-EMERGENCE HERBICIDE, PRETILACHLOR

Pretilachlor application at 1 day after sowing (DAS) halted germination in resistant (R), susceptible (S), and MR220CL2 populations (Table 5). At 3 and 5 DAS, germination resumed with healthy seedlings showing no injury symptoms (Figure 2(A)). Seeds treated at 1 DAS developed roots but no shoots, while those treated later exhibited normal growth. Shoot and leaf development increased with time: 32-61% leaf presence at 3 DAS and 61-89% at 5 DAS. No leaf growth was observed at 1 DAS due to complete control. Among the populations, R exhibited the tallest plants, followed by S and MR220CL2 (Figure 2(A)). Pretilachlor, a VLCFA inhibitor, effectively halted early development in all populations by disrupting fatty acid biosynthesis in plants (Nazir et al. 2022; Strom et al. 2020).

MR220CL2 and weedy rice failed to survive treatment at 1 DAS, while delayed applications (3 and 5 DAS) resulted in stunted growth and fewer leaves (Figure 2(A)). Weedy rice maintained greater height and leaf number than MR220CL2, likely due to its faster early growth (Sudianto et al. 2016). Optimal application timing such as 2 DAS enhances pretilachlor's effectiveness on weedy rice while minimizing impact on cultivated varieties (Shen et al. 2013). Though resistance to pretilachlor is rare, its performance can be influenced by soil and environmental conditions (Nazir et al. 2022; Strom et al. 2020), which can be beneficial in integrated weed management programs.

THE GROWTH RESPONSE OF WEEDY RICE TOWARDS DIFFERENT HERBICIDE MODE OF ACTION (MOA): POST-EMERGENCE HERBICIDES CLETHODIM, IMAZETHAPYR AND QUINCLORAC

Clethodim completely controlled all populations, with no survival recorded (Table 6). In contrast, quinclorac-treated plants showed over 90% survival across all populations, while imazethapyr-treated susceptible (S) plants showed just 30% survival, compared to >90% survival in resistant (R) and MR220CL2. Dry weight reductions in the populations varied across herbicide treatments.

