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Article

Cross-Resistance to Imazapic and Imazapyr in a Weedy Rice (*Oryza sativa*) Biotype Found in Malaysia

*Biótipos de Arroz Vermelho (**Oryza sativa**) com Resistência Cruzada a Imazapic e Imazapyr Encontrados na Malásia*

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ABSTRACT - The Clearfield® rice production system is an effective management tool for weedy rice and other weeds in the direct-seeded rice culture. However, if farmers cultivating the Clearfield® rice disregard stewardship recommendations, the industry could face a problem of herbicide-resistant weedy rice which would occur through the selection of outcrosses. This study aimed to confirm imidazolinone-resistant weedy rice in Malaysia. The resistant weedy rice (R-WR) was found to be 67 fold more resistant to OnDuty® (premix of imazapic and imazapyr) than the susceptible weedy rice (S-WR) based on the GR_{50} values (rate that causes 50% inhibition of shoot growth). The Clearfield® rice cultivar was 32-fold more tolerant to OnDuty® than the S-WR. Furthermore, the R-WR was 54 and 89 fold more resistant to imazapic and imazapyr applied separately than the S-WR, respectively. The Clearfield® rice was 140- and 40-fold more tolerant to imazapic and imazapyr, respectively than the S-WR. The R-WR biotype was susceptible to non-selective herbicides glyphosate and glufosinate, as well as the selective graminicide quizalofop. Oxadiazon controlled the R-WR biotype, but pretilachlor was ineffective. The present study documented the first case of weedy rice that was cross-resistant to imazapic and imazapyr in Malaysian Clearfield® rice field.

Keywords: Clearfield® system, imidazolinone-resistance, direct-seeded rice, ALS-inhibitor herbicide, ACCase-inhibitor herbicide.

RESUMO - O sistema de produção de arroz Clearfield® é uma ferramenta de manejo efetiva para as plantas de arroz vermelho, bem como para outras plantas daninhas na cultura do arroz proveniente de semeadura direta. No entanto, se os agricultores negligenciarem as recomendações de manejo do sistema Clearfield®, a indústria enfrentará sérios problemas nos cultivos de arroz com o estabelecimento de plantas daninhas resistentes aos herbicidas, em razão da seleção de biótipos por cruzamentos externos. Assim, este estudo teve por objetivo confirmar a ocorrência de plantas de arroz vermelho resistentes às imidazolinonas na Malásia. O arroz vermelho resistente (R-WR) foi 67 vezes mais resistente ao OnDuty® (pré-mistura de imazapic e imazapyr) do que o arroz vermelho suscetível (S-WR), com base nos valores de GR50 (taxa que causa 50% de inibição do crescimento de plântulas). O cultivar de arroz Clearfield® foi 32 vezes mais tolerante ao OnDuty® do que os biótipos S-WR. Além disso, o biótipo R-WR foi 54 e 89 vezes mais resistente ao imazapic e imazapyr aplicados isoladamente do que o biótipo S-WR, respectivamente. O arroz Clearfield® foi 140 e 40 vezes mais tolerante ao imazapic e ao imazapyr, respectivamente, do que o biótipo S-WR. O biótipo R-WR foi suscetível a herbicidas não seletivos, como o glyphosate e o glufosinate-ammonium, seletivo ao graminicida quizalofop. O oxadiazon controlou o biótipo R-WR, mas o

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pretilachlor foi ineficaz. O presente estudo documentou o primeiro caso de arroz com plantas daninhas resistente ao imazapic e ao imazapyr em campos de arroz Clearfield® da Malásia.

Palavras-chave: sistema Clearfield®, resistência às imidazolinonas, semeadura direta de arroz, herbicidas inibidores da ALS, herbicidas inibidores da ACCase.

