Weedy Rice Infestation in Malaysia: What Do We Know and Where Do We Go?

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Abstract: Weedy rice is a notorious weed in the rice agroecosystem. Its infestation has caused significant economic loss in many rice-production countries, including Malaysia. In Malaysia, weedy rice consists of a genetic admixture of cultivated and wild traits, resulting in a highly competitive ability against cultivated rice for resources. The morphology and genetic plasticity of weedy rice provide several adaptive advantages, enabling it to thrive in rice agroecosystems and become a serious weed infesting Malaysian rice granaries. Previous strategies in combating weedy rice have often been less effective since weedy rice is easily adapted to a wide range of environments. In this review, we address the weedy rice issue in Malaysia and the progress of weedy rice studies. A future direction on weedy rice research via omics study is also discussed to propose solutions for improvement in weed control, good agronomic practices, and food security.

Keywords: weedy rice; Oryza spp.; omics technology; metabolomics; weed management; food security

1. Introduction

Weedy rice infestation in rice fields has been reported in many countries, including Malaysia. The estimated loss of yield per season in 2004 due to weedy rice infestation in Peninsular Malaysia was about USD 24 million [1]. In 1996, there were more than 19,900 ha of rice granaries in Malaysia infested by weedy rice [2]. Studies found that a weedy rice infestation at 10% of the rice field can cause up to an 80% yield loss, thus affecting the economy and threatening food security [3–5]. Until now, weedy rice has continuously been an unsolved weed problem in the rice industry.

Weedy rice (Oryza spp.) is a conspecific weed of cultivated rice (Oryza sativa L.). It is well known as red rice in many temperate countries due to the red pigmentation of the pericarp. In Malaysia, weedy rice is locally known as ‘padi angin’ (literal translation as ‘wind rice’) due to the ease of seed shattering attributed to the wind. It has diverse morphological traits resembling the admixture of wild rice and cultivated rice [6,7]. Weedy rice primarily constitutes the diploid AA-genome species (2n = 24) with perfect synapsis and high sexual compatibility, as well as the pollen and panicle fecundity of F1 hybrids [8,9]. At the same time, wild rice and cultivated rice can hybridize, thus producing competitive weedy rice in nature [4,7,10].

Weedy rice is descended from several groups of rice: indica, japonica, and aus, which are domesticated from different parts of Asia [11,12]. Regardless of the number of geographically different domestication events, all evidence suggests that indica and japonica rice are descended from pre-differentiated ancestral Oryza rufipogon populations [13–15]. The Malaysian rice landraces encompassed a mixture of indica and japonica subspecies before being changed by elite rice cultivars [16]. Additionally, these elite cultivars were introduced during the industrialized farming era of Malaysia and were derived from the
Weedy rice was discovered in rice-growing regions in 1846 in North and South America, Europe in the 1970s, and various Asian countries in the 1990s [19]. The direct seeding method was reported to be the main cause of the emergence of weedy rice in the majority of rice agroecosystems in the world [2,5,20]. In Malaysia, weedy rice infestation was first reported in 1988 at Tanjung Karang, Selangor, and then spread sporadically to the northern part (Muda area, Kedah, Malaysia) in 1990 [20,21]. Five years later, weedy rice was reported in Besut, Terengganu [22] and subsequently in Seberang Perai, Penang, and Kerian, Perak.

In this review, we address the weedy rice issue in Malaysia and the progress in rice agronomic practices in the country. We then explore the adaptive traits of weedy rice that contribute to its resilience and persistence in crop fields. Building on this foundation, the review discusses the extensive research efforts in Malaysia aimed at addressing these challenges. The manuscript then transitions to the potential of omics studies as future-oriented, knowledge-based strategies for weed management, highlighting the importance of genomics in revealing the genetic underpinnings of weedy rice. Furthermore, we consider the potential use of metabolomics to elucidate the biochemical processes that support weedy rice’s survival and competitiveness. Finally, the review envisions the future of weedy rice research in Malaysia, emphasizing the integration of multi-omics approaches and the adoption of cutting-edge artificial intelligence (AI) technologies to enhance precision agriculture and develop innovative management solutions.

2. Weedy Rice Issues in Malaysia

Weedy rice was discovered in rice-growing regions in 1846 in North and South America, Europe in the 1970s, and various Asian countries in the 1990s [19]. The direct seeding method was reported to be the main cause of the emergence of weedy rice in the majority of rice agroecosystems in the world [2,5,20]. In Malaysia, weedy rice infestation was first reported in 1988 at Tanjung Karang, Selangor, and then spread sporadically to the northern part (Muda area, Kedah, Malaysia) in 1990 [20,21]. Five years later, weedy rice was reported in Besut, Terengganu [22] and subsequently in Seberang Perai, Penang, and Kerian, Perak.

The evolution of weedy rice is fundamentally entwined with the domestication history of its cultivated relative (Figure 1). There are four hypotheses of the origin of weedy rice or its evolution: (1) weedy rice may have evolved from the adaptation of wild rice that continuously disturbed cultivated rice habitats; (2) weedy rice may have originated from escaped cultivated rice seeds; (3) natural hybridization between cultivated and wild rice, which could have shared characteristics from both to form weedy rice; (4) hybridization between cultivated rice [17,18].

Figure 1. A schematic illustration of the evolutionary relationship among cultivated rice, progenitor (wild rice), and conspecific weed (weedy rice).
in 1996 [23,24]. Within a decade from the first reported incidence, weedy rice was recorded in rice fields in MADA (Kedah, Malaysia), Ketara (Terengganu, Malaysia), and Seberang Perak and Kerian Sg. Manik, (Perak, Malaysia) [1,25–27]. By the year 2000, the spread of weedy rice almost covered all of Peninsular Malaysia, with at least a 10% infestation in every rice granary [5,28]. By this time, weedy rice was identified as the most pervasive weed in the Muda area, while the infestation level in Kedah ranges from less than 10% to more than 20% coverage [27,29]. In Besut, Terengganu, and Tanjung Karang, Selangor, more than 50% of rice granaries have been reported to be infested with weedy rice [1,23].

The practice of traditional transplanting has provided an early growth advantage for the rice cultivar to compete with weedy rice, together with the careful selection of cultivars without any weedy rice contamination. Therefore, the agronomic shift of rice cultivation practice in Peninsular Malaysia from rice transplanting to direct seeding has increased the possibility of seed contamination during the sowing [2,5,20,30]. The changes in agronomic practices in Malaysia are illustrated in Table 1. As a result of the cultivation of weak, semi-dwarf indica-type varieties, weedy rice infestation has become apparent.

Poor land preparation contributed to the development and rapid spread of weedy rice, consequently enhancing its survival rate in seed banks [3,5,31]. In addition, most farmers did not follow the suggested standard operating procedures for land preparations due to the high investment in cost and time, which consequently contributed to the continuous infestation of weedy rice throughout the country [30,32]. In 2019, weedy rice finally reached east Malaysia (Borneo of Malaysia), where it was first reported in Sabah in rice fields across the Kota Belud and Kota Marudu regions [6,33]. The agronomic shift, coupled with modern mechanization in farming, contributed to the accidental introduction of weedy rice from Peninsular Malaysia into Borneo [33].

Table 1. The progress of rice agronomic practices in Peninsular Malaysia from 1970–2020.

