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Source: Weed Science, 69(5) : 598-608

Published By: Weed Science Society of America

URL: https://doi.org/10.1017/wsc.2021.33

Weed Science

www.cambridge.org/wsc

Evolution of imidazolinone-resistant weedy rice in Malaysia: the current status

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Special Issue Article

Cite this article: Ruzmi R, Ahmad-Hamdani MS, Abidin MZZ, Roma-Burgos N (2021) Evolution of imidazolinone-resistant weedy rice in Malaysia: the current status. Weed Sci. **69**: 598–608. doi: 10.1017/wsc.2021.33

Received: 23 December 2020
Revised: 5 March 2021
Accepted: 9 April 2021
First published online: 20 April 2021

Associate Editor:
William Vencill, University of Georgia

Keywords:
AHAS mutation; Clearfield® rice; herbicide resistance; imidazolinone herbicides; Malaysian rice fields

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Abstract

Weedy rice (*Oryza sativa* f. *spontanea* or *O. sativa* complex) has become a severe threat to Malaysian rice (*Oryza sativa* L.) granaries after the direct-seeding method of rice cultivation was introduced in the late 1980s. Since then, researchers have studied the biology and ecology of weedy rice and espoused the evolutionary theory of the origin of Malaysian weedy rice. This review paper aimed to synthesize the body of knowledge about weedy rice and the evolution of herbicide-resistant (HR) weedy rice in Malaysia. The imidazolinone (IMI) herbicide component of the Clearfield® Production System (CPS) rice package is among the most effective tools for weedy rice control. However, dependence solely on this technology and farmers' ignorance about the appropriate use of IMI herbicides with the CPS rice package have resulted in the evolution of IMI-resistant (IMI-R) weedy rice. This has reduced the efficacy of IMI herbicides on weedy rice, ultimately nullifying the benefit of CPS rice in affected fields. At present, it is assumed that IMI-R weedy rice populations are widely distributed across the rice granaries in Malaysia. Therefore, it is important that integrated management measures be adopted comprehensively by Malaysian rice growers to curb the spread of IMI-R weedy rice problem in Malaysia, especially in fields planted with CPS rice. This review focuses on the biology of Malaysian weedy rice, the history of the establishment of weedy rice in Malaysian rice fields, the impact of HR rice technology on the evolution of IMI-R weedy rice in Malaysia, the distribution of resistant weedy rice populations across Peninsular Malaysia rice granaries, the weedy rice resistance mechanisms, and weedy rice management. The synthesis of all this information is helpful to researchers, policy makers, the private agricultural industry, advisers to farmers, and proactive farmers themselves with the goal of working toward sustainable rice production.

Introduction

In Malaysia, rice (*Oryza sativa* L.) is believed to have been cultivated since the 10th century in the state of Kedah (Hamid 2010). However, structured rice cultivation began in 1664, patterned after production practices in Thailand (Hamid 2010). In 2019, Malaysia had a total rice production area of 684,416 ha, of which 425,613 ha was under the government-scheme for irrigated cultivation granaries (Department of Agriculture Malaysia 2019). A rice granary is a vast arable land with a centralized canal irrigation system under the management of federal agriculture and considered by the national Agriculture Policy of Malaysia as a major rice-producing area. Twelve rice granary areas have been developed; 10 of these are in Peninsular Malaysia; the other 2 are located in East Malaysia (Sabah and Sarawak states). The rice granaries are Muda Agricultural Development Authority (MADA), Kemubu Agricultural Development Authority (KADA), Integrated Agricultural Development Area (IADA) Kerian, IADA Barat Laut Selangor, IADA Pulau Pinang, IADA Seberang Perak, IADA Ketara, IADA Kemasin Semerak, IADA Pekan, IADA Rompin, IADA Kota Belud and IADA Batang Lupar.

Cultivated rice can be categorized into three major subspecies: (1) tropical and subtropical Asia long-grained *indica* rice; (2) short/medium-grained *japonica* rice in the temperate regions of northern China and Japan; and (3) medium-grained *javanica* rice in the Philippines, the Madagascar mountain ranges, and Indonesia (Muthayya et al. 2014). Historically, the Malaysian rice landraces comprised a mixture of *indica* and *japonica* subspecies before being replaced by "elite" rice cultivars (Song et al. 2014). These elite cultivars, derived from the exotic germplasm of the *Indica* line, were introduced during the industrialized farming era of Malaysia (Song et al. 2014).

Rice is a primary staple food that has been produced for hundreds of years in Malaysia, but rice production is yet to meet the national demand; 20% to 24% is imported from nearby countries (FAO 2018). This is due to several challenges faced by the Malaysian rice industry, namely water scarcity, extreme weather, loss of agricultural land, unfavorable soil conditions, poor crop management practices, insect pests, diseases, and weeds. Undeniably, weed infestation is one of the significant contributors to rice yield losses, not only in Malaysia, but also in most rice-growing nations. Savary et al. (2000) ranked weeds above pathogens and insects as the most devastating pest in many tropical Asian rice fields; nearly 95% rice yield loss occurs globally under severe weed competition (Rabbani et al. 2011). Of several weeds infesting rice fields, weedy rice (*Oryza sativa* f. *spontanea* or *O. sativa* complex) has been recognized as the major rice weed in Asia and can cause 5% to 100% yield loss (Azmi and Abdullah 1998; Azmi and Karim 2008; Ruzmi et al. 2017). Taxonomically, weedy rice belongs to the genus *Oryza*. The genus *Oryza* comprises various species and subspecies, including wild rice (*Oryza rufipogon* Griff.), cultivated rice, and weedy rice (Shrestha et al. 2019). In contrast to wild rice, weedy rice is believed to have evolved from (1) outcrossing between the wild type and rice cultivars with subsequent genetic exchange between the outcrosses and wild type or the rice cultivars or among various outcrosses (Kanapeckas et al. 2016; Londo and Schaal 2007; Qiu et al. 2014); (2) de-domestication events of cultivars (Kanapeckas et al. 2016; Li et al. 2017; Olsen et al. 2007; Sun et al. 2019); and (3) direct establishment from wild rice populations (Huang et al. 2017; Shrestha et al. 2019).