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of Malaysians, so Malaysia needs to become more self-sufficient in rice production. In 2015, the country's self-sufficiency level for rice production was estimated at 72% whereas the National Food Security Policy aims to attain 100% sufficiency in rice production. High demand for rice in Malaysia caused the shifting of rice seeding method from transplanting to direct-seeding in the 1980s (Azmi and Johnson, 2009). The current percentage of direct-seeded rice (DSR) cultivation in Peninsular Malaysia is believed to be highest compared to that of other Asian countries (Azmi et al., 2005). Yields are higher in DSR also, require less labour and consume less water compared to the puddled transplanting system (Sarkar et al., 2003; Mahajan et al., 2006; Bhushan et al., 2007; Zhu, 2008); thus, farmers adopted DSR rapidly. However, weeds are the major problem in direct seeded fields especially at the early stages of crop growth because of the absence of flooded condition that suppress weed growth (Chauhan and Johnson, 2010). A number of studies have shown greater rice yield losses in DSR due to high weed infestation (Baltazar and De Datta, 1992; Rao et al., 2007; Chauhan, 2012).

Weedy rice is a threat to rice production worldwide (Ziska et al., 2015). Its ability to compete against rice in all development stages (Burgos et al., 2006) has resulted in large losses of rice yield and reduction in grain quality in Malaysia (Azmi and Abdullah, 1998; Bakar et al., 2000; Chauhan, 2013). Prior to the introduction of Clearfield® rice technology, there were no selective herbicides suitable for supressing weedy rice; management was done only through land preparation, mechanical tools, and other cultural practices. Before rice cultivation, non-selective herbicides like glyphosate or glufosinate can be applied in the stale seedbed to control weedy rice (Maneechote et al., 2007). In Malaysia, the pre-emergence herbicide, pretilachlor has been commonly applied immediately after tillage, followed by allowing irrigation water to stand in the field for 7 to 10 days before broadcasting pre-germinated rice seeds (Dilipkumar et al., 2012). Other pre-emergence herbicides including oxadiazon and s-metolachlor are effective on weedy rice, but these herbicides must be applied at least 15 days before rice sowing to avoid any phytotoxic effects (Eleftherohorinos and Dhima, 2002).

Weedy rice infestation had been a major problem for rice production in Malaysia, until the Clearfield® rice cultivars were commercialized in 2010. The Clearfield® rice technology was first commercialized in 2002 in the USA to control weedy rice (Croughan, 2003; Bond and Walker, 2011). Other countries rapidly adopted the technology and these include Brazil, Colombia, Nicaragua, and Panama in 2003 (Sudianto et al., 2013), Costa Rica in 2004 (Valverde, 2005), Uruguay, Argentina, Paraguay and Bolivia in 2005 (Delouche et al., 2007; Kharkwal and Shu, 2009) and Italy in 2006 (Busconi et al., 2012). In Malaysia, two Clearfield® rice cultivars were developed by the Malaysian Agricultural Research and Development Institute (MARDI) namely, MR220CL1 and MR220CL2. MR220CL2 is the most widely planted cultivar in Malaysia because it is shorter and matures earlier than MR220CL1. The Clearfield® rice technology in Malaysia consists of three components, namely Clearfield® certified seeds, the OnDuty® herbicide (premix formulation of imazapic and imazapyr at 3:1 ratio) and the stewardship guidelines. OnDuty® is recommended to be applied at 0 to 7 days after sowing (DAS) at 150 g a.i. ha^{-1}.

Overall, the Clearfield® technology has benefitted the rice industry in many countries by reducing the weedy rice infestation and increasing rice yields. For instance, weedy rice-infested rice fields in Malaysia have shown increased rice yields from 3.5 to 7 metric tons/ha (Sudianto et al., 2013). Despite these advantages, there is concern regarding the escape of the resistance trait from Clearfield® rice cultivar to weedy or wild rice in cases where weedy rice control is less than 100%. Natural hybridization between the Clearfield® and weedy rice is well documented. In North America this could range from 0.003 to 0.46% depending on the cultivar, weedy rice type, and planting date (Zhang et al., 2006; Shivrain et al., 2007; Shivrain et al., 2009a). These numbers may be low, but this equates to hundreds of herbicide-resistant outcrosses per hectare. The risk of outcrossing is expected to be higher in Malaysia where two consecutive rice crops are planted per year. Moreover, Malaysian weedy rice is highly diverse in morphology and phenology. Molecular (Song et al., 2014) and morphological (Sudianto et al., 2016) studies collectively have claimed that the local conventional *indica* rice cultivars and wild rice (*Oryza rufipogon)* populations genetically contributed to the complexity of weedy rice in Malaysia. Therefore, synchronization in flowering period between the weedy and cultivated rice and genetic compatibility are expected to support intermittent gene flow across these *Oryza* groups.