<table>
<thead>
<tr>
<th>Years</th>
<th>Agronomical Practices</th>
<th>Rice Morphology</th>
<th>Problems</th>
<th>Rice Varieties Development</th>
<th>Weedy Rice Issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-1970</td>
<td>Manual transplanting</td>
<td>Tall</td>
<td>High lodging resistance, Sheath blast</td>
<td>Mahsuri, Murni, Masria,</td>
<td>No reports of weedy rice</td>
</tr>
<tr>
<td></td>
<td>Manual harvesting</td>
<td>Easy shattering</td>
<td></td>
<td>Jaya, Sri Malaysia 11,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Single crop per year</td>
<td>Later maturity</td>
<td></td>
<td>Pulut Malaysia 1,</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Heavy shattering</td>
<td></td>
<td>Setanjang, Sekembang,</td>
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<td></td>
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<td></td>
<td>Sekencang</td>
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<tr>
<td>1971–1975</td>
<td>Double cropping</td>
<td>Moderate height</td>
<td>Bacteria leaf blight, Panicle blight,</td>
<td>Kadaria, Pulut Siding,</td>
<td>First report of weedy rice</td>
</tr>
<tr>
<td></td>
<td>Intensified research on cultural practices</td>
<td>Non-shattered, Short maturity</td>
<td>Stem borer, Plant hopper</td>
<td>Manik, Muda, Seberang,</td>
<td>Weedy rice mimicking cultivated rice</td>
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<td></td>
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<td></td>
<td></td>
<td>Makmur, MR81, MR84</td>
<td></td>
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<tr>
<td></td>
<td>Improved infrastructure for irrigation</td>
<td>High germination rate</td>
<td></td>
<td>MR127, MR159, MR167, MR185,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Full use of combined harvester</td>
<td>Short maturity</td>
<td></td>
<td>MR211, MRQ80</td>
<td></td>
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<tr>
<td>1986–1997</td>
<td>Integrated weed management</td>
<td>Long and slender rice</td>
<td></td>
<td>First report of weedy rice</td>
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<tr>
<td>1998–2010</td>
<td>Modern cultivation</td>
<td>Heavy grain weight, Quality fragrance</td>
<td></td>
<td>MR219, MR220, MRQ74, MR232,</td>
<td>Weedy rice resistance to herbicide and tolerance to</td>
</tr>
<tr>
<td></td>
<td>Fully mechanized</td>
<td></td>
<td></td>
<td>MR200CL1, MR200CL2, MBM16,</td>
<td>stress conditions</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MR253, MR263</td>
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<tr>
<td>2011–2020</td>
<td>Breeding expansion</td>
<td>Semi-dwarf</td>
<td>Lack of desired phenotypes</td>
<td>MR269, MR284, MARDI Sempadan 303, MARDI Sebernas 207,</td>
<td></td>
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<tr>
<td></td>
<td>Aerobic rice</td>
<td>High germination rate</td>
<td></td>
<td>MR1A, MRQ76</td>
<td></td>
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<tr>
<td></td>
<td>System of rice intensification (SRI)</td>
<td>Short maturity</td>
<td></td>
<td>Weedy rice resistance</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Target to achieve 80% self-sufficiency</td>
<td>High yield inbred and hybrid</td>
<td></td>
<td>to herbicide and tolerance</td>
<td></td>
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<tr>
<td></td>
<td>level (SSL)</td>
<td>Good resistance</td>
<td></td>
<td>to stress conditions</td>
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<td></td>
<td></td>
<td>Colored rice and milled rice grade</td>
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In addition to different agronomic techniques, environmental conditions also have an impact on the rate of resistance in the evolution of weedy rice [34,35]. In addition to the shift to direct seeding, which influences the spread of weedy rice with a high-shattering biotype (increased contaminated seeds), remnants from old cultivars (seedbanks) and the use of large sharing harvesters also contribute to the proliferation and dominance of weedy
rice in Malaysia [5]. Thus, developing management measures thoroughly is necessary to delay the evolution and spread of weedy rice. Despite the rapid and continuous process of changing the rice ecosystems and, consequently, the weedy rice landscape, the knowledge about weed biology and ecology, specifically Malaysian weedy rice, is still inadequate to understand its fast adaptation.

Weedy rice, in general, is taller in stature than the modern semi-dwarf cultivated rice varieties in Malaysia. Mechanical control by removing the weedy rice panicle before maturity is the only method to manage weedy rice in the rice fields [5,36]. As a result, this occurrence has led to the emergence of new biotypes of weedy rice where it has a similar stature to cultivated rice. These new biotypes were first documented in Selangor in 2010 [2]. In addition, the existence of the new biotypes of weedy rice (NBWR) has also contributed to a complex situation that does not alert most farmers because the weed can be hidden in the rice fields, making it difficult to control [2].

This serious weedy rice infestation in Malaysia prompted the Malaysian Agricultural Research and Development Institute (MARDI) to introduce the Clearfield® Rice Production System (CPS) in 2010 [37]. MR220CL1 and MR220CL2 are CPS varieties tolerant to the imidazolinone (IMI) herbicides. CPS was originally developed in the United States of America (USA) by mutation breeding and then adopted in Malaysia by crossing an IMI-TR Line No. 1770 from the USA with a Malaysian rice variety MR220. MR220CL1 and MR220CL2 are tolerance to imazapic and imazethapyr, which are acetohydroxyacid synthase (AHAS)-inhibiting herbicides from the imidazolinone family [35,38,39].

This successful strategy for weed management and high-yield production gained popularity among farmers with its new technology [5,35]. The continuous use of CPS allowed the replacement of the soil seed bank with herbicide-resistant populations in the future. However, the CPS system was not achieving the target of managing weedy rice due to the failure of farmers to strictly follow the CPS stewardship guidelines [39,40]. This has caused new problems where weedy rice had ‘escaped’ the CPS treatment and became resistant to the imidazolinone herbicide [5,35,39,40]. Herbicide resistance is an inherited trait for weedy rice to survive, and its population reproduces more extensively, thus increasing the resistant allele frequency in populations [7].

3. Adaptive Traits of Weedy Rice

Weedy rice adapts to the rice agroecosystem by having complex characters, constituting the admixture between wild- and cultivated-like traits [7,17,41]. Its biotypes can have wild-like traits such as black hull, long awn, red or pigmented pericarp, tall stature, and easy shattering, or crop mimic traits, including straw hull, white pericarp, and semi-dwarf height [42]. At the vegetative stage, weedy rice is difficult to distinguish in the rice field due to its close morphological characteristics with cultivated rice. However, weedy rice is perceptible at flowering due to its relatively shorter maturation period (90–120 days) than cultivated rice (115–120 days), in addition to its distinctive characteristics in the field such as tall stature, open panicle, and easy shattering [43,44].

To compete in the rice agroecosystem, especially in Malaysia, weedy rice generally grows higher (90–150 cm) than cultivated rice (60–70 cm). Tall stature is one of the weedy rice strategies to compete for light while creating shade for its competitors, i.e., cultivated rice [4,45]. Weedy rice also produces a high number of tillers to adapt in the rice fields, which then helps to produce more panicles and seeds [4,46]. A high tiller number at the vegetative stage also gives an advantage to weedy rice, as a high leaf area index accumulates more nitrogen and sucrose, leading to increased biomass production [47]. Mahmod et al. [42] found that weedy rice morphology is highly diverse and dispersed across Peninsular Malaysia. They also found a cluster of weedy rice biotypes that have a set of morphological characters that resemble cultivated rice, especially in terms of shorter stature, awnless, and straw-colored seeds. In line with Sudianto et al. [7], the awnless biotypes are easily found in all rice granaries across Peninsular Malaysia compared to the
awned biotype [42]. These prove the existence of the mimicry adaptation in the weedy rice population.

Weedy rice is locally known as ‘padi angin’ (wind rice) in Malaysia, attributed to its phenotypic characteristic, i.e., the easy seed shattering by the wind [2,5]. The inheritance of several wild-like traits in weedy rice, such as smaller grains with awn, facilitates seed dispersion by wind and animals [48,49]. Easy-shattering seeds enhance the capacity of weedy rice to be widely dispersed in the field and enrich the seed bank [7,50]. The easy shattering trait, along with seed dormancy, increases the survivability of weedy rice seeds in the seed bank [5,51,52]. Furthermore, seed dormancy is highly correlated with the pigmentation of the seed pericarp, which supports the quality decrement of cultivated rice production in the country [2,34]. Therefore, preserving the wild traits enables weedy rice to infest extensively in various rice agroecosystems.