Despite many initiatives on weedy rice management, weedy rice has remained the leading weed problem in rice for many years in Asia, the Americas, and Africa (Chauhan 2013; Delouche et al. 2007; Mortimer et al. 2000; Second 1991; Wahab and Suhaimi 1991). The adoption of herbicide-resistant (HR) rice varieties initially controlled weedy rice well, but occasional survivors, or escapes, occur due to various reasons (unintentional or otherwise) and limitations in the supply, technical support, and farm conditions. The remaining weedy rice can outcross with the crop, eventually resulting in HR weedy rice outcrosses and populations (Burgos et al. 2008; Busconi et al. 2012; Espinoza-Esquivel and Arrieta-Espinoza 2007; Hoyos et al. 2019; Kaloumenos et al. 2013; Roso et al. 2010; Sudianto et al. 2013). There is renewed interest among researchers to understand and solve the HR weedy rice problem in a sustainable manner, or at least mitigate the evolution of resistance in weedy rice populations.

Scientists have defined herbicide resistance as the inherited ability of plants to survive the application of herbicide at a dose that normally would control the wild/susceptible populations (Vencill et al. 2002; Weed Science Society of America 1998). In the context of herbicide resistance in weeds, the herbicide-susceptible weed population becomes a population that could survive herbicide application (Moss 2017). By February 2021, a total of 263 HR weed species have been reported worldwide; 52 of them are weeds in rice, including weedy rice (Heap 2021). HR weedy rice has been documented in seven countries, namely the United States, Brazil, Costa Rica, Italy, Greece, Colombia, and Malaysia (Heap 2021). All of these countries have been adopting HR rice technology (Burgos et al. 2008; Busconi et al. 2012; Espinoza-Esquivel and Arrieta-Espinoza 2007; Hoyos et al. 2019; Kaloumenos et al. 2013; Roso et al. 2010; Sudianto et al. 2013).

In Malaysia, imidazolinone-resistant (IMI-R) rice varieties (MR220CL1 and MR220CL2) in the Clearfield® Production System (CPS) (BASF Malaysia Sdn. Bhd., 40706 Shah Alam, Selangor, Malaysia) were introduced in late 2010 and since then have received positive response from farmers due to the potential of these varieties to yield five to eight times more than conventional varieties, boosting rice yields up to 9,700 kg ha^{-1} (Azmi et al. 2012b; Mispan et al. 2019; Ruzmi et al. 2017; Svizzero 2020). However, rice growers' continuous dependence on and inappropriate application of the IMI herbicide OnDuty™ (BASF Malaysia Sdn. Bhd.) in the CPS package has tarnished the success story of CPS. Reports of IMI-R weedy rice in CPS are mounting (Ahmad-Hamdani et al. 2015; Dilipkumar et al. 2018; Ruzmi et al. 2017, 2020) and are expected to continue to rise in the near future.

Resistance of weedy rice to IMI herbicides may evolve via gene flow from CPS rice accompanied by repeated selection with IMI herbicides, favoring increased resistant allele frequency in populations (Rajguru et al. 2005; Sudianto et al. 2013). To a lesser extent, resistance to IMI herbicides could also arise from selection of rare de novo mutation(s) among populations, as evidenced by the findings of Sales et al. (2008). There is also concern about the persistence and accumulation of herbicide residue in soil as a result of continuous use of CPS. This residual activity can cause carryover injury to rotational crops (rice or other crops) that are susceptible to IMI herbicides (de Lima Fruet et al. 2020). Thus, the sustainability of CPS rice technology is impeded by the evolution of HR weedy rice populations and possible buildup of herbicide residue in soil. The rate of resistance evolution is influenced by biological, ecological, agronomic, and environmental factors (Moss et al. 2019). Understanding the resistance problem better and developing comprehensive management strategies to delay the evolution and spread of resistant weeds has taken almost 60 yr of research across many disciplines in plant science (Baucom 2019). The quest to understand the nuances of herbicide resistance in the hope of finding novel tools for weed management is a continuing challenge.

Biology and History of Weedy Rice in Malaysia

Generally, weedy rice can be defined as the unwanted plant types of the genus *Oryza* (Delouche et al. 2007; Suh 2008) as it pertains to interference with rice production and reduction of rice grain quality. The most widespread weedy rice is of the same species as cultivated rice, *Oryza sativa*, and hence is most similar to the crop (Delouche et al. 2007). *Oryza* spp. are primarily self-pollinated with low levels of hybridization (Shivrain et al. 2007), which could account for the wide diversity of weedy rice populations worldwide (Delouche et al. 2007).

Weedy rice types are most distinguishable by their spikelet characteristics. Malaysian weedy rice spikelets may be awned or awnless with various hull colorations, including strawhull, intermediate strawhull, brownhull, and blackhull (Sudianto et al. 2016). The weedy rice hull has parallel trichomes, providing a better anchorage of seeds on soil surface to facilitate seed burial and prevent being washed away by heavy rain (Abraham and Jose 2015). Morphologically, the Malaysian weedy rice populations of today can be divided into four groups (Sudianto et al. 2016). Group 1 is comprised of the majority of awned, blackhull, and brownhull types. These plant types appeared to have descended from wild rice, lending support to the population genetics data. The majority of strawhull types fall under Group 2, which supports the second possible origin of weedy rice types: these are feral plants, or variants from Malaysian elite *indica* rice cultivars, which are high shattering. The third cluster is primarily composed of brownhull morphotypes. The fourth cluster contains a mixture of other weedy morphotypes, lending credence to the natural outcrossing between crop and weed and among weedy populations of various types, resulting in an admixture of plant traits (Sudianto et al. 2016). The complexity of the genesis of Malaysian weedy rice remains to be fully understood (Ruzmi et al. 2017).