Recently, many complaints have been received at MARDI from local farmers regarding the failure of OnDuty® to control weedy rice in Clearfield® rice fields (Dilipkumar et al., 2017). The occurrence of imidazolinone-resistant weedy rice has not been documented in Malaysia. Therefore, this study aimed to verify whether the reduced efficacy of OnDuty® on weedy rice is due to the occurrence of resistant weedy rice and to evaluate the potential of using other herbicides with different modes of action to control the imidazolinone-resistant weedy rice.

MATERIAL AND METHODS

Plant and herbicide materials

Weedy rice seeds from a putative imidazolinone-resistant biotype (R-WR) were collected from Clearfield® rice fields in Pendang, Kedah, Malaysia (GPS coordinates: 5°58'56.9" N, 100°26'34.9" E) in July 2016. This location was surveyed because of complaints received from local farmers about failure to control weedy rice in Clearfield® rice system. The field had been planted with the Clearfield® rice cultivar, MR220CL2 for seven consecutive seasons, which was a violation of the stewardship recommendations. Three types of weedy rice were present in the field. The predominant weedy rice type was tall with open panicle and straw-coloured hull. This was chosen for investigation. Samples were collected following a zig-zag pattern. Panicles of 40 mature plants were harvested separately and a pooled subsample of caryopses was used for the dose-response bioassays. The collected weedy rice seeds were air-dried, threshed, placed in paper bags and stored at 3-5 °C prior to use. The S-WR, conventional rice (MR220) and Clearfield® rice MR220CL2 (CL) seeds were obtained from the Rice Genebank of MARDI Seberang Perai, Pulau Pinang, Malaysia. The S-WR seeds were initially collected from a rice field in Kepala Batas, Pulau Pinang, Malaysia (GPS coordinates: 5°32'59.9" N, 100°26'28.5" E). The response of plant samples to imazapic, imazapyr, premix of imazapic and imazapyr, glyphosate, glufosinate, pretilachlor, oxadiazon, and quizalofop was evaluated (Table 1).

Table 1 - List of herbicide common names, trade names, formulation rates and manufacturers

Common name	Trade name	Mode of action	Formulation rate % (w/w)	Manufacturer
Imazapic	Cadre®	ALS inhibitor	70	BASF (M) Pvt. Ltd., Selangor, Malaysia.
Imazapyr	Chopper®	ALS inhibitor	11.9	BASF (M) Pvt. Ltd., Selangor, Malaysia.
Imzapic + imazapyr	OnDuty®	ALS inhibitor	52.5 + 17.5	BASF (M) Pvt. Ltd., Selangor, Malaysia.
Glyphosate	Ken-Phosate®	EPSP synthetase inhibitor	41	Kenso Corporation (M) Pvt. Ltd., Selangor, Malaysia.
Glufosinate	Tepat®	glutamine synthetase inhibitor	5.66	Bayer Co. (M) Pvt. Ltd., Penang, Malaysia.
Pretilachlor	Sofit®	Cell division inhibitor	28.7	Syngenta Crop Protection, Selangor, Malaysia.
Oxadiazon	Revolt®	PPO inhibitor	13	Hextar Chemicals Pvt. Ltd., Selangor, Malaysia.
Quizalofop*		ACCase inhibitor	10.4	

* Herbicide information kept confidential as requested by the manufacturer.