In the rice agroecosystem, weedy rice is a highly polymorphic group of plants. Weedy rice biotypes encompass variations in every morphological trait compared to the cultivated rice varieties [7,53]. Mimicking cultivated rice is one of the adaptive mechanisms of weedy rice in the rice agroecosystem [42,54,55]. Due to the extensive manual weeding of weedy rice [28,56], weedy rice with short or similar height to cultivated rice emerged in Malaysian rice fields, indicating the capability of weedy rice to select favorable traits for survival [2]. Short stature also gives weedy rice erect flag leaves to increase photosynthesis capability [57,58]. Erect leaves improve the light-intercepting of plant architecture to induce plant growth. At the same time, the semi-dwarf trait is correlated with high tillering, thus preventing the plant from lodging [44,59]. The crop-mimicking traits promoting aggressive growth and development create unequal competition between cultivated and weedy rice biotypes [53,57,60,61].

The ability to adapt by mimicry helps weedy rice survive in diverse rice agroecosystems [53]. Certain weedy ‘red’ rice biotypes mimic the cultivar by having low seed shattering and/or straw hull phenotypes, which are involuntarily harvested together with cultivated rice, resulting in the contamination of the seed stock due to the red pericarp [5]. Straw hull seeds with a red pericarp can mostly be found in every rice granary in Peninsular Malaysia [7,42]. The contamination of the seed stock has a negative impact on yield quality, reduces rice yield production, and increases the dispersion of weedy rice.

Several studies have suggested that weedy rice can adapt to current climate change [62,63]. High tolerance to abiotic (drought, cold, salinity) and biotic (diseases) stresses is a powerful defense system in weedy rice [58,64,65]. It can also easily adapt to changes in agronomic practices [5,58]. For example, weedy rice reportedly ‘escaped’ and became resistant to imidazolinone herbicide [35,39,66]. Since 2010, Malaysia has implemented the Clearfield® Production System (CPS) to reduce the infestation of weedy rice and, at the same time, increase rice production [37,38,67–69]. However, resistance to the imidazolinone herbicide indicates the adaptation dynamic of the weedy rice. The high correlation between resistance and various morphological and physiological traits shows the ability of weedy rice to adapt to agronomic practices and environmental changes [70]. These polymorphic traits in weedy rice are remarkable and need to be studied for a better understanding of the plant’s interactions with its environment, which is useful for weed management and crop improvement.

4. Weedy Rice Research in Malaysia

The study of weedy rice in Malaysia has become a highlight after the serious weedy rice infestation in the early 1990s [20,25,71]. Table 2 summarizes several research aspects that have been conducted on weedy rice in Malaysia, including morphological, physiological, ecological, genetics, weed management, and metabolomic studies.
Table 2. Research progress of weedy rice in Malaysia.

<table>
<thead>
<tr>
<th>Category</th>
<th>Methods</th>
<th>Findings</th>
<th>Area/Samples Coverage</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ecology</strong></td>
<td>Field samplings, field surveys</td>
<td>More than 19,900 ha of rice farms were damaged by weedy rice. New biotypes of weedy rice (NBWR) with shorter plant height were first reported. Most farm blocks showed clump or under-dispersed spatial distribution.</td>
<td>Peninsular Malaysia</td>
<td>[25]</td>
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<tr>
<td></td>
<td></td>
<td>The relative abundant indices of annual weeds were more dominant than perennial weeds. The Lloyd patchiness index revealed that more than 50% of surveyed field blocks in five states displayed uniform distribution.</td>
<td>Kedah</td>
<td>[72]</td>
</tr>
<tr>
<td><strong>Genomic</strong></td>
<td>PCR, SSR</td>
<td>The high genetic diversity showed that Malaysian weedy rice had diverse origins: de-domestication from cultivated rice, adaptation from wild rice, and hybridization between cultivated and wild rice and between cultivated rice and weedy rice.</td>
<td>Peninsular Malaysia</td>
<td>[16,20,53,71,73,74]</td>
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<td></td>
<td></td>
<td>Sabah weedy rice was shaped by accidentally introducing Peninsular Malaysia’s weedy rice strains. Cultivated rice was highly diverse compared to Peninsular, which supported the progenitor of Sabah cultivar-like weedy rice.</td>
<td>Sabah</td>
<td>[6,33]</td>
</tr>
<tr>
<td><strong>Morphology and</strong></td>
<td>Greenhouse and field experiment</td>
<td>All weedy rice populations showed cross-resistance to the IMI herbicides imazapic and imazapyr due to a Ser-653-Asn mutation in the AHAS gene resulting from an herbicide-insensitive AHAS enzyme.</td>
<td>Not applicable</td>
<td>[8]</td>
</tr>
<tr>
<td><strong>Physiology</strong></td>
<td></td>
<td>The multiplex PCR assays employed a standard agarose-based gel electrophoresis system to simultaneously disclose at least two major grain quality (amylose content and fragrance) and biotic stress (blast, sheath blight, and bacterial leaf blight) genes in rice.</td>
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<tr>
<td><strong>Weed Management</strong></td>
<td>Field samplings and surveys</td>
<td>Heterogenous plant height, hull color, pericarp color, awn, and maturity period. Most weedy rice had seed shattering. Greater in growth development, tiller number, LAI</td>
<td>Peninsular Malaysia</td>
<td>[1,7,42–44,73,76,77]</td>
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<tr>
<td></td>
<td></td>
<td>The rice and weed dry matter, rice plant height, chlorophyll content, leaf area, number of tillers, filled grain, 1000 grain weight and grain yield were reduced with an increased crop–weed competition period.</td>
<td>Selangor, Pahang</td>
<td>[53,61]</td>
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<td></td>
<td></td>
<td>The germination rate was found to be related to the degree of dormancy, but it had no influence on the range of cardinal temperatures.</td>
<td>Peninsular Malaysia</td>
<td>[78]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A high degree of seed dormancy retained their viability for more than 200 days once imbibed.</td>
<td>Selangor</td>
<td>[70]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sites that practiced integrated weed management with regular surveillance and monitoring had less weedy rice infestation and higher rice yields and vice versa with control. Major weed populations are F. miliacea, L. Hyssopifolia and O. sativa.</td>
<td>Perlis, Kedah</td>
<td>[79]</td>
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<td></td>
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<td>Most farmers ignored the technology and deliberately disregarded stewardship guidelines. Their perceptions of the weedy rice issue varied from region to region, leading to differing methods for controlling weedy rice. Farmers were more likely to use herbicides than mechanical to control weedy rice.</td>
<td>Peninsular Malaysia</td>
<td>[80,81]</td>
</tr>
</tbody>
</table>
### Table 2. Cont.

<table>
<thead>
<tr>
<th>Category</th>
<th>Methods</th>
<th>Findings</th>
<th>Area/Samples Coverage</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weed Management</td>
<td>Greenhouse and field experiment</td>
<td>Weedy rice populations were effectively controlled, resulting in an average 15% yield increment under the Clearfield&lt;sup&gt;®&lt;/sup&gt; Production System (CPS) in direct seeded fields. The critical period for weed control ranged from approximately 12–16 to 53–60 DAS.</td>
<td>Perak, Penang [28,37]</td>
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<td></td>
<td>Weedy rice was found to be more resistant to OnDuty&lt;sup&gt;®&lt;/sup&gt; (premix of imazapic and imazapyr) than the susceptible weedy rice.</td>
<td>Perlis, Kedah Penang [39]</td>
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<tr>
<td></td>
<td></td>
<td>Weedy rice in Malaysia developed various degrees of resistance toward IMI herbicide.</td>
<td>Selangor [66]</td>
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<tr>
<td>Metabolomic</td>
<td>Field sampling, NMR</td>
<td>Weedy rice and cultivated rice can be clearly distinguished based on metabolome profiles. It was found that the metabolite profiles of weedy rice from the east coast are different from the west coast area of Peninsular Malaysia.</td>
<td>Peninsular Malaysia [82]</td>
<td></td>
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</tbody>
</table>

Weedy rice infestations in Malaysia were temporally dynamic [2,25,29]. For instance, surveys on weedy rice in 1995 revealed the existence of weedy rice in the majority of rice granaries in Peninsular Malaysia. Yet, it did not significantly affect the rice industry. However, the infestation rapidly increased a year later, with more than 19,900 ha of rice granaries affected [25,29]. Moreover, weedy rice was discovered in 82% of Muda rice fields in 2001, but a year later, only about half the fields had at least a 10% infestation rate [25]. But then, in 2005, the infestation tremendously increased when 91% of the rice fields were infested [25,83]. This temporal dynamic infestation might be due to the early shift to direct seeding from manual transplanting and the adoption of modern machinery, causing cross-contamination between rice fields. This ‘roller-coaster’ dynamic of weedy rice infestation caused difficulties in managing weedy rice in the country [2].