Weedy rice grain generally has a red or brown, rough pericarp, although a white pericarp is also found among some African and Asian weedy rice strains where weedy red rice is the predominant type (Delouche et al. 2007; Suh 2008). Regardless of awn presence, the majority of Malaysian weedy rice types across the granaries (40% to 100%) have a red pericarp. The white-kernel rice types represent 3% to 60% of the populations. Most of the weedy rice kernels are either long/slender or round/short (Sudianto et al. 2016). The majority (77%) of weedy rice types are tall (~100 to more than 150 cm), while only a small group (23%) are of the same height as cultivated rice (85 to 110 cm) (Dilipkumar et al. 2021). Malaysian weedy rice ecotypes mature early, with almost 80% maturing less than 100 d after emergence (Dilipkumar et al. 2021). Taller weedy rice plants have the advantage of aboveground competition over the shorter rice cultivars (Akasaka et al. 2009; Diarra et al. 1985; Rathore et al. 2016; Ratnasekera et al. 2014; Shivrain et al. 2010; Zhang et al. 2012). Weedy rice being taller than the rice crop contributes to a significant rice yield loss, especially when lodging occurs. Another important weedy trait that contributes to the persistence of weedy rice is high grain shattering (Azmi et al. 2012b; Chauhan 2013; Shivrain et al. 2010). Gripping the mature weedy rice panicle gently by hand can release more than 60% of the seeds (Zhu et al. 2012). Because many types mature early and shatter, a large proportion of seeds would have already dropped to the ground at rice harvest. Wind gusts can also shatter weedy rice grains, giving rise to the local Malay name for weedy rice: *padi angin*. *Padi* generally translates to "paddy" or "rice," and *angin* means "wind" (Ruzmi et al. 2017).

The increased infestation of weedy rice in Southeast Asia, including Malaysia, began after wide-scale adoption of the direct-seeding method. Direct seeding was introduced in the late 1980s; thereafter, weedy rice became a major problem wherever direct seeding was practiced across rice granaries in Malaysia (Azmi and Abdullah 1998). Weedy rice infestation was first reported in the Northwest Selangor Project rice fields in 1988 (Wahab and Suhaimi 1991) followed by the Muda area, Kedah State in 1990 (Mohammed Zuki and Kamarudin 1994). In 1995, weedy rice was reported in Besut in Terengganu State, and in 1996, weedy rice was found in the rice granaries in Seberang Perai of Pulau Pinang State and Kerian in Perak State (Azmi et al. 2000). In 2004, weedy rice infestation in the Muda area had spread in every district, with at least 10% cover (Azmi and Karim 2008). Weedy rice variants are the new, prevalent, and most complex weed species in the rice granaries of Peninsular Malaysia (Azmi and Baki 2007; Ruzmi et al. 2017), with at least 50% infestation levels in the east and west coast granaries and up to 20% in the northern granaries (Mispan et al. 2019). In late 2018, a comprehensive survey on geographic distribution of resistant weedy rice populations across all 12 Peninsular Malaysia rice granaries was conducted. From the CPS IMI-herbicide screening, it was discovered that 79.4% of the surveyed populations have evolved resistance to the IMI–herbicide premix (MS Ahmad-Hamdani, unpublished data).

Planting high-shattering rice varieties, direct seeding, and the use of large combine harvester that shatters rice grains are considered the major contributing factors to the spread and dominance of weedy rice in Malaysia (Suh 2008). Researchers have proposed several hypotheses to explain the introduction and proliferation of weedy rice in Malaysia. One of these is that weedy rice in Malaysia arose from hybridization between wild rice and the rice cultivars and between the different varieties of cultivated rice. Wild rice exists in Malaysia. Outcrossing between cultivated rice and wild rice is believed to have resulted in weedy rice variants that are morphologically similar to wild rice among weedy rice populations in the Muda area (Abdullah et al. 1996; Azmi and Karim 2008). This hypothesis is supported by the fact that in several rice-planting zones in Muda, weedy rice and wild rice have been observed to coexist in the same sites (Azmi and Karim 2008). Thus, outcrossing among wild, weedy, and cultivated rice seems the most plausible origin of weedy rice in Malaysia (Azmi and Karim 2008). However, the flowering time of wild rice and weedy rice does not normally synchronize, casting some doubt on this hypothesis (Azmi and Karim 2008). Others proposed that weedy rice has evolved through natural mutations (Lu et al. 2003) and through simultaneous interbreeding and mutations among the *Oryza* spp. (Azmi and Karim 2008). Yet another hypothesis is the "impractical but unavoidable" rice seeding establishment, causing reversion of some varietal traits to the weedy allele and producing phenotypes that would thrive better under certain stress conditions (Azmi and Karim 2008). This topic has been reviewed by Ruzmi et al. (2017). Indeed, some weedy rice types arose from de-domestication (Kanapeckas et al. 2016; Sun et al. 2019). The incidental de-domestication of weedy rice has thus resulted in high physical and physiological similarities between weedy rice and cultivated rice, rendering chemical control difficult. Today, such arguments about weedy rice origins can be settled using molecular and population genetics studies.

A population genetics study of Malaysian weedy rice using simple sequence repeat (SSR) markers revealed three possible origins: (1) traditional Malaysian rice cultivars or landraces (a mixture of *japonica* and *indica* varieties), (2) Malaysian elite rice cultivars that are genetically different from the traditional landraces, and (3) the wild rice *O. rufipogon*. (Song et al. 2014). This finding is congruent with the origin of weedy rice in Thailand, where the weedy rice populations form two distinct groups based on SSR marker analysis (Pusadee et al. 2013). The first group includes populations that are genetically similar to the companion cultivated rice variety, while the second group consists of populations that are admixtures of cultivated rice variety and *O. rufipogon* (Pusadee et al. 2013). The South Asian weedy rice has high genetic diversity contributed to by both cultivated varieties and *O. rufipogon* (Huang et al. 2017). The contribution of crop–weed gene flow to weedy rice evolution is also demonstrated in other regions in Southeast Asia (Huang et al. 2017). In China, weedy rice collected from Liaoning and Guangdong provinces is more closely related to the cultivated rice varieties that have been planted in the same fields where the weedy rice samples were collected than to other rice varieties planted elsewhere in China and the common Chinese wild rice (Zhang et al. 2012).