Dose-response test

The study was conducted at the weed science glasshouse, Rice Research Centre, MARDI Seberang Perai, Pulau Pinang, Malaysia. The resistance levels of R-WR, CL, and MR220 were compared to the S-WR, respectively in a rate titration of selected imidazolinone herbicides, imazapic, imazapyr, and the premix OnDuty®. The recommended field rate for the respective herbicides was 150 g a.i. ha^{-1}. Twenty pre-germinated seeds from each plant material were sown in plastic pots (19 cm diameter x 26 cm height) containing 1.6 kg moist silty loam field soil/pot (21% clay, 75% silt, 4% sand, pH 6, organic carbon 1.6%). The seeds were sown at a depth of 0.5 cm and then the seedlings were thinned to 15 per pot prior to herbicide treatment, except for the treatment at 0 day after sowing (DAS). Pots were placed on the centre benches in a glasshouse with a day/night temperature of 35/20 °C and natural sunlight. Herbicides rates for S-WR and MR220 were 0, 1/8, 1/4, 1/2, 1 and 2 times the recommended field rate (Table 1). For the imazapic treatment on S-WR or MR220, 1/16 of the recommended rate was included and the double rate was dropped. On the other hand, the herbicides rates for R-WR or CL were 0, 1, 2, 4, 8, 16 and 32 times of the labelled rate (Table 1). Imazapic was sprayed as a pre-emergence herbicide (0 DAS) while OnDuty® and imazapyr were sprayed at early post-emergence (5 DAS or 1 to 2 leaf stage) and late post-emergence (12 DAS or 3 to 4 leaf stage), respectively. A non-ionic surfactant was added to all post-emergence treatments at 0.15% (v/v). Herbicides were sprayed using a knapsack sprayer with a flat-fan nozzle, delivering 200 L ha^{-1} at a spray pressure of 100 kPa. Pots were irrigated as required throughout the duration of the experiment. No fertilizer was applied as it might interact with the imidazolinone herbicides (Geier and Stahlman, 2009). The experiment was arranged in a randomized complete block design with five replications and the experiment was repeated. At three weeks after treatment, the healthy (no injury symptoms) weedy rice survivors were counted. Shoot dry weight was determined by harvesting the aboveground living tissues of healthy plants, followed by drying at 80 °C for 72 h. Plant survival and shoot dry weight data were expressed as percentages of their respective controls.

Herbicide efficacy test

Dry seeds of S-WR or R-WR were treated at the recommended field rates of glyphosate (615 g e.a. ha^{-1}), glufosinate (420 g a.i. ha^{-1}), pretilachlor (500 g a.i. ha^{-1}), oxadiazon (500 g a.i. ha^{-1}) or quizalofop (80 g a.i. ha^{-1}), respectively. Pretilachlor and oxadiazon were sprayed as pre-emergence treatments (0 DAS) while glyphosate, glufosinate and quizalofop were sprayed post-emergence (12 DAS or 3 to 4 leaf stage). Quizalofop was applied with the recommended surfactant. BASF is currently developing a new rice cultivar tolerant to quizalofop (Lancaster et al., 2014; Harden et al., 2014). Therefore, quizalofop was included in the study to verify its potential in controlling imidazolinone-resistant weedy rice. Non-treated checks were included. The planting, spraying and maintenance procedures were as described previously. All treatments were arranged in a randomized complete block design with five replications and the whole experiment was repeated. Three weeks after treatment, the weedy rice survivors and shoot dry weight were recorded as described.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SPSS 20.0 to test treatments for experimental run interaction. Data from each experimental run were combined since no significant interactions were detected. Survivors (%) and biomass (% of nontreated check) data from dose-response tests were fitted to a logistic regression model, as follows (Kuk et al., 2002):

$$Y = d/(1 + [x/x_0]^b)$$

where, Y is percentage of plant survival or shoot dry weight, d is the coefficients corresponding to the upper asymptotes, x is herbicide rate, x_0 is herbicide rate required to inhibit survival or shoot dry weight by 50% relative to the control, and b is the slope of the line. Regression analyses were conducted and the values of x_0 were calculated from the regression equations. The T-test was used to compare the x_0 values between R-WR and CL or S-WR and MR220 at the 5% of significant level. The x_0-based resistant level (RL) was calculated as follows:

$$RL = Rx_0 / Sx_0$$

where, Rx_0 is x_0 value for R-WR or CL or MR220 and Sx_0 is for S-WR. For the herbicide efficacy experiments, data were subjected to one-way ANOVA in SPSS 20.0. Means were separated using the Tukey's test at the 5% level of significance.