Research on the morphological aspect of weedy rice has mainly been focused on the variation in the morphology and physiology of weedy rice. Weedy rice in Malaysia has vegetative and reproductive superiority compared to cultivated rice, including taller stature and higher tiller number [1,44,73,76]. It has also been reported to morphologically mimic and have a similar height to local cultivars [2,42]. Comprehensive surveys of weedy rice across Peninsular Malaysia based on more than 20 trait descriptors have shown that the weeds are morphologically diverse [7,42]. The morphological variation in Malaysian weedy rice traits includes different pigmentation of grains, grain types, panicle types, grain weight, maturity period, number of tillers, plant height, and percentage of seeds shattering. Sudianto et al. [7] identified four major clusters of weedy rice: (1) resembles wild *Oryza* populations, (2) mimic elite *indica* rice cultivar, (3) brown-hull biotypes, and (4) admixture of weedy traits.

Weedy rice from different geographical locations comprises various morphological characters; for instance, the awned seeds were more abundant in the west coast region of Peninsular Malaysia, while the black hull color was prevalent in the east coast region [7]. Mahmod et al. [42] found that most weedy rice from the east coast of Peninsular Malaysia are represented with traits similar to cultivated rice, especially in terms of shorter stature and awnless seeds, while weedy rice with high tiller and panicle number were mainly from the west coast region.

Studies on the origin of weedy rice in Malaysia have received little attention from weed scientists. A molecular study on weedy rice origin in the mid-90s based on the randomized amplified polymorphic DNA (RAPD) markers indicated that weedy rice in Malaysia was genetically diverse, which permits the selection of adaptive traits required by cultivation practices [20]. Based on further genetic comparisons among cultivated rice, wild rice (*Oryza rufipogon*), and weedy rice variants, weedy rice was closely related to cultivated
rice compared to wild rice, indicating that Malaysian weedy rice may derive from local cultivars [20,71]. However, no extensive research was conducted to advance the study on the origin of weedy rice in Malaysia until Song et al. [16] investigated 207 weedy rice samples from 17 populations in Peninsular Malaysia, using 24 polymorphic microsatellite markers. In contrast with the study by Abdullah et al. [71], Song et al. [16] showed that the diverse weedy rice strains were shaped by the introgression of weed-adaptive alleles (sh4 and Bh4) from the *Oryza rufipogon* population, which evolved from the hybridization process with modern-bred elite rice cultivars.

Despite having a close morphological resemblance, Malaysian weedy rice strains are genetically different from weedy rice in the USA, indicating the independent evolutionary origin of weedy rice in the country. Further investigation of weedy rice in east Malaysia (Borneo) conducted using genome-wide SNPs reported that weedy rice in the region may have originated from two paths. First, weedy rice in east Malaysia has similarities with the local cultivars, indicating a hybridization event between cultivars, and second, the weedy rice displayed a wild-like genetic makeup, which indicates the possibility of cross-contamination with weedy rice from Peninsular Malaysia [33].

The shift towards industrialized rice farming and the continuous development of modernized elite cultivars will favor the continued proliferation of weedy rice in Malaysia [5,16]. Therefore, continuous and large-scale genetic screening and high throughput analysis of weedy rice are required to understand and predict future infestations and potential management of weedy rice. Using cultivated and weedy rice samples from Malaysia, Mohd Hanafiah et al. [8] successfully developed a PCR-based multiplex assay for large-scale genotyping of rice genetic resources. The universal multiplex protocol is effective in detecting rice grain quality (amylose content and fragrance) and biotic stress resistance traits (blast, sheath blight, and bacterial leaf blight) in both cultivated and weedy rice. Moreover, the metabolomics study of weedy rice in Malaysia was first reported by analyzing weedy rice samples collected from different areas in Peninsular Malaysia together with several cultivated rice [82]. This study showed a clear distinction between weedy rice and cultivated rice, in which the former contains higher levels of γ-aminobutyric acid (GABA), α-glucose, fumaric acid, and phenylalanine. Interestingly, the study also found that the metabolite profiles of weedy rice from the east coast region are different from those of the west coast area of Peninsular Malaysia, which could be influenced by genotype and environmental conditions.

5. Omics Study as Knowledge-Based Weed Management Strategies for the Future

Various omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, have emerged as attractive approaches in plant science. The principal of an omics study is aimed at comprehensively profiling and characterizing a biological system to interpret and understand its function or mechanism [84]. Rapid data generation through the advancement of high-throughput platforms, e.g., next-generation DNA sequencing, SNP-chip profiling, and state-of-the-art analytical instruments, such as liquid chromatography–tandem mass spectrometry (LC-MS/MS) and nuclear magnetic resonance (NMR) spectroscopy, has accelerated these omics studies. Each layer of these omics studies offers a wealth of knowledge and information that can provide a better understanding of the functions and mechanisms of biological systems (Figure 2) [85,86].

The applications of omics study in plant science and agriculturally important crops, including rice, have increased over the years [85]. In weed science, genomics study is frequently employed, but other omics areas, such as transcriptomics, proteomics, and metabolomics, have also been explored in recent years. The omics approach could assist researchers in understanding weedy traits as well as the mechanisms of herbicide resistance, adaptation to stress environments, and crop–weed interactions [85,87].
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(GABA), α-glucose, fumaric acid, and phenylalanine. Interestingly, the study also found that the metabolite profiles of weedy rice from the east coast region are different from those of the west coast area of Peninsular Malaysia, which could be influenced by genotype and environmental conditions.

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Figure 2. Omics study involves genomics, transcriptomics, proteomics, metabolomics and phenomics toward a better understanding of systems biology.

In weedy rice studies, genomic research has been extensively investigated (Table 3). The genetic studies of weedy rice were conducted through a simple sequence repeats (SSR) marker or microsatellite to identify the quantitative traits locus (QTL), DNA sequencing, single nucleotide polymorphisms (SNPs), and genome-wide association study (GWAS) [88–90]. Genomic studies in weedy rice also focused on herbicide-resistance traits, including target site resistance and non-target site resistance, using genotyping-by-sequencing (GBS) to identify candidate genes involved in ALS-inhibitor [91]. Transcriptomic and proteomic studies of weedy rice were concentrated on abiotic (cold, drought, temperature, and nutrients) and biotic (pathogen) stresses [92–96]. A metabolomics study was applied to uncover different metabolite profiles between cultivated and weedy rice, as well as weedy rice samples, from different geographical areas [82].

Compared to cultivated rice, omics studies in weedy rice are still limited. Therefore, the application of omics technology in weedy rice is important to improve knowledge-based weed management strategies in the future.

Table 3. Application of omics study in weedy rice.