CPS Rice and Its Impact on the IMI-Herbicide Resistance Evolution in Malaysian Weedy Rice

The genetic and morphological similarity between weedy rice and cultivated rice has made chemical, physical, and cultural control difficult, because any herbicide that can inhibit the growth of weedy rice will be detrimental to cultivated rice. Hence, only HR rice herbicides in tandem with IMI-R cultivated rice work best for weedy rice (Barber et al. 2021). As an option for chemical control of weedy rice, IMI-R rice has been used for weedy rice management in Malaysia since 2010 (Azmi et al. 2012b). CPS rice was first developed in the United States by mutation breeding and was first commercialized in 2002. This technology was introduced quickly to Central and South America and then to Europe (Sudianto et al. 2013). In Malaysia, the IMI-R rice varieties were developed by crossing an IMI-R variety (IMI-TR) from the United States (mutant line harboring a mutation at Ser-653 in the *AHAS* gene) with a Malaysian rice variety MR220. The collaboration between the Malaysian Agricultural Research and Development Institute (MARDI) and BASF (Malaysia) Sdn. Bhd. resulted in the release of two IMI-R rice varieties, 'MR220CL1' and 'MR220CL2' (Azmi et al. 2012b). MR220CL1 and MR220CL2 have 98.5% and 92.5% similarity with the local parent MR220, respectively (Azmi et al. 2012a; Sudianto et al. 2013), and exhibit resistance to the IMI herbicides imazapic and imazapyr, which are then used with CPS rice to control weedy rice.

CPS rice cultivation practice consists of certified seeds of IMI-R rice varieties, the premix of the IMI herbicides imazapic (52.5%) and imazapyr (17.5%) (OnDuty™ WG,), and surfactant (Tenagam, BASF Malaysia Sdn. Bhd.) to increase herbicide sorption to the soil (Azmi et al. 2012b). The adjuvant is intended to increase the residual activity of herbicides, preventing weedy rice escapes. CPS also comes with the prescribed stewardship guideline. The Clearfield® stewardship guideline is important to ensure proper use of the Clearfield® technology by rice growers. The CPS IMI herbicides are recommended PRE and are most effective when applied within 5 d of sowing. Application later than this is not effective.

The IMIs (imazamethabenz, imazamox, imazapic, imazapyr, imazaquin, and imazethapyr) are acetohydroxyacid synthase (AHAS)-inhibiting herbicides. The IMIs are extensively used in agricultural fields because of their minimal impact on the environment, good crop selectivity, and broad range of target weeds (Ramezani 2008). Thus, besides controlling weedy rice, the mixture of imazapic and imazapyr (e.g., OnDuty™) also controls other major rice weeds, including barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], Chinese sprangletop [*Leptochloa chinensis* (L.) Nees], ricefield flatsedge (*Cyperus iria* L.), and fimbry [*Fimbristylis littoralis* Gaudich.].

The implementation of CPS rice technology has given the Malaysian rice industry an upper hand in controlling weedy rice effectively (Azmi et al. 2012b). Nonetheless, there has been a rising concern about gene flow from CPS rice to weedy rice, as has been documented in the first few years of planting Clearfield®® rice in the United States (Shivrain et al. 2007) and Brazil (Roso et al. 2010). The risk of gene flow and subsequent introgression of resistance traits into the weedy population is expected to be higher in tropical regions such as Malaysia, where farmers plant up to three rice crops in 1 yr or five rice crops in 2 yr. The benefit of winterkill, which is helpful in temperate regions, is absent. This situation is exacerbated by the fact that many farmers in developing countries do not have the knowledge, resources, infrastructure, technical support, and institutional support that are needed to follow the CPS stewardship guideline sufficiently (Dilipkumar et al. 2021). The genetic and morphological data by Song et al. (2014) and Sudianto et al. (2016) demonstrate that introgression of crop traits into weedy populations has occurred in Malaysia (although introgression of the mutant *AHAS* gene is not yet quantified) due to the previously mentioned mitigating factors. As evidence, SSR marker analysis also shows the occurrence of gene flow between Malaysian CPS rice to weedy rice, although the *AHAS* gene has not been sequenced (Engku et al. 2016). The samples from Pahang, Malaysia, revealed an outcrossing rate of up to 20.38% based on the survival rate of putative weedy rice outcrosses. The HR gene from CPS rice can be transferred to weedy rice with 0.1% to 3.2% probability (Cao et al. 2006; Clegg et al. 1993; Shivrain et al. 2007, 2009; Singh et al., 2017; Zhang et al. 2006). If that situation occurs, the continued selection of resistant outcrosses with IMI herbicides will facilitate the introgression of resistant alleles into weedy populations, forming stabilized HR weedy rice populations (Burgos et al. 2014; Gealy et al. 2003; Singh et al. 2017; Sudianto et al. 2013). Natural hybridization between cultivated rice and its weedy relatives or between wild rice and weedy rice has been documented by many research groups across the globe (Chen et al. 2004; Majumder et al. 1997; Messeguer et al. 2004; Oka and Chang 1961; Song et al. 2002, 2003).

Other than gene flow, resistance evolution in weedy rice populations may also be contributed by selection of resistance-conferring de novo mutation(s). This was evidenced by two populations of weedy rice in Arkansas, USA, containing IMI-R individuals that harbor one de novo resistance-conferring *ALS* mutation, Gly-654-Glu. These populations were sampled before the adoption of Clearfield® rice (Sales et al. 2008). However, because the occurrence of natural mutations is orders of magnitude rarer than the rate of effective gene flow, what we are witnessing in CPS fields is the evolution of HR populations via crop–weed outcrossing. Farmers in Malaysia, and apparently also in other world regions, are unable to follow all aspects of the CPS stewardship guideline, for various reasons. This is a problem. The inability to rotate rice with other crops, for example, will accelerate the selection of resistant outcrosses by the continued use of the same herbicides. Thus far, selection pressure with IMI herbicides in CPS has not yet been reported to select for de novo *ALS* mutations other than the one reported in the United States. However, while *ALS* mutations may not have occurred in weedy rice, other weed species in rice are prone to such selection for resistance to IMI herbicides, as has occurred with *Echinochloa* spp. (Matzenbacher et al. 2013; Panozzo et al. 2013; Rouse et al. 2018), *Cyperus* spp. (Chiapinotto et al. 2017; Yu et al. 2020), *Fimbristylis* spp. (Ortiz et al. 2017; Schaedler et al. 2015), and many others.