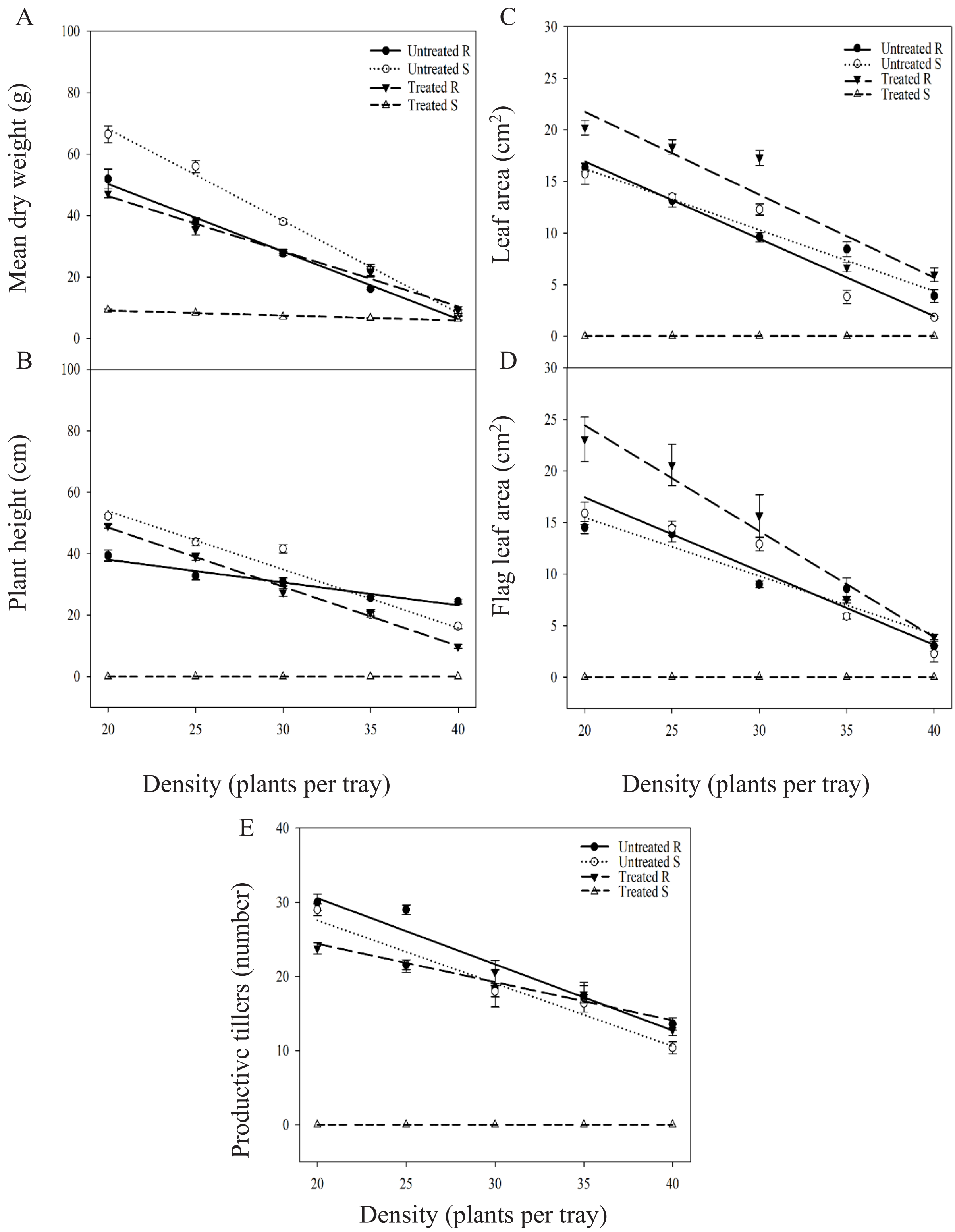

FIGURE 1. Relationship between dry weight (A), plant height (B), leaf area (C), flag leaf area (D), and productive tillers (E) of resistant (R) and susceptible (S) weedy rice in four different planting densities, in the absence and presence of herbicide treatment. Bars indicate the standard error of the mean. The means are significant at P<0.001

TABLE 4. Physiological parameters of resistant (R) and susceptible (S) weedy rice populations in different planting densities in the absence and presence of herbicide treatment at 30, 45, 60 and 75 days after sowing (DAS)

DAS	Treatment	A (µmol m^{-2} s^{-1})	g_{sw} (mmol m^{-2} s^{-1})	C_i (µmol mol^{-1})	E (mmol m^{-2} s^{-1})
30	NR0	16.46±0.06a	634.05±2.953c	331.92±0.41d	12.86±0.04c
	NR1	15.86±0.02b	853.45±1.41a	342.64±0.08b	17.32±0.02b
	NR2	10.48±0.06c	681.18±1.42b	350.12±0.13a	17.46±0.03a
	NR3	7.62±0.04d	286.18±0.06e	326.94±0.25e	6.63±0.00e
	NR4	7.19±0.03e	313.01±0.56d	341.38±0.26c	7.82±0.01d
	NS0	18.33±0.12a	676.59±2.66b	326.14±0.55c	16.06±0.05a
	NS1	16.75±0.03b	743.01±1.04a	336.52±0.12b	15.24±0.02b
	NS2	10.57±0.20c	291.15±15.01d	317.64±4.83d	7.77±0.31d
	NS3	8.59±0.03d	502.13±0.83c	349.54±0.14a	12.42±0.02c
	NS4	4.35±0.01e	154.83±0.31e	329.20±0.16c	4.23±0.01e
	HR0	20.48±0.03a	526.09±4.67b	310.42±0.42e	10.61±0.07b
	HR1	17.34±0.05b	547.89±0.80a	319.23±0.26d	13.78±0.01a
	HR2	9.70±0.15c	356.03±1.21d	330.89±0.20c	10.18±0.03c
	HR3	7.70±0.02d	401.71±2.37c	389.78±0.21b	8.91±0.04d
	HR4	1.63±0.03e	270.41±0.44e	389.78±0.21a	8.19±0.01e
	HS0	n.a.	n.a.	n.a.	n.a.
	HS1	n.a.	n.a.	n.a.	n.a.
	HS2	n.a.	n.a.	n.a.	n.a.
	HS3	n.a.	n.a.	n.a.	n.a.
	HS4	n.a.	n.a.	n.a.	n.a.
45	NR0	17.40±0.03a	376.87±2.02b	299.36±0.52d	8.90±0.04b
	NR1	15.32±0.07b	243.71±1.65c	277.02±0.17e	5.47±0.03d
	NR2	14.64±0.36c	536.02±0.59a	333.65±1.14b	9.29±0.01a
	NR3	9.20±0.05d	239.86±2.66d	317.97±0.32c	5.29±0.06c
	NR4	2.87±0.12e	219.37±2.14e	361.83±1.22a	5.27±0.05e
	NS0	20.66±0.09a	654.88±0.57a	318.93±0.25d	14.43±0.01a
	NS1	13.88±0.04b	258.86±2.44e	291.36±0.59e	6.12±0.05e
	NS2	10.34±0.05c	410.90±3.88c	339.37±0.53c	8.73±0.06d
	NS3	7.73±0.53d	464.36±9.82b	351.62±2.85a	11.29±0.18b
	NS4	7.13±0.15d	351.81±1.42d	345.79±0.92b	9.31±0.03c
	HR0	27.85±1.16a	623.48±11.72a	293.24±5.30b	14.16±0.11a
	HR1	22.02±0.01b	434.09±2.43b	288.34±0.41bc	9.93±0.04b
	HR2	15.54±0.02c	280.02±2.41c	286.08±0.88c	6.79±0.05c
	HR3	9.87±0.05d	253.55±0.57d	305.39±0.56a	5.57±0.01d
	HR4	9.36±0.02d	159.99±0.53e	273.75±0.16d	3.77±0.01e
	HS0	n.a.	n.a.	n.a.	n.a.
	HS1	n.a.	n.a.	n.a.	n.a.
	HS2	n.a.	n.a.	n.a.	n.a.
	HS3	n.a.	n.a.	n.a.	n.a.
	HS4	n.a.	n.a.	n.a.	n.a.