<table>
<thead>
<tr>
<th>Omics Study</th>
<th>Field of Research</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomics</td>
<td>Evolution: origin, genetic variation, adaptation</td>
<td>[6,11,18,74,89,97–105]</td>
</tr>
<tr>
<td>Genomics</td>
<td>Phenotypic variation</td>
<td>[106]</td>
</tr>
<tr>
<td>Genomics</td>
<td>Herbicide resistance</td>
<td>[53]</td>
</tr>
<tr>
<td>Genomics</td>
<td>Anthocyanin nutrition</td>
<td>[107]</td>
</tr>
<tr>
<td>Genomics</td>
<td>Abiotic stress: temperature</td>
<td>[108]</td>
</tr>
<tr>
<td>Transcriptomics</td>
<td>Evolution: origin, genetic variation, adaptation</td>
<td>[109]</td>
</tr>
<tr>
<td>Transcriptomics</td>
<td>Phenotypic variation, hormone level</td>
<td>[110]</td>
</tr>
<tr>
<td>Transcriptomics</td>
<td>Abiotic stress: cold stress</td>
<td>[65,94,111,112]</td>
</tr>
<tr>
<td>Transcriptomics</td>
<td>Abiotic stress: drought</td>
<td>[113,114]</td>
</tr>
<tr>
<td>Transcriptomics</td>
<td>Abiotic: nutrient stress</td>
<td>[95]</td>
</tr>
<tr>
<td>Transcriptomics</td>
<td>Abiotic stress: temperature</td>
<td>[93]</td>
</tr>
<tr>
<td>Proteomics</td>
<td>Abiotic stress: drought</td>
<td>[114]</td>
</tr>
<tr>
<td>Proteomics</td>
<td>Biotic stress: insects</td>
<td>[92]</td>
</tr>
<tr>
<td>Metabolomics</td>
<td>Chemical profiles: accession, geographical origin</td>
<td>[82]</td>
</tr>
</tbody>
</table>
6. Genomics Aspect of Weedy Rice

As an intermediate conspecific weedy rice, their unique characteristics, such as competitive ability, adaptive evolution to stress tolerance, increased seed dispersal, seed dormancy, and variation in morphology, are the key factors in exploring its genetic background and chemical compositions [93,115]. Identifying important genes with the chemical compounds of weedy rice may provide additional features for crop improvements. Table 4 summarizes several important genes in weedy rice associated with important morphological traits and their tolerance to abiotic and biotic stress conditions.

Seed shattering is one of the important characteristics in the evolution of weedy rice. For instance, *sh4* is a functional gene that controls seed shattering and is driven by spatiotemporal promoter *pSH4* [116]. Studies showed that *sh4* is linked with higher seed dormancy (*DOG1, Sdr4, qSD7-1* genes) and the variant of germination time (*CYP707A, NCED* genes) [117,118]. These activities involved abscisic acid (ABA) signaling at the various growth stages of weedy rice, which is promoted under hypoxic conditions [119]. Such a mechanism ensures effective seed dissemination, paving the way for persistent soil seed bank development and sustainability over multiple seasons.

The red pericarp is the most distinct trait that differentiates between weedy rice and cultivated rice. *Rc* is the regulatory gene responsible for seed pigmentation, in which red pigments are produced by activating the flavonoid biosynthesis pathway in the pericarp tissue’s lower epidermal cell layer [107,120,121]. The association of *qSD7-1/Rc* (seed dormancy/red pericarp) developed pleiotropic effects in some variations of germination events by enhancing the ABA biosynthesis [120].

Nonetheless, the function of the pigmented hull is still vague. The black hull color can be originated from the *aus* crop [122] and/or from other wild rice species [123]. Meanwhile, *Bh4* was documented for black pigmentation and encoding an amino acid transporter, whereas

<table>
<thead>
<tr>
<th>Gene(s)</th>
<th>Important Morphological Traits with Response to Abiotic and Biotic Stress</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>sh4, qSH1</em></td>
<td>Seed shattering</td>
<td>[116,124,125]</td>
</tr>
<tr>
<td><em>Rc</em></td>
<td>Pigmented pericarp</td>
<td>[54,74,107]</td>
</tr>
<tr>
<td><em>Bh4, Phr1</em></td>
<td>Hull color (black)</td>
<td>[119,122]</td>
</tr>
<tr>
<td><em>An-1, LABA1</em></td>
<td>Awn length and awn barb</td>
<td>[126,127]</td>
</tr>
<tr>
<td><em>Sdr4, DOG1, qSD7-1, CYP707A, NCED</em></td>
<td>Dormancy and germination</td>
<td>[51,128]</td>
</tr>
<tr>
<td><em>sd-1</em></td>
<td>Plant height</td>
<td>[89,129]</td>
</tr>
<tr>
<td><em>Prog1</em></td>
<td>Erect leave and erect panicles</td>
<td>[89,99,130]</td>
</tr>
<tr>
<td><em>OsLG1</em></td>
<td>Closed panicle</td>
<td>[99,131]</td>
</tr>
<tr>
<td><em>Hdl</em></td>
<td>Early flowering</td>
<td>[132]</td>
</tr>
<tr>
<td><em>AtCYP78A7</em></td>
<td>Drought tolerance</td>
<td>[113]</td>
</tr>
<tr>
<td><em>HKT, NHX1, SOS1</em></td>
<td>Salinity stress</td>
<td>[115,133]</td>
</tr>
<tr>
<td><em>RAB16, OVP1, APX1</em></td>
<td>Cold tolerance</td>
<td>[111]</td>
</tr>
<tr>
<td><em>HSF2a, HSFA7</em></td>
<td>Heat tolerance</td>
<td>[134]</td>
</tr>
<tr>
<td><em>ADH, PDC, OsB12D1</em></td>
<td>Flooding tolerance</td>
<td>[135]</td>
</tr>
<tr>
<td><em>AHAS, ALS, EPSP</em></td>
<td>Herbicide resistance (imazapic–imazapyr, glyphosate)</td>
<td>[35,136]</td>
</tr>
<tr>
<td><em>Pi-ta</em></td>
<td>Blast resistant (Magnaporthe oryza B.)</td>
<td>[137]</td>
</tr>
</tbody>
</table>

*Phr1* encodes polyphenol oxidase that is only accumulated in ripened hulls [121,122]. *Phr1* has been reportedly associated with long-term grain storage, which improves seed
dormancy [9]. The evolution from a black hull color into a straw hull color in weedy rice involved different loss-of-function alleles, a 22-bp frameshift deletion in exon 3, about 4% carrying a 1-bp frameshift deletion in exon 1 [138]. The black hull color significantly correlated with high seed shattering, while the straw hull color was associated with non-shattering seeds. The brown hull color indicated hybridization between the black and straw hull weedy rice [122]. The hull color variation represented multiple rice domestication and de-domestication occurrences in weedy rice.

Awn length denotes the strategy to increase seed dispersion in weedy rice. The awn protects seeds from bird and mammal predation, aiding in seed dispersal by clinging to animal fur and enabling self-planting. The upregulation of An-1 and LABA1 corresponds to Histone H1 expression, which may directly or indirectly promote cell division for awn length [126]. Furthermore, An-1/LABA1 on chromosome 4 downregulates meristematic activity by reducing OSH1 and cytokinin levels, which are responsible for reducing the grain number per panicle [126,127]. The awn trait is generally related to seed dispersal, seed shattering, dormancy, and adaptation in stress conditions [45,58,139].

To escape being eliminated, the plant architecture of weedy rice has evolved under natural conditions and mimicked cultivated rice, such as from taller to shorter plants with erect leaves (PROG1 gene), pigmented hull to straw hull color, non-shattering (qSH1 gene), and open to closed panicles (OsLG1 gene). Closed panicles have more seeds than open panicles and can retain maturity longer than open panicles. Long awns with closed panicles have also changed seed-shedding and pollinating behaviors [131]. These genes were targeted for selection in the early stages of rice domestication, and they have similar haplotypes with weedy rice at these loci [9].

The variation in flowering helps weedy rice improve its adaptation to different environments. For instance, differences in the Hd1 protein, Hd3a promoters, and Ehd1 expression contribute to the variation in flowering time [140]. Mutations in hd1 during rice domestication affected the insensitivity of the photoperiod in tropical and subtropical areas, which benefited the reproduction of weedy rice [132].

Through multiple de-domestications, weedy rice carries valuable genetic resources, with several varietal-specific improvement genes, under abiotic conditions (drought, flooding, cold, heat, and salinity), biotic stress-tolerant (diseases), and herbicide-resistance [58,141]. Evidently, the overexpression of certain genes during stress conditions stimulates stress-responsive pathways (sucrose, metabolism, antioxidant defenses). For example, these include the overexpression of the AtCYP78A7 gene during drought [113], HKT, NHX1, and SOS1 genes in terms of tolerance to salinity [133], HSF2a and HSF47 genes under heat stress [134] and ADH, PDC, and OsB12D1 genes during flooding [135].

Rice blast is one of the main diseases in cultivated rice. Weedy rice survival in blast disease is a potential resource for the novel blast R gene for resistance breeding. The pi-ta gene was found to successfully control rice blast disease caused by *Magnaporthe oryzae* [137,142]. R genes produce a cytoplasmic protein with a nucleotide-binding domain leucine-rich repeat (NLR), indicating their functions as receptors and/or in protein–protein interaction [137]. As documented by Lee et al. [142], the resistant Pi-ta allele is mostly found in the awnless straw hull of weedy rice.