Entering the 10th year of its implementation in Malaysia, CPS rice technology has received mixed feedback from various sectors in agriculture. Growers who have been adopting CPS for at least eight planting seasons started to experience the reduced efficacy of IMI herbicides on weedy rice. In a single-dose CPS IMI-herbicide screening, Ahmad-Hamdani et al. (2015) observed a moderate to high level of survival in the three weedy rice populations collected from rice fields in Kedah State. Dilipkumar et al. (2018) later confirmed that the weedy rice population collected from Pendang, Kedah, has evolved up to 67-fold resistance to IMI herbicides. More recently, resistance to IMI herbicides was also confirmed in weedy rice populations collected from three CPS rice fields in Kedah and Perlis states (Ruzmi et al. 2020). Therefore, although the innovation of HR rice offers the ability to selectively control weedy rice, this benefit can be nullified by the evolution of HR weedy rice populations. Rice farmers will then have to revert to traditional, time-consuming practices of managing weedy rice. Herbicides, including the herbicide package in HR crops, are highly effective tools to manage weeds. Nonetheless, to sustain herbicide efficiency, one must integrate herbicides with cultural, mechanical, and biological methods. With the CPS rice technology, farmers are advised to rotate CPS rice with non-CPS rice every two planting seasons to reduce the risk of herbicide persistence and resistance evolution in weedy rice.

Distribution of Resistant Weedy Rice in Malaysia

In Malaysia, rice is grown in granary areas comprising more than 60% of the total rice-planted areas (Department of Agriculture Malaysia 2019). CPS rice has been widely grown in several major rice granaries following its introduction in Malaysia because of its effectiveness in controlling weedy rice. The CPS rice varieties MR220CL1 and MR220CL2 mature early at around 95 to 98 d after sowing and yield higher than other inbred varieties, making them highly popular among rice growers (Harun et al. 2018; Rosnani et al 2013; Sudianto et al. 2013). The adoption of CPS rice rose sharply from 0.9% in 2011 to 56% in 2015 in Peninsular Malaysia (Harun et al. 2018), following a similar trajectory of adoption in the United States (Hardke 2020). Notwithstanding its popularity among rice growers, the CPS rice technology will not be sustainable if the various constraints to proper adoption are not alleviated and general failure to follow the stewardship guideline continues.

Jaafar et al. (2014) surveyed weedy rice plants that survived/escaped the IMI herbicides in four selected CPS rice-cropping zones in Peninsular Malaysia. The survey targeted rice fields with a history of CPS rice technology for at least four consecutive planting seasons. Across all surveyed fields (approximately 50 ha, comprising 40 rice fields), 1,240 weedy rice plants survived/escaped the IMI herbicides applied in the dry/off season and 813 weedy rice plants survived/escaped in the main season of 2012. Even though weedy rice resistance to IMI herbicides escaped plants was not verified at that time, our latest nationwide survey indicated that the majority of sampled weedy rice populations are now IMI-R (MS Ahmad-Hamdani, unpublished data). With a continuous CPS rice cycle that allows these survivors to reproduce, these remaining plants will gradually replace the soil seedbank and form the contemporary HR weedy rice populations (Shivrain et al. 2008; Singh et al. 2017). The high number of consecutive CPS rice plantings and the inadequate removal of weedy rice survivors from CPS rice fields led to the increasing abundance of resistant weedy rice populations in Malaysia, as has been the story in Italy (Busconi et al. 2012), the United States (Burgos et al. 2014), Greece (Kaloumenos et al. 2013), and Brazil (Avila et al. 2021). Of late, several rice fields in Malaysia have been planted with CPS rice for more than eight consecutive seasons; thus, it is expected that IMI-R weedy rice occurs broadly in all the rice granaries in Malaysia. A publication of an extensive study on the in situ geographic distribution of resistant weedy rice populations in CPS rice fields in all rice granaries across Peninsular Malaysia is in progress (MS Ahmad-Hamdani, unpublished data).

Mechanisms Endowing IMI-Herbicide Resistance in Malaysian Weedy Rice

Generally, the mechanisms of resistance to herbicides can be divided into two categories: (1) target-site resistance (TSR) and (2) non–target site resistance (NTSR) (Gaines et al. 2020). TSR pertains to reduced target-site sensitivity as a consequence of alteration(s) in amino acid sequence(s) of the target enzyme, resulting in reduced herbicide binding. Other than that, resistance involving the target site might be conferred by the overexpression of a target enzyme through modification(s) in the gene promoter or transcription factor (Powles and Yu 2010). Most cases of AHAS-inhibitor resistance in weed species are associated with reduced sensitivity of *AHAS* to herbicide due to amino acid substitutions in the catalytic site that alter the topology of the binding pocket (Menne and Kocher 2007; Powles and Yu 2010; Tranel and Wright 2002). NTSR generally arises from multiple mechanisms that prevent the herbicide from reaching the target site, limit the amount of herbicide that reaches the target site to a nonlethal dose, or protect the plant from lethal effects of the herbicide. Among these mechanisms are reduced herbicide entry, reduced herbicide translocation, and enhanced herbicide detoxification in the plant (Gaines et al. 2020).