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60	NR0	12.08±0.07a	577.00±0.29a	341.18±0.21b	14.08±0.00a
	NR1	6.09±0.04b	408.39±3.83b	355.27±0.32a	9,93±0.07b
	NR2	5.96±0.01c	208.32±0.17d	328.88±0.18e	4.50±0.00d
	NR3	5.87±0.02c	241.17±3.72c	339.18±0.47c	6.63±0.09c
	NR4	4.46±0.02d	181.07±1.27e	337.16±0.50d	4.11±0.02e
	NS0	28.66±0.05a	668.47±4.52a	295.28±0.57d	16.30±0.08a
	NS1	13.69±0.14b	451.87±12.67c	323.05±1.93c	12.23±0.26b
	NS2	12.79±0.08c	528.76±6.13b	336.52±0.27b	12.36±0.11b
	NS3	5.89±0.11d	184.61±0.25e	321.20±1.36c	4.78±0.00d
	NS4	2.07±0.04e	317.59±1.82d	370.84±0.21a	8.04±0.04c
	HR0	10.86±0.07a	280.96±2.96a	302.80±1.19c	6.61±0.04a
	HR1	9.03±0.02b	217.67±2.17c	301.48±0.87cd	5.37±0.04c
	HR2	8.47±0.02c	249.21±0.85b	314.42±0.34b	5.89±0.01b
	HR3	8.39±0.02c	195.59±1.56d	300.99±0.40d	4.70±0.03d
	HR4	2.51±0.03d	67.92±0.43e	317.08±0.41a	2.16±0.01e
	HS0	n.a.	n.a.	n.a.	n.a.
	HS1	n.a.	n.a.	n.a.	n.a.
	HS2	n.a.	n.a.	n.a.	n.a.
	HS3	n.a.	n.a.	n.a.	n.a.
	HS4	n.a.	n.a.	n.a.	n.a.
75	NR0	12.51±0.02a	241.44±0.11a	291.60±0.19b	6.30±0.00a
	NR1	11.07±0.06b	179.68±0.80b	277.32±0.98d	5.36±0.02b
	NR2	9.50±0.01c	125.64±0.38c	254.41±0.27e	4.21±0.01c
	NR3	6.63±0.01d	125.49±0.21c	285.42±0.10c	3.81±0.00d
	NR4	3.80±0.14e	0.00±0.00d	418.46±1.58a	0.00±0.00e
	NS0	14.19±0.12a	303.98±0.97a	293.64±0.56c	10.24±0.03a
	NS1	12.91±0.28b	113.54±0.17d	194.12±4.63d	3.58±0.01d
	NS2	10.09±0.20c	230.22±0.83b	302.67±1.80b	8.10±0.02b
	NS3	6.11±0.01d	135.63±1.99c	302.56±0.96b	4.81±0.06c
	NS4	0.96±0.06e	0.00±0.00e	391.24±0.81a	0.00±0.00e
	HR0	13.52±0.02a	112.09±1.06b	181.61±1.58d	4.01±0.04b
	HR1	12.53±0.03b	129.38±0.97a	218.12±1.47c	4.61±0.03a
	HR2	10.83±0.13c	131.36±11.15a	236.88±7.77b	4.79±0.37a
	HR3	9.66±0.04d	87.88±0.25c	220.29±0.27c	3.08±0.01c
	HR4	8.08±0.01e	43.20±0.45d	297.68±0.31a	1.63±0.02d
	HS0	n.a.	n.a.	n.a.	n.a.
	HS1	n.a.	n.a.	n.a.	n.a.
	HS2	n.a.	n.a.	n.a.	n.a.
	HS3	n.a.	n.a.	n.a.	n.a.
	HS4	n.a.	n.a.	n.a.	n.a.