The application of herbicides for a long period has had a negative impact on weed management. The gene flow occurrence between cultivated and weedy rice was revealed through the mutation of EPSP (5-enilpyruvoylshikimate-3-phospate) for resistance to glyphosate, ALS (acetolactate synthase), and AHAS (acetohydroxyacid synthase) for IMI resistance [35,136,143]. The gene flow between cultivated and weedy rice results in hybrids by selection, as they are primarily self-fertilizing. In the future decade, the weedy rice genome will be reshaped by the widespread selection for crop introgression.

The availability of rice reference genomes and well-characterized genes relevant to domestication provides an effective platform to investigate the genetic mechanisms underlying weedy rice evolution. However, research into the genes related to weediness functional identification is still far behind that of cultivated rice. Recent comparative
genomics studies have encouraged the understanding of the development of weediness traits in weedy rice. Therefore, further investigations into the molecular mechanism of weediness candidate genes would be useful in understanding the adaptation, diversity, and evolution of weedy rice.

The first genomic-associated study of Malaysian weedy rice was conducted via random amplified polymorphic DNA (RAPD), and this study revealed that the weedy rice origin was distinct from wild rice [20]. Next, through simple sequence repeats (SSR) analysis, weedy rice was shaped by contributions of genetic materials from local wild rice Oryza rufipogon populations at sh4 and Bh4 domestication genes [16]. Therefore, to gain insights into various biological aspects to understand the entire genetic makeup and cellular processes of weedy rice, omics technology could provide a better option. Omics study may also bridge the gap in information on the functional identification of weediness-related genes.

7. The Potential Use of Metabolomics in Weedy Rice Study

Metabolites are chemical phenotypes that serve as the products of biological processes for plant growth, development, and adaptation toward biotic and abiotic changes [12,144,145]. Metabolomics is a branch of omics disciplines investigating the dynamics, compositions, and interactions of metabolites with internal and external environments [146]. It aims to understand biological responses in organisms involving the identification and quantification of metabolites and chemical footprints of cellular regulatory processes [147,148]. Metabolomics plays an important role in elucidating the metabolic pathways and processes, understanding plant interaction with biotic or abiotic factors, identifying biomarkers, and relating those specific compounds to growth, disease, stress, and environmental changes to investigate the functions of unknown genes and mutant characterization in plants, as well as for quality control of plants and crops [85,149,150].

There are already quite a number of papers published on rice research employing the metabolomics approach, such as in exploring chemical diversity in rice varieties [150,151], understanding crop responses to oxidative stress [152], salinity tolerance studies [115], and rice–pathogen interactions [153]. Table 5 summarizes comprehensive studies on rice metabolomics from 2009 to 2021. In general, metabolite compositions in rice vary according to varying rice cultivars and environmental influences [154–156].

Weedy rice, as a conspecific weed in the rice agroecosystem, has morphological similarities with wild rice and, at the same time, shares the primary gene pool with cultivated rice [7,55]. Exploring the metabolomics of weedy rice can enhance our understanding of the adaptation mechanism of the weed in nature, which may be useful if these characteristics can be utilized for the enhancement of current rice cultivars. This holds true for other important traits that are potentially useful for rice breeding programs [137,157–159]. For example, various studies have reported that weedy rice has developed tolerance to extreme abiotic stresses, such as drought, high salinity, and low and high temperature [93,112,114,115]. Rice breeders may turn directly to weedy rice because this conspecific counterpart of rice may provide additional features for crop improvement. Selection from weedy rice strains as one of the parents rather than using a wild rice progenitor is very helpful in terms of recovering some genetic diversity that has been removed during the domestication process [55].

Seed dormancy is one of the important adaptive traits of weedy rice. In general, weedy rice has much stronger seed dormancy than cultivated rice [55]. Rapid and uniform seed germination has been selected in crops, but a moderate degree of dormancy is desirable for rice to resist preharvest sprouting. Reducing the degree of seed dormancy could promote germination uniformity and make weeds relatively easy to eliminate by agronomic practices or mitigate the risk of transgene flow into wild/weed relatives [117,160]. Thus, understanding the metabolism and complex network of genes and metabolites during the seed dormancy and germination stages could be realized by integrating metabolomic and transcriptomic approaches.
Table 5. Metabolomics studies on rice from 2009 to 2021.