RESULTS AND DISCUSSION

Confirmation of imidazolinone-resistant weedy rice

Responses of the weedy rice biotypes and rice cultivars to increasing rates of tested imidazolinone herbicides varied (Tables 2 and 3). Results from the paired samples t-test indicated that there was no significant difference ($p \geq 0.05$) between the LD_{50} (herbicide rate that caused 50% mortality) or GR_{50} (herbicide rate that caused 50% reduction in shoot growth) values of susceptible weedy rice (S-WR) and rice cultivar MR220, respectively for all tested herbicides (Tables 2 and 3). Conventional rice and weedy rice generally have similar responses to imidazolinone herbicides as was reported also in Italy, involving the conventional rice cultivar 'Claudio' (Kaloumenos et al., 2013). In the USA, Kuk et al. (2008) reported that the conventional cultivars Drew, Bengal, Dongjin, and Wells were equally susceptible to imazethapyr as the standard susceptible weedy rice accession 82. On the other hand, the LD_{50} or GR_{50} values of putative R-WR were significantly ($p \leq 0.05$) higher or lower than those of Clearfield® rice cultivar depending on the herbicide evaluated (Tables 2 and 3). The R-WR was 50- to 67-fold more resistant to OnDuty® than S-WR, confirming that the sampled weedy rice was resistant to imidazolinone herbicides. Cases like this have been reported in other countries adopting the technology such as in Rio Grande do Sul, Brazil where the same premix is used (Cassol et al., 2015). In their location, the resistance level among weedy rice outcrosses was generally low, ranging from 3.5 to 4.5.

Table 2 - Estimated imidazolinone herbicides LD_{50} and RL values for putative resistant weedy rice (R-WR), Clearfield® rice cultivar (CL) and conventional rice cultivar (MR220) compared to the standard susceptible weedy rice (S-WR)

Herbicide	S-WR	R-WR	MR220	CL	RL (R-WR/S-WR)	RL (CL/S-WR)	RL (MR220/S-WR)
	LD_{50}						
OnDuty®	47 (3.7)	2372 (94.0)*	43 (2.7)	823 (101.2)	50	18	0.9
Imazapic	7 (0.6)	477 (57.8)*	8 (0.5)	1341 (95.2)	68	192	1.1
Imazapyr	70 (5.2)	4995 (261.3)*	96 (12.2)	3617 (514.3)	71	52	1.4

Abbreviations: LD_{50} is the herbicide rate corresponding to 50% mortality. RL is the resistant level on the basis of the ratio between LD_{50} value from the R-WR or CL or MR220 and the LD_{50} value of S-WR. The values in parentheses are the standard error of the mean. *Denotes significant difference between R-WR and CL in LD_{50} after subjected to t test at 5% of significance level.

Table 3 - Estimated imidazolinone herbicides GR_{50} and RL values for putative resistant weedy rice (R-WR), Clearfield® rice cultivar (CL) and conventional rice cultivar (MR220) compared to the standard susceptible weedy rice (S-WR)

Herbicide	S-WR	R-WR	MR220	CL	RL (R-WR/S-WR)	RL (CL/S-WR)	RL (MR220/S-WR)
	GR_{50}						
OnDuty®	17 (2)	1131 (191.6)*	16 (1.5)	538 (60)	67	32	0.9
Imazapic	7 (0.3)	376 (32.2)*	7 (0.3)	979 (45.7)	54	140	1.0
Imazapyr	56 (1.7)	4993 (787.1)*	65 (3.7)	2246 (352.3)	89	40	1.2

GR_{50} is the herbicide rate corresponding to 50% shoot growth reduction. RL is the resistant level on the basis of the ratio between GR_{50} value from the R-WR or CL or MR220 and the GR_{50} value of S-WR. The values in parentheses are the standard error of the mean. *Denotes significant difference between R-WR and CL in GR_{50} after subjected to t test at 5% of significance level.