Data is expressed as mean ± standard error. The means are significant at P<0.001. A = Photosynthesis rate, g_{sw} = stomatal conductance, C_i = intercellular CO_2, E = transpiration rate n.a. = not available

TABLE 5. The germination, number of leaves, and plant height of resistant, susceptible weedy rice and MR220CL2 rice populations treated with pretilachlor at 1, 3, and 5 days after sowing (DAS)

Parameters	Population	DAS 1	DAS 3	DAS 5
Germination (% of control)	R	0.00±0.00a	100.00±0.00a	100.00±0.00a
	S	0.00±0.00a	100.00±0.00a	100.00±0.00a
	MR220CL2	0.00±0.00a	100.00±0.00a	100.00±0.00a
Number of leaves (% of control)	R	0.00±0.00a	61.5±4.47a	89.4±6.68a
	S	0.00±0.00a	60.4±6.30a	95.0±1.72a
	MR220CL2	0.00±0.00a	32.5±4.53b	61.4±1.73b
Plant height (% of control)	R	0.00±0.00a	40.3±2.22a	74.6±7.72a
	S	0.00±0.00a	25.2±2.81b	73.4±4.53a
	MR220CL2	0.00±0.00a	32.5±4.53ab	73.7±2.10a

Data is expressed as mean±standard error of the populations. The means are significant at P<0.001