<table>
<thead>
<tr>
<th>Organ Parts</th>
<th>Category</th>
<th>Experimental Methodology</th>
<th>Metabolomics Acquisition Methods</th>
<th>Bioinformatics Tools</th>
<th>Statistical Analysis</th>
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<th>Source of Samples</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grains</td>
<td>Natural variation</td>
<td>Field</td>
<td>LC-MS, GC-MS</td>
<td>R, SIMCA-P, Cytoscape, MeV, MetATT</td>
<td>PCA, Heatmap, LASSO, Two-way ANOVA, ASCA, Pearson correlation</td>
<td>Japonica and indica subspecies were significantly different in relative abundances of metabolites and metabolic association networks.</td>
<td>China</td>
<td>[151]</td>
</tr>
<tr>
<td>Grains</td>
<td>Natural variation</td>
<td>Laboratory</td>
<td>SPME-GC-MS, NMR, LC-ESI-MS, GC-TOF-MS</td>
<td>MetAlign, AMIX, SIMCA-P, TagFinder</td>
<td>PCA, PLS-DA, HCA</td>
<td>Cultivars of jasmine and basmati showed different metabolic profiles. Storage grains had a significant effect on the metabolome in both cultivars.</td>
<td>Iran, Pakistan, Malaysia, Thailand, Indonesia, Philippines, Cambodia, Australia, Japan</td>
<td>[154]</td>
</tr>
<tr>
<td>Grains</td>
<td>Natural variation</td>
<td>Laboratory</td>
<td>NMR</td>
<td>Chenomx, SIMCA-P, SPSS</td>
<td>PCA, PLS-DA, ANOVA, Tukey’s t-test, HCA, Heatmap, metabolic pathway</td>
<td>Increment in hexose phosphates, tricarboxylic acid cycle intermediates, and γ-aminobutyric acid after one hour of water imbibition. Later enrichment in carbohydrate, amino acid, and cell wall metabolism.</td>
<td>Thailand</td>
<td>[155]</td>
</tr>
<tr>
<td>Grains</td>
<td>Natural variation</td>
<td>Greenhouse</td>
<td>GC-MS</td>
<td>MetaMiner, AMDIS, PageMan, MapMan</td>
<td>t-test, HCA, Heatmap, metabolic pathway</td>
<td>Significant differences in the contents of the anthocyanins, cyanidin-3-glucoside and peonidin-3-glucoside of red, black and non-colored indica and japonica rice subspecies.</td>
<td>None</td>
<td>[161]</td>
</tr>
<tr>
<td>Grains</td>
<td>Natural variation</td>
<td>Field</td>
<td>HPLC</td>
<td>Chrompare, XLSTAT</td>
<td>PCA, HCA</td>
<td>Hyperin, isoquercitrin, quercetin, gentiobioside were isolated from the grains of sugary rice.</td>
<td>China</td>
<td>[162]</td>
</tr>
<tr>
<td>Grains</td>
<td>Natural variation</td>
<td>Field, genebank</td>
<td>LC-MS, NMR</td>
<td>None</td>
<td>None</td>
<td>Brown and white rice contained lower quantities of phytochemicals compared to black and red rice.</td>
<td>Korea</td>
<td>[163]</td>
</tr>
<tr>
<td>Grains</td>
<td>Natural variation</td>
<td>Field, genebank</td>
<td>HPLC</td>
<td>Statistix 8.0</td>
<td>ANOVA</td>
<td></td>
<td>France</td>
<td>[164]</td>
</tr>
<tr>
<td>Grains</td>
<td>Natural variation</td>
<td>Field, genebank</td>
<td>LC-MS, GC-MS</td>
<td>Chroma TOF, MassLynx, SIMCA-P, MeV, Statistica 18</td>
<td>PCA, PLS-DA, box-whisker, ANOVA, Duncan’s test, Pearson’s correlations</td>
<td>Antioxidant compounds (cyanidin-3-glucoside, peonidin-3-glucoside, proanthocyanidin dimers, proanthocyanidin trimers, and catechin) mostly found in black and red rice seeds.</td>
<td>Korea</td>
<td>[165]</td>
</tr>
<tr>
<td>Grains</td>
<td>Natural variation</td>
<td>Greenhouse</td>
<td>NMR</td>
<td>Mestrenova</td>
<td>OPLS-DA</td>
<td>Metabolites of valine, threonine, alanine, glutamate, galactitol, β-glucose, α-glucose, raffinose, and fumaric acid influenced the separation of red rice and black rice.</td>
<td>Indonesia</td>
<td>[166]</td>
</tr>
<tr>
<td>Grains</td>
<td>Natural variation</td>
<td>Field</td>
<td>LC-MS, GC-MS</td>
<td>MeV, SIMCA-P, Cytoscape, SPSS, R</td>
<td>PCA, Heatmap, ANOVA, Metabolic pathway, t-test, Pearson’s correlation</td>
<td>The chalky endosperm had lower levels of metabolites compared to the translucent upper part.</td>
<td>China</td>
<td>[167]</td>
</tr>
<tr>
<td>Organ Parts</td>
<td>Category</td>
<td>Experimental Methodology</td>
<td>Metabolomics Acquisition Methods</td>
<td>Bioinformatics Tools</td>
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</tr>
<tr>
<td>Grains</td>
<td>Natural variation</td>
<td>Field, genebank</td>
<td>GC-MS</td>
<td>TraceFinder</td>
<td>Heatmap</td>
<td>Identified 66 metabolites in the rice samples and cultivar Jaya showed the highest number of metabolites.</td>
<td>India</td>
<td>[168]</td>
</tr>
<tr>
<td>Grains</td>
<td>Natural variation</td>
<td>Field, genebank</td>
<td>HR-MAS NMR</td>
<td>MATLAB, SIMCA-P, SAS</td>
<td>PCA, OPLS-DA, ANOVA, t-test</td>
<td>Waxy rice cultivars accumulated lipids and had high levels of glutamate, aspartate, asparagine, alanine, and sucrose compared to nonwaxy rice cultivars.</td>
<td>Korea</td>
<td>[169]</td>
</tr>
<tr>
<td>Cooked grains</td>
<td>Natural variation</td>
<td>Laboratory</td>
<td>LC-MS</td>
<td>MarkerLynx, SIMCA-P</td>
<td>PLS-DA, Kruskal-wallis test</td>
<td>Chemical diversity among the varieties clustered according to subspecies classifications: indica, japonica, and aus.</td>
<td>Philippines</td>
<td>[170]</td>
</tr>
<tr>
<td>Rice bran</td>
<td>Natural variation</td>
<td>Field, genebank</td>
<td>LC-MS</td>
<td>Metabolon, SIMCA-P</td>
<td>Metabolic pathway, PCA</td>
<td>The 71 rice bran compounds of significant variation by cultivar included 21 amino acids, 7 carbohydrates, 2 metabolites from cofactors and vitamins, 33 lipids, 6 nucleotides, and 2 secondary metabolites. Tryptophan, α-ketoglutarate, Y-tocopherol/β-tocopherol, and Y-tocotrienol were among the metabolites.</td>
<td>Cambodia, India, Kenya, Mali, Nepal, Nicaragua and USA</td>
<td>[156]</td>
</tr>
<tr>
<td>Grains and seedlings</td>
<td>Natural variation</td>
<td>Field, growth chamber</td>
<td>HPLC</td>
<td>Analyst 1.5</td>
<td>Heatmap, metabolic pathway</td>
<td>24 candidate genes were associated with various metabolic quantitative trait loci by data mining.</td>
<td>China</td>
<td>[171]</td>
</tr>
<tr>
<td>Seedlings</td>
<td>Natural variation</td>
<td>Laboratory</td>
<td>GC-MS</td>
<td>ChemStation, XLSTAT</td>
<td>PCA, MANOVA</td>
<td>Thai black and purple rice contained higher levels of metabolites than the red and colorless samples.</td>
<td>Thailand</td>
<td>[172]</td>
</tr>
<tr>
<td>Seedlings</td>
<td>Natural variation</td>
<td>Laboratory</td>
<td>GS-MS, LC-MS</td>
<td>SIMCA-P, SPSS</td>
<td>PCA, PLS-DA, metabolic pathway</td>
<td>25 metabolites, including acidic compounds, amino acids, sugars, lipids, and secondary metabolites were identified as the components that contributed to the variations in the germinated brown rice group.</td>
<td>Korea</td>
<td>[173]</td>
</tr>
<tr>
<td>Seedlings</td>
<td>Natural variation</td>
<td>Laboratory</td>
<td>LC-ESI-MS</td>
<td>R</td>
<td>OPLS-DA</td>
<td>Phenylpropanoid biosynthesis and glutathione metabolism were continuously enriched during the seed germination and young seedling growth stages.</td>
<td>China</td>
<td>[174]</td>
</tr>
<tr>
<td>Leaves</td>
<td>Abiotic stress (Plant–drought–flood)</td>
<td>Field</td>
<td>LC-MS</td>
<td>XCMS, Mconverser, Excel, SIMCA-P, R, SPSS</td>
<td>PCA, PLS-DA, OPLS-DA, t-test, Heatmap, Euclidean distance, ANOVA</td>
<td>102 different metabolites were identified from the rice spike between T1 (abrupt drought–flood alternation) and control treatment, 104 different metabolites were identified between T1 and CK1 (drought) treatment, and 116 different metabolites were identified between T1 and CK2 (flood) treatment.</td>
<td>China</td>
<td>[96]</td>
</tr>
<tr>
<td>Organ Parts</td>
<td>Category</td>
<td>Experimental Methodology</td>
<td>Metabolomics Acquisition Methods</td>
<td>Bioinformatics Tools</td>
<td>Statistical Analysis</td>
<td>Findings</td>
<td>Source of Samples</td>
<td>References</td>
</tr>
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</tr>
<tr>
<td>Leaves</td>
<td>Abiotic stress</td>
<td>Field</td>
<td>LCMS</td>
<td>R, ggplot2</td>
<td>PCA, Venn diagram, heatmap</td>
<td>Decrement of some metabolites in stressed plants at specific development stages and organs: glycerophosphoryl, isocitric acid, ribitol, A116014 (Flag leaves/flowering), A214004, dehydroascorbic acid, dimer, glyceric acid, glycine, malic acid, phosphoric acid (flag leaves/early grain-filling), A147011, A180002, arabinit, aspartic acid, erythronic acid, galactonic acid, phosphoric acid (flowering spikelets).</td>
<td>Philippines</td>
<td>[158]</td>
</tr>
<tr>
<td>Leaves</td>
<td>Abiotic Stress</td>
<td>Greenhouse</td>
<td>GC-MS, NMR</td>
<td>AMDIS, MarkerLynx XS, MestReNova, Topspin, MiniTab 15, SPSS</td>
<td>PCA, t-test</td>
<td>Metabolites of 5-methyl methionine and the dippeptide alanylglycine were only detected by ¹H NMR.</td>
<td>USA</td>
<td>[175,176]</td>
</tr>
<tr>
<td>Leaves</td>
<td>Natural variation</td>
<td>Chamber</td>
<td>GS-MS, LC-MS</td>
<td>Vx Capture, MassLynx DataBridge, MetAlign, SIMCA-P, STATISTICA, Cytoscape</td>
<td>PCA, PLS-DA, OPLS-DA, Heatmap, ANOVA, Pearson’s correlation, metabolic pathway</td>
<td>Antioxidant activities of rice leaves were high in blue, white, and green light, followed by red and shade light of LED.</td>
<td>Korea</td>
<td>[177]</td>
</tr>
<tr>
<td>Leaves</td>
<td>Abiotic stress</td>
<td>Chamber</td>
<td>GC-MS</td>
<td>Chroma TOF, R</td>
<td>PCA, HCA, Heatmap correlation</td>
<td>Metabolite levels were mainly negatively correlated with performance parameters under drought stress.</td>
<td>Philippines, Vietnam</td>
<td>[178]</td>
</tr>
<tr>
<td>Leaves</td>
<td>Biotic Stress</td>
<td>Greenhouse</td>
<td>NMR</td>
<td>Chenomx, SIMCA-P, SPSS</td>
<td>PCA, Tukey’s test, ANOVA</td>
<td>The concentration of 10 metabolites was significantly altered between the infestation by planthopper and the control groups.</td>
<td>Thailand</td>
<td>[179]</td>
</tr>
<tr>
<td>Leaves</td>
<td>Abiotic stress</td>
<td>Greenhouse</td>
<td>GC-MS, CE-MS, LC-MS</td>
<td>None</td>
<td>PCA, Heatmap, Pathway</td>
<td>The accumulation of glucose, fructose, and sucrose involved in starch degradation, sucrose metabolism, and the glyoxylate cycle were upregulated in rice plants exposed to cold or dehydration.</td>
<td>Japan</td>
<td>[180]</td>
</tr>
<tr>
<td>Leaves</td>
<td>Biotic Stress</td>
<td>Greenhouse</td>
<td>GC-MS, LC-MS</td>
<td>XCMS, AMDIS, PeakView, MArkView, MetaboAnalyyst, MetPA, SPSS</td>
<td>PCA, t-test, metabolic pathway, ANOVA</td>
<td>Alteration of metabolites in pathway analysis in both resistant cultivars compared to the control. Cyanomino acids and lipid metabolism were induced in IR36, while changes in thiamine, taurine and hypotaurine metabolism in IR56.</td>
<td>Philippines</td>
<td>[181]</td>
</tr>
<tr>
<td>Leaves</td>
<td>Abiotic stress</td>
<td>Greenhouse</td>
<td>GC-MS</td>
<td>SAS, R</td>
<td>Heatmap, HCA, ANOVA</td>
<td>Sugars and amino acids increased significantly in the leaves and roots of both genotypes under salt stress, while organic acids increased in roots and decreased in leaves.</td>
<td>None</td>
<td>[182]</td>
</tr>
</tbody>
</table>
Weedy rice is also known as red rice due to the red pigmentation on the pericarp. The pericarp of weedy rice seeds had much greater quantities of anthocyanin, beneficial trace elements (Ca, Fe, Zn, and Se), free amino acids, and unsaturated fatty acids than cultivated rice [107]. These nutritional values of weedy rice can be of great interest for the development of nutritious and high antioxidant content rice. Metabolomics study can be applied to chemically profile and screen the high nutritional line of weedy rice. Moreover, pericarp pigmentation could conceivably provide camouflage for weed seeds in crop fields or protection from biotic and abiotic stresses [74].