Spontaneous mutation(s) could occur in weedy rice populations (Sales et al., 2008), which could confer herbicide resistance (Kuk et al., 2008) under selection pressure. However, the evolution of herbicide-resistant weedy rice has occurred faster via gene flow than via selection of *de novo* resistance-conferring mutation(s) in the USA (Zhang et al., 2006; Shivrain et al., 2009b), Brazil (Roso et al., 2010), Colombia (Gressel and Valverde, 2009), Costa Rica (Valverde, 2005, 2007), Italy (Busconi et al., 2012), Greece (Kaloumenos et al., 2013) and Uruguay (Sudianto et al., 2013). The R-WR populations that evolved via gene flow are expected to have the same resistance level as the Clearfield® rice cultivar that was involved in the hybridization process, upon reaching a homozygous state. The study by Kaloumenos et al. (2013) demonstrated this, wherein the weedy rice outcrosses homozygous for the resistance-conferring $Ser_{653}Asn$ mutation in the acetolactate synthase (ALS) gene were as resistance as the Clearfield® rice cultivar that donated the resistance trait. In the present study, the Malaysian CL rice was 18- to 32-fold more tolerant to OnDuty® than the S-WR based on LD_{50} and GR_{50} values (Tables 2 and 3). Interestingly, the R-WR was 2.1 to 2.8 times more resistant than the CL rice, relative to the S-WR. The R-WR in this study had been subjected to seven consecutive seasons of selection pressure with imidazolinone herbicides as well as pollen flow from the Clearfield® rice. Thus, it is possible that this R-WR population harbors an additional resistance-conferring target-site mutation or an additional resistance mechanism.

Besides resistance evolution via gene flow, resistance development in weedy rice populations can also occur through spontaneous mutations. In Arkansas, USA, a weedy rice accession 84 collected before the adoption of the Clearfield® rice technology was found to be 48-fold more resistant to imazethapyr than the S-WR (Kuk et al., 2008). In this case, the selection pressure was from exposure to ALS-inhibitor herbicides used in the primary rotational crop, soybean, including chlorimuron, flumetsulam, imazaquin, and imazethapyr. In addition, similar to the present study, Kuk et al. (2008) also found a significant difference in the resistance level between weedy rice accession 84 and Clearfield® cultivar CL161 planted in the southern USA at the time. Further study has revealed that weedy rice accession 84 harbored $Gly_{654}Glu$ and $Val_{669}Met$ mutations (Sales et al., 2008) while CL161 carries the $Ser_{653}Asn$ single mutation (Tan et al., 2005).

Cross-resistance to imazapic and imazapyr

The R-WR showed low level of resistance to imazapic compared to CL while the opposite response was observed with imazapyr (Tables 2 and 3). The LD_{50} and GR_{50} values of imazapic in R-WR were 477 and 376 g a.i. ha^{-1}, respectively (Tables 2 and 3). These rates were 54- to 68-fold higher than those observed in S-WR. The LD_{50} and GR_{50} values for CL were 1,341 and 979 g a.i. ha^{-1} of imazapic, respectively, and these were 140- to 192-fold greater than those of the S-WR. On the other hand, the LD_{50} and GR_{50} values for imazapyr in R-WR were 4,995 and 4,993 g a.i. ha^{-1} respectively, while CL required 3,617 and 2,246 g a.i. ha^{-1} respectively, to achieve the same. Present study demonstrated that, the Malaysian CL rice was more tolerant towards imazapic (pre-emergence herbicide) whereas the R-WR evolved more resistance towards imazapyr (post-emergence herbicide).

Lack of adherence to stewardship guidelines is the most likely explanation for this phenomenon. The composition ratio of imazapic and imazapyr in OnDuty® is 3:1 and it should be sprayed at 0 to 7 DAS at the recommended rate of 150 g a.i. ha^{-1}. In the sampled field, OnDuty® had been applied at 10 to 15 DAS for the past seven consecutive seasons (personal communication with farmer). The low composition of imazapyr in OnDuty® will increase the survival of weedy rice seedlings if sprayed later than 7 DAS. This is evident in the present study where the S-WR treated with imazapyr at 12 DAS required 70 and 56 g a.i. ha^{-1} to achieve 50% mortality and shoot growth reduction, respectively (Tables 2 and 3). Meanwhile, the field rate of OnDuty® contains about 37.5 g a.i. ha^{-1} imazapyr. With repeated exposure to this low rate, the susceptible weedy rice might be able to expedite its resistance evolution to the herbicide. This is in agreement with a previous study reporting that, rigid ryegrass biotypes were selected 6 to 50 times more resistant than the original non-selected biotype, when the tested biotypes were exposed to reduced rates of diclofop-methyl continuously for 3 generations (Neve and Powles, 2005).