Among weed species, the first reported case of IMI-herbicide resistance was in rigid ryegrass (*Lolium rigidum* Gaudin) infesting spring barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) expressing resistance to imazapyr (Heap 2021; Preston et al. 1996). Meanwhile, the first IMI- HR weedy rice was detected within two seasons of planting CPS rice in the United States (Heap 2021; Rajguru et al. 2005). The majority of reported resistance to IMI herbicides is target-site based (Heap 2021). Currently, as many as 22 *AHAS* gene mutations associated with IMI-herbicide resistance have been reported in 94 weed species worldwide (Table 1). These include Ala-122 (8 species), Pro-197 (16 species), Ala-205 (6 species), Asp-376 (8 species), Trp-574 (30 species), Ser-653 (10 species), and Gly-654 (2 species). Of all these, three loci (Ala-122, Ser-653, and Gly-654) are associated with resistance in weedy rice. IMI resistance in weedy rice has been generally attributed to gene flow from HR rice in countries adopting CPS rice technology. Thus far, mutations in weedy rice conferring resistance to IMI herbicides include: Ser-653-Asn in the United States (Rajguru et al. 2005; Sales et al. 2008), Italy (Scarabel et al. 2012), and Greece (Kaloumenos et al. 2013), and Gly-654-Glu, Ser-653-Asn, Ser-653-Asp, and Ala-122-Thr alone or in combination in Brazil (Roso et al. 2010). In Malaysia, IMI-R weedy rice populations collected from CPS rice fields in Kedah and Perlis states harbor a single-nucleotide polymorphism (AGT to AAT) resulting in a Ser-653-Asn substitution in *AHAS* (Ruzmi et al. 2020) as has been reported elsewhere. Regardless, without population genetics data, we cannot rule out the possibility that continuous use of IMI herbicides has selected for this mutation de novo in some cases. Until then, we have to say that IMI-R weedy rice in Malaysia may not all be outcrosses with CPS rice. More recently, based on *AHAS* gene sequence analysis, an enzyme colorimetric assay, and a genome-wide association study, the coexistence of both TSR and NTSR was speculated in the resistant weedy rice populations collected from CPS rice fields across seven states in Peninsular Malaysia (Yean et al. 2021). Studies to determine NTSR mechanisms are warranted.

Management of HR Weedy Rice in Malaysia

After several years of wide-scale, intensive adoption of HR rice technology in Malaysia, the problem of how to manage HR weedy rice populations took center stage. The basic principle of managing HR weeds is to prevent the spread of HR populations and to delay the evolution of resistance by integrating alternative chemical tools and nonchemical practices (Beckie 2006).

The first step is prevention, for which the most effective practice is the use of uncontaminated, certified seeds (Andres et al. 2014; Dilipkumar et al. 2018). Just as this practice prevents the introduction of weedy rice into a field, it also prevents spreading of HR weedy rice to other fields. The sharing of contaminated seeds between farmers has contributed to the spread of weedy rice from infested areas to clean areas (Azmi and Karim 2008; Chauhan 2013). Unless rice farmers can be convinced to abandon this custom of seed sharing or access to certified seed can be improved,

Table 1. Summary of acetohydroxyacid synthase amino acid substitution in field-evolved resistant weed species to IMI herbicides worldwide (modified from Heap 2021; Tranel et al. 2021).

Amino acid and position	Resistance substitution	Species	Year	IMI herbicides to which resistance is conferred
Ala-122	Thr	*Xanthium strumarium*	1995	Imazethapyr
	Thr	*Solanum ptycanthum*	2000	Imazethapyr, imazamox
	Thr	*Amaranthus retroflexus*	2005	Imazethapyr
	Thr	*Amaranthus powellii*	2005	Imazethapyr
	Thr	*Amaranthus hybridus* (syn: *quitensis*)	2006	Imazethapyr
	Thr[a]	*Oryza sativa* var. *sylvatica*	2006	Imazethapyr, imazapic
	Tyr	*Raphanus raphanistrum*	2012	Imazamox, imazapyr
	Val	*Echinochloa crus-galli* var. *crus-galli*	2013	Imazethapyr, imazamox
	Thr	*Echinochloa crus-galli* var. *crus-galli*	2013	Imazethapyr, imazamox
	Asn	*Echinochloa crus-galli* var. *crus-galli*	2017	Imazamox
Pro-197	His	*Lactuca serriola*	1992	Imazethapyr
	Ile	*Sisymbrium orientale*	1999	Imazethapyr
	Leu	*Amaranthus retroflexus*	2001	Imazamethabenz, imazamethapyr, imazapyr, imazethapyr
	His	*Papaver rhoeas*	2004	Imazethapyr
	Thr	*Papaver rhoeas*	2004	Imazamox
	Ser	*Papaver rhoeas*	2004	Imazamox
	Thr	*Chrysanthemum coronarium*	2004	Imazethapyr
	Thr	*Lactuca serriola*	2006	Imazapyr, imazethapyr
	Leu	*Thlaspi arvense*	2007	Imazethapyr
	Arg	*Papaver rhoeas*	2009	Imazamox
	Leu	*Papaver rhoeas*	2009	Imazamox
	Ala	*Papaver rhoeas*	2009	Imazamox
	Leu	*Anthemis cotula*	2011	Imazethapyr
	Gln	*Anthemis cotula*	2011	Imazethapyr
	Thr	*Anthemis cotula*	2011	Imazethapyr
	Ser	*Anthemis cotula*	2011	Imazethapyr
	Leu	*Sonchus asper*	2012	Imazethapyr, imazamox
	Thr	*Hordeum murinum* ssp. *leporium*	2012	Imazamox
	Glu	*Myosoton aquaticum*	2015	Imazethapyr
	Thr	*Alopecurus aequalis*	2015	Imazamox, imazapic
	His	*Cyperus difformis*	2015	Imazamox
	Leu	*Senecio vulgaris*	2016	Imazamox
	Ser	*Cyperus compressus*	2016	Imazapic
	Leu	*Galium aparine*	2016	Imazethapyr
	Ser	*Galium aparine*	2019	Imazethapyr
	His	*Galium aparine*	2019	Imazethapyr
Ala-205	Val	*Xanthium strumarium*	1996	Imazethapyr
	Val	*Helianthus annuus*	2003	Imazethapyr
	Val	*Amaranthus retroflexus*	2005	Imazethapyr
	Val	*Solanum ptycanthum*	2007	Imazethapyr, imazamox
	Val	*Conyza canadensis*	2015	Imazapyr
	Phe	*Poa annua*	2016	Imazamox
Asp-376	Glu	*Amaranthus hybridus* (syn: *quitensis*)	2004	Imazethapyr
	Glu	*Amaranthus powellii*	2009	Imazethapyr
	Glu	*Conyza canadensis*	2011	Imazethapyr
	Glu	*Raphanus raphanistrum*	2012	Imazamox, imazethapyr, imazapyr
	Glu	*Schoenoplectus juncoides*	2013	Imazaquin
	Glu	*Amaranthus retroflexus*	2016	Imazethapyr
	Glu	*Lolium perenne*	2016	Imazamox
	Glu	*Galium aparine*	2019	Imazethapyr
Trp-574	Leu	*Xanthium strumarium*	1995	Imazethapyr
	Leu	*Amaranthus tuberculatus* (= *A. rudis*)	1996	Imazethapyr
	Leu	*Amaranthus hybridus* (syn: *quitensis*)	1997	Imazethapyr
	Leu	*Kochia scoparia*	1999	Imazethapyr
	Leu	*Sisymbrium orientale*	1999	Imazethapyr
	Leu	*Ambrosia artemisiifolia*	2001	Imazaquin
	Leu	*Ambrosia trifida*	2002	Imazethapyr
	Leu	*Amaranthus blitoides*	2003	Imazamethabenz, imazapyr, imazaquin, imazethapyr