Weedy rice has a high adaptability to environmental stresses, which enables it to survive in harsh conditions. Weedy rice has been reported to have higher tolerance to salinity, flooding, drought, heat, and cold temperatures compared with the commercial rice varieties [111,113,133–135]. Resistance traits of weedy rice toward rice diseases (e.g., rice blast, bacterial leaf blight, panicle blight) [137] could be due to the production of phytoalexins, a group of metabolites produced upon disease infection as a defense mechanism. Thus, it can be an effective option to elucidate the valuable chemicals and screen the beneficial specific genes controlling important metabolites to be incorporated into rice cultivars to produce new disease-resistant rice varieties or higher tolerance toward abiotic stresses such as drought and salinity.

8. Study of Weedy Rice in Malaysia: Where Do We Go?

In addition to the application of a single omics platform, the incorporation of multi-omics studies could dive into insights and provide a deeper understanding of the molecular and physiological mechanisms of the targeted organisms [85]. Single large-scale omics studies described previously have accumulated data in various organelles, cells, tissues, and organs. While these omics data are becoming more accessible and cost-friendly, scientists are now leveraging more comprehensive multi-omics studies integrating the various omics layers, which have promise in bridging the relationship between these different omics and their phenotypes.

Combining two or more omics approaches, e.g., metabolomics with genomics, transcriptomics, or proteomics, has been shown to provide an understanding of the molecular
phenotype by providing a holistic view into the mechanisms controlling cellular processes, molecular functions, and interactions. Metabolomics studies are particularly important in multi-omics studies due to their closeness to phenotype and, therefore, have been selected as the ‘common denominator’ to guide many study designs [185].

In rice, integrated metabolomics and transcriptomics approaches have been used in landraces and cultivated rice [186] and studies in rice under different stress conditions [187–189] in which the metabolome and transcriptome profiles have shed light into their molecular mechanism, metabolite–gene correlation networks and prediction of diverse biosynthetic pathways. The outcome of such studies can help in the understanding of rice metabolism and how it responds to various environmental stimuli. Similarly, proteomics and metabolomics integration studies have been performed in rice [190,191], providing a protein–metabolite interaction network.

Despite the immense importance and potential outcome of multi-omics research, the application of this study in weedy rice is yet to gain momentum. The issues with multi-omics data integration remain a challenge that hinders the progress of multi-omics research. However, one notable study has emerged recently in which Zhang et al. [192] incorporated transcriptomics, proteomics, and metabolomics analysis studies that describe the metabolism of hypoxic germination tolerance in weedy rice embryos. It is hopeful that in the future, the outcome of multi-omics research studies will be able to shed light on plant metabolism, and the integration of various molecular-level data can provide a systems biology view of metabolism, especially the pathways and networks in weedy rice.

The rise in deep learning (DL), a subfield of machine learning (ML) and artificial intelligence (AI), marks a significant advancement in the automation of precision agriculture [193]. These AI tools have the potential to effectively differentiate weedy rice from cultivated rice, addressing a critical challenge in crop management. Ancillary technologies, such as the Internet of Things (IoT), unmanned aerial vehicles (UAVs), field robots, and AI-enhanced herbicides, further augment AI’s capabilities, providing holistic and data-driven approaches to weed control [194]. For example, a computer vision and meta-heuristic hybrid NNICA classifier has demonstrated high accuracy in distinguishing between different weed varieties and rice plants under both natural light (NLC) and controlled light conditions (CLC) [195]. Leveraging AI in weedy rice management holds great promise for the future, potentially transforming agricultural practices and improving crop yields.

9. Conclusions

The research on weedy rice in Malaysia has revealed its complex dynamics and significant impact on rice agriculture. The morphological, physiological, and genetic diversity of weedy rice has been documented, especially in its superior adaptability and its ability to mimic cultivated rice. Moreover, genetic analyses have traced the origins of Malaysian weedy rice to both local cultivars and wild rice populations, demonstrating the intricate evolutionary pathways and hybridization events that have shaped its current form. This understanding underscores the need for continuous monitoring and advanced management strategies to mitigate weedy rice infestations.

The application of omics technologies offers promising avenues for enhancing our understanding and management of weedy rice. Genomic, transcriptomic, proteomic, and metabolomic studies have begun to uncover the molecular mechanisms behind the traits that make weedy rice a formidable competitor. These studies not only provide insights into weedy rice’s stress tolerance, seed dormancy, and adaptive evolution but also highlight potential genetic resources that could be harnessed for crop improvement. Furthermore, integrating multi-omics approaches will be crucial for a systems-level understanding of metabolism. The revolution of advanced omics technologies with artificial intelligence (AI) in weedy rice studies is hoped to accelerate the development of effective, knowledge-based weed management strategies and ensure the sustainability of rice agriculture in Malaysia.
Author Contributions: Conceptualization, I.F.M., M.S.M. and M.Z.S.; formal analysis, I.F.M., M.S.M. and M.Z.S.; data curation, I.F.M., M.S.M. and M.Z.S.; writing—original draft preparation, I.F.M.; writing—review and editing, I.F.M., S.N.S.B., M.S.M., F.S., Z.M. and M.Z.S.; supervision, M.S.M. and M.Z.S.; project administration, M.S.M., F.S. and M.Z.S.; funding acquisition, M.S.M., F.S., Z.M. and M.Z.S. All authors have read and agreed to the published version of the manuscript.

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