Efficacy of herbicides with other modes of action

Imidazolinone-resistant weedy rice biotypes could diminish the advantage of Clearfield® rice technology; thus integration with other chemical control methods is required to minimize the infestation by resistant weedy rice. The present study was carried out to evaluate efficacies of glyphosate, glufosinate, pretilachlor, oxadiazon and quizalofop on the imidazolinone-resistant weedy rice. All tested herbicides at label rate provided 100% control of S-WR (Table 4). Similarly, the R-WR also was completely controlled by all herbicides, except pretilachlor (Table 4). Although many studies have been reported on multiple resistance involving imidazolinones and glyphosate or quizalofop herbicides, to date such cases have not been documented for weedy rice (Heap, 2017). Therefore, management of the imidazolinone-resistant weedy rice by the stale seedbed technique followed by application of glyphosate or glufosinate is an option. Quizalofop was highly effective to both S-WR and R-WR (Table 4), indicating that the new ACCase-inhibitor-tolerant rice technology developed by BASF (Lancaster et al., 2014; Harden et al., 2014) will help rice growers manage imidazolinone-resistant weedy rice. However, this new herbicide-tolerant rice technology should be used carefully and wisely, taking into account all the experiences gained from the Clearfield® rice technology. Ignoring past experiences could result in the evolution of multiple-resistant weedy rice populations.

Table 4 - Efficacy of selected herbicides at recommended rates on survival rate and shoot growth of susceptible (S-WR) and putative resistant (R-WR) weedy rice

Herbicide	S-WR		R-WR	
	Survival rate	Shoot biomass	Survival rate	Shoot biomass
	% relative to control			
Pretilachlor	0	0	48 (3.1)	39 (4.6)
Oxadiazon	0	0	0	0
Glyphosate	0	0	0	0
Glufosinate	0	0	0	0
Quizalofop	0	0	0	0

The values in parentheses are the standard error of the mean.

Pretilachlor at the recommended rate reduced survival and shoot growth of R-WR by only 52 and 61%, respectively, while S-WR was controlled completely (Table 4). Pretilachlor used in the present study was co-formulated with fenclorim (safener) which protects rice seeds by inducing glutathione-S-transferase activity, resulting in rapid detoxification of pretilachlor (Deng and Hatzios, 2002; Scarponi et al., 2003). However, dry seeds of the S-WR used in the study could not survive following pretilachlor treatment because fenclorim is principally absorbed at the radicle of pre-germinated seed (Taylor, 2004). Surprisingly, this was not observed in the R-WR. This is the first report demonstrating the tolerance of imidazolinone-resistant weedy rice towards pretilachlor. Further research is needed to understand this phenomenon. On the other hand, oxadiazon exhibited excellent control of R-WR (Table 4). This herbicide can be used to manage R-WR, but needs to be applied at least 15 days before rice sowing (Eleftherohorinos and Dhima, 2002).

Clearfield® rice cultivars are still the mainstay in Malaysian rice production (Dilipkumar et al., 2017). To maintain the viability of the technology, it is essential to follow the three basic principles stated in the stewardship guidelines, namely, (1) planting Clearfield® rice for not more than two consecutive seasons, (2) using certified seed, and (3) spraying the registered imidazolinone herbicide at the specified rate plus correct timing. Rapid PCR-based assays are essential to elucidate the mechanisms involved in the evolution of imidazolinone-resistant weedy rice in Malaysia. Furthermore, the frequency of outcrossing from Clearfield® rice to weedy rice under Malaysian rice production systems warrants a strong guideline for the development of future new herbicide-tolerant rice varieties. To date, only Engku et al. (2016) reported gene flow rate from MR220CL1 and MR220CL2 to different weedy rice biotypes in Malaysia. However, the output of the study is questionable because outcrossing rate was determined by evaluating weedy rice survivors after treatment with OnDuty® at 14 DAS. As discussed earlier, the low rate of imazapyr in OnDuty® might result in high survival rate of weedy rice if sprayed beyond 7 DAS. Strict adherence to stewardship guidelines is not only prescribed for the Clearfield® rice growers but also for the researchers involved in the Clearfield® rice production system.

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