(*Continued*)

Table 1. (*Continued*)

Amino acid and position	Resistance substitution	Species	Year	IMI herbicides to which resistance is conferred
	Leu	*Camelina microcarpa*	2004	Imazethapyr, Imazamox
	Leu	*Amaranthus retroflexus*	2005	Imazethapyr
	Leu	*Amaranthus powellii*	2005	Imazethapyr
	Leu	*Sinapis arvensis*	2005	Imazethapyr
	Leu	*Schoenoplectus juncoides*	2007	Imazaquin
	Leu	*Lolium rigidum*	2007	Imazapyr
	Leu	*Bidens subalternans*	2009	Imazethapyr
	Leu	*Schoenoplectus mucronatus* (= *Scirpus mucronatus*)	2010	Imazethapyr
	Leu	*Galium spurium*	2012	Imazethapyr
	Leu	*Echinochloa phyllopogon* (= *E. oryzicola*)	2013	Imazamox
	Leu	*Poa annua*	2013	Imazaquin
	Leu	*Echinochloa crus-galli* var. *crus-galli*	2013	Imazamox
	Leu	*Alopecurus aequalis*	2015	Imazamox, Imazapic
	Leu	*Conyza canadensis*	2015	Imazapyr
	Leu	*Cyperus esculentus*	2015	Imazethapyr, imazamox
	Leu	*Cyperus iria*	2015	Imazamox
	Leu	*Raphanus sativus*	2016	Imazethapyr, imazapyr, imazamox
	Leu	*Amaranthus palmeri*	2016	Imazethapyr
	Leu	*Descurainia sophia*	2016	Imazethapyr
	Arg	*Digitaria sanguinalis*	2017	Imazethapyr
	Leu	*Galium aparine*	2019	Imazethapyr
	Leu	*Lithospermum arvense*	2019	Imazethapyr
Ser-653	Thr	*Amaranthus powellii*	2001	Imazethapyr
	Asn	*Amaranthus tuberculatus* (= *A. rudis*)	2001	Imazethapyr
	Thr	*Amaranthus tuberculatus* (= *A. rudis*)	2001	Imazethapyr
	Asn[b]	*Oryza sativa* var. *sylvatica*	2002	Imazethapyr
	Asn	*Amaranthus hybridus* (syn: *quitensis*)	2006	Imazethapyr
	Asp[a]	*Oryza sativa* var. *sylvatica*	2006	Imazethapyr, imazapic
	Ile	*Setaria viridis*	2009	Imazethapyr
	Asn	*Setaria viridis*	2009	Imazethapyr
	Thr	*Setaria viridis*	2009	Imazethapyr
	Asn[c]	*Oryza sativa* var. *sylvatica*	2010	Imazamox
	Asn	*Galium spurium*	2012	Imazethapyr
	Thr	*Avena fatua*	2012	Imazamethabenz, imazamox
	Asn	*Avena fatua*	2012	Imazamethabenz, imazamox
	Asn[d]	*Oryza sativa* var. *sylvatica*	2013	Imazethapyr, imazamox
	Thr	*Amaranthus retroflexus*	2015	Imazethapyr
	Thr	*Sorghum bicolor*	2017	Imazethapyr
	Asn	*Bromus tectorum*	2017	Imazamox
	Asn[e]	*Oryza sativa* var. *sylvatica*	2020	Imazapic, imazapyr
Gly-654	Glu[a]	*Oryza sativa* var. *sylvatica*	2006	Imazethapyr
	Glu[f]	*Oryza sativa* var. *sylvatica*	2008	Imazethapyr, imazapic
	Asp	*Setaria viridis*	2009	Imazethapyr

[a]Additional data from Rajguru et al. (2005); not available in Heap (2021).
[b]Additional data from Roso et al. (2010); not available in Heap (2021).
[c]Additional data from Scarabel et al. (2012); not available in Heap (2021).
[d]Additional data from Kaloumenos et al. (2013); not available in Heap (2021).
[e]Additional data from Ruzmi et al. (2020); not available in Heap (2021).
[f]Additional data from Sales et al. (2008); not available in Heap (2021).

weedy rice will continue to be spread by farmers among themselves. Similarly, comprehensive sanitation practices surrounding farm equipment and field edges help minimize weed seed immigration into the field (Beckie and Harker 2017). Implementation of these practices may be may even more challenging than planting certified seed. The time for land preparation and the planting season are short. With severe constraints on time, labor, and machinery, farmers will not be able to practice equipment sanitation.

Cultural practices such as the stale seedbed technique, crop residue burning, the flooding technique, crop rotation, and controlling weedy rice escapes are recommended by most researchers to control weedy rice (Arya and Ameena 2015; Chauhan 2013; Chin 2001; Rathore et al. 2013). The same practices can likewise prevent the proliferation of resistant weedy rice. Stale seedbed practice is said to be the most effective cultural practice to control weedy rice, because it involves a series of operations, including crop residue burning and preparation of soil to favor weed seed germination for 12 to 15 d, followed by herbicide application to kill the emerged seedlings (Arya and Ameena 2015). Nonselective herbicides such as glyphosate and glufosinate are highly effective chemical tools in stale seedbed technique, applied 30 to 35 d before rice planting (MSA-H, personal observations). The stale seedbed technique and repeated plowing will reduce the weedy rice seedbank, resistant or not (Chauhan 2012; Dilipkumar et al. 2018).