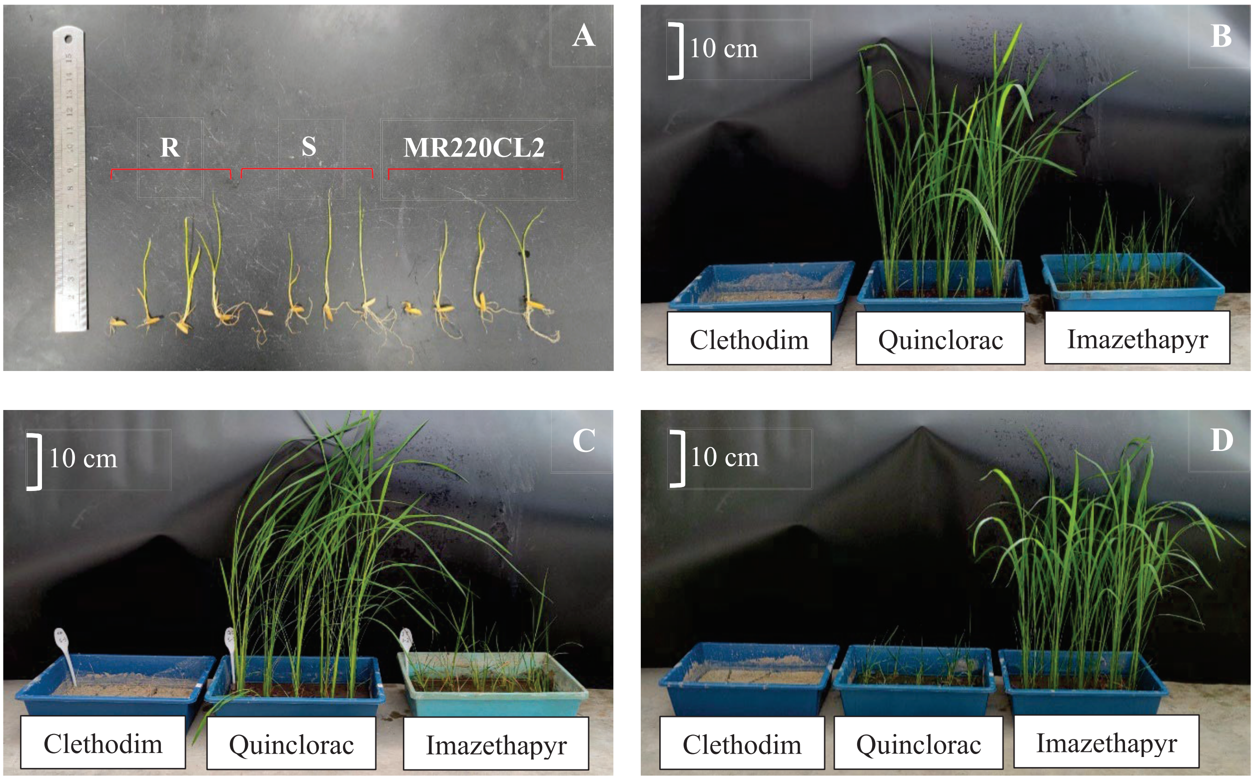

FIGURE 2. The germination and growth of resistant (R), susceptible (S) and MR220CL2 populations 14 days after sowing (DAS) treated with pretilachlor (A). The first seeds of each population were treated at 1 DAS, the second at 3 DAS, the third at 5 DAS, and the fourth with no treatment (control). The survival and growth of R (B), S (C), and MR220CL2 (D) populations at 21 DAS treated with clethodim, quinclorac and imazethapyr

TABLE 6. Survival and mean dry weight of resistant, susceptible weedy rice and MR220CL2 rice treated with imazethapyr, quinclorac, and clethodim

Parameters	Population	Imazethapyr	Quinclorac	Clethodim
Survival (% of control)	R	92.50±1.44b	98.75±1.25a	0.00±0.00a
	S	30.00±2.04c	96.25±2.39a	0.00±0.00a
	MR220CL2	98.75±1.25a	97.50±2.50a	0.00±0.00a
Mean dry weight (% of control)	R	91.74±1.60a	70.72±2.34a	0.89±0.08a
	S	11.17±1.65c	76.12±2.65a	0.74±0.16a
	MR220CL2	76.67±6.16b	6.62±0.66b	1.04±0.27a

Data is expressed as mean±standard error. The means are significant at $P<0.001$

Imazethapyr caused the most significant biomass reduction in R (91.7%) and MR220CL2 (76.7%) populations, but only 11.2% in the S population. Quinclorac was more effective in controlling S (76.1%) than R (70.7%) and ineffective to control MR220CL2 (6.6%). Clethodim-treated populations retained initial biomass due to complete mortality (Figure 2). These outcomes align with previous studies confirming cross-resistance of IMI-resistant weedy rice to other imidazolinones like imazapic and imazapyr (Ruzmi et al. 2020).

Clethodim, an ACCase inhibitor widely used in dicot crops like soybean and canola, effectively controlled weedy rice in this study, consistent with earlier findings (Gomes, Sambatti & Dalazen 2020; Yuan et al. 2021). It remains a strong candidate in resistance management programs due to its efficacy and lower risk of resistance development (Saini et al. 2015). However, its performance can be influenced by application timing and environmental factors. Imazethapyr mostly affect the growth of S weedy rice since it has no resistance to IMI compared to R and MR220CL2 (Ruzmi et al. 2021). Quinclorac, an auxin mimic herbicide, is used in rice cultivation including those using the Clearfield® system for weedy rice and other weed control (Liu et al. 2021; Rangani et al. 2022). Despite its use, the results in this study showed poor control of both R and S populations (Table 6). The effectiveness of quinclorac against weedy rice may be contingent upon the growth stage at the point of application, owing to its morphological similarity to cultivated rice (Brabham et al. 2022).

CONCLUSION

The impact of the Ser-653-Asn mutation, which confers resistance to IMI-herbicides in weedy rice on plant fitness was evaluated. Physiological similarities persist in treated and untreated resistant weedy rice with untreated susceptible ones, having minimal differences in leaf areas and plant height. Effective pre-emergence control of weedy rice is achieved with pretilachlor, though caution is needed to avoid IMI-rice damage. Post-emergence herbicides have varied efficacy, with R weedy rice and MR220CL2 resisting imazethapyr. VLCFA and ACCase inhibitors control weedy rice, while synthetic auxin requires combination with other action modes for efficacy.

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