Manual removal of weedy rice panicles is also practiced by some farmers in Malaysia. As outlined in MARDI's cultural practices guidelines for direct-seeded rice, weedy rice must be rouged between 70 and 80 d after planting to prevent new seed deposit and gradually diminish the soil seedbank (Badrulhadza et al. 2013). In Malaysia, where the average individual rice farm size is small (1.2 to 1.5 ha), rouging weedy rice panicles before grain filling is doable. Rouging has been included as one of the key checks in the "Farmers Current Agriculture Practices on Paddy Cultivation and Relationship with Work Performance in IADA Batang Lupar, Sarawak, Malaysia" that must be followed by farmers to achieve a yield of 10,000 kg ha^{-1} (Hassan et al. 2019). Earlier, Azmi and Abdullah (1998) proposed a holistic management program to eliminate weedy rice in rice fields that promoted rouging as one of the effective approaches. Today, rouging is still practiced by many farmers in all rice granaries in Malaysia.

Water-seeding and flooding culture has been the primary cultural practice to reduce weedy rice infestation (Chauhan 2013). Managing water by providing optimum flood depth, duration, and timing will not only reduce the germination of weedy rice but also that of other weed species, especially in a direct-seeded rice system (Arya and Ameena 2015). The adoption of a 20- to 40-cm water depth in acid sulfate soil areas in Vietnam during the winter–spring season has reduced the weedy rice infestation (Chin 2001). In Malaysia, a 5- to 10-cm flooding depth inhibits weedy rice establishment (Azmi and Karim 2008). Flooding can delay the establishment and proliferation of HR weedy rice populations (Dauer et al. 2018). At present, water seeding or transplanting is practiced by up to 71.9% of rice farmers (Dilipkumar et al. 2021). The majority practice flooded-rice culture.

Farmers are also encouraged to rotate the crop establishment methods in their rice fields, for example, rotating between wet-seeded rice to transplanted rice to reduce the weedy rice infestation, especially in most Asian countries, where wet-seeding of rice has been practiced indefinitely (Arya and Ameena 2015). Weedy rice emerging in wet-seeded fields is practically indistinguishable from cultivated rice, because rice seeds are broadcast so the farmers cannot rely on rows to discriminate weedy rice (Chauhan 2013). In a field that has been severely infested by weedy rice (resistant or not), reverting to the transplanting method can be the only recourse to reduce the infestation (Chauhan 2013), because it allows farmers to identify and manually remove weedy rice. This method has been recommended by MARDI as reported in the local *Sinar Harian* newspaper (March 13, 2018, not available online).

Rotation with different crops is highly effective in reducing weedy rice infestation and has been practiced traditionally by rice farmers when they run out of options (Arya and Ameena 2015; Burgos et al. 2008, 2014). Among the best rotational crops with rice are legumes (Burgos et al. 2008; Labrada 2006). There are many practical reasons why farmers practice rice monoculture just as in Malaysian rice granaries. The rice granaries of Malaysia are arable lands that are purposely reserved by the Malaysian government through the National Agricultural Policy as main paddy producing areas. Malaysia has not been self-sufficient in rice production for a long time; thus the establishment of rice granary areas that are monocropped with rice is intended to ensure national food security. To facilitate high production, these rice granaries are centrally irrigated through a systematic and scheduled rice irrigation system by the Department of Irrigation and Drainage, making these areas not suitable for planting other crops. Rice farmers in the granary areas also receive production input (fertilizers and pesticides) subsidies. Thus, the Malaysian CPS stewardship guideline does not recommend rotation of CPS rice variety with other crops, but rather with non-CPS rice varieties. In such situations, farmers have to diversify the herbicide modes of action they use.

As an option for controlling the spread of resistant weedy rice chemically, the non-selective herbicides glyphosate and glufosinate can be applied in the stale seedbed technique (Dilipkumar et al. 2018). Other than that, PRE herbicides like pretilachlor and oxadiazon can also control weedy rice before emergence, provided the herbicides are applied at least 15 to 7 d before sowing (Dilipkumar et al. 2018; Eleftherohorinos and Dhima 2002; Malaysian Agricultural Research and Development Institute 2020). These herbicides mainly function to reduce the weedy rice seedbank before rice planting. This integrated chemical control method is crucial to avoid overreliance on IMI herbicides in CPS rice.

Intensive monitoring or scouting of CPS rice fields is necessary throughout the growing season. Weedy rice escapes need to be removed manually or chemically (by spot spraying) in every season CPS rice is planted. Outcrosses need to be detected early by observing telltale traits of F_1 outcrosses (Burgos et al. 2008). It is important to report resistance cases immediately so that precautions can be taken to prevent seed production from outcrosses and introgression. Using herbicide tactics in conjunction with cultural practices is foremost to thoroughly control resistant weedy rice, either to prevent its spread or to delay resistance evolution. Above all, farmers' adherence to the stewardship guideline is necessary to ensure the viability and sustainability of HR rice technology. There is a great need to empower and enable the farmers in this regard.

Conclusion

The sole dependence on herbicides for rice weed control, particularly IMI herbicides in the CPS package, has resulted in the evolution of HR weedy rice in Malaysia. Despite the advantages of the CPS package, the evolution and rapid spread of resistant weedy rice has diminished the benefit of CPS and compromised the sustainability of the technology. Molecular and morphological data strongly indicate that HR weedy rice arose from gene flow, but population genetics data are needed to rule out the possibility of selection of de novo mutations. The incidence of HR weedy rice is increasing in Malaysia because of pervasive inability to follow the stewardship guideline. It is necessary to diversify the approach in managing weedy rice by integrating various management strategies, and farmers need to be empowered to do so.

Acknowledgments. The authors would like to express their sincere gratitude to the Ministry of Higher Education Malaysia, under the Fundamental Research Grant Scheme (FRGS/1/2018/WAB01/UPM/02/1: 07-01-18-1961FR; 5540086), for providing financial support for research activities and findings discussed in this review. No conflicts of interest have been declared.

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