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Morphology of Malaysian Weedy Rice (*Oryza sativa*): Diversity, Origin and Implications for Weed Management

Edi Sudianto, Ting-Xiang Neik, Sheh May Tam, Tse-Seng Chuah, Akmal Adilah Idris, Kenneth M. Olsen, and Beng-Kah Song*

Weedy rice is one of the most dominant and competitive weed species found throughout rice planting areas worldwide. In Malaysia, a combination of agricultural practices such as direct seeding and shared use of machinery has contributed to the rapid proliferation of weedy rice across paddy fields in recent decades. Here, we report on the morphological characterization of weedy rice populations and inferred origin(s) of different morphotypes present in Peninsular Malaysia. Eight weedy rice morphotypes were distinguished based on a combination of traits such as awn presence/absence and hull color, from 193 accessions collected in 17 locations. Results showed a high proportion of awnless accessions (strawhull-, intermediate strawhull-, and brownhull-colored morphotypes, together composing 65 % of sampled accessions), with awned accessions represented by strawhull, brownhull, and blackhull forms. Clustering and PCA analyses revealed four major clusters: (1) *O. rufipogon* and the majority of awned, blackhull, and brownhull—suggestive of a type of weedy rice originating from wild *Oryza* populations; (2) elite *indica* cultivar rice and the majority of strawhull weeds—supporting a previous proposal that weedy rice from Malaysia mainly evolved from indirect selection on cultivars for easy-shattering feral forms; (3) the majority of brownhull; and (4) a mixture of other weedy morphotypes—potentially reflecting multiple origins and subsequent admixture. The combination of key morphological descriptors will be useful for advising farmers appropriately in strategies for controlling the spread of weedy rice, including periodic manual weeding to reduce buildup of the seed bank in the fields.

Nomenclature: Weedy rice, *Oryza sativa* L. ORYSA; rice cultivars, *Oryza sativa* L. ssp. *indica* S. Kato ORYSA; wild rice, *Oryza rufipogon* Griffiths.

Key words: Morphological characterization; rice; seed traits; weed control, weedy rice.

Weedy rice (*Oryza sativa* L.), known locally as “padi angin” in Malaysia (in reference to its easily shattering seeds), is one of the most dominant and competitive weed species found in rice planting areas worldwide. This conspecific relative of domesticated rice is difficult to detect in rice fields, particularly at early growth stages, and successfully competes with cultivated rice for space and resources. In the past two decades, many severe incidences of weedy rice infestation have been

reported globally, ranging from countries in North and South America (Delouche et al. 2007) to Europe (Fogliatto et al. 2012) and various Asian countries (Bakar et al. 2000; Chung and Park 2010; Watanabe et al. 2000). The growth and spread of weedy rice in Southeast Asia has been reported to be closely associated with a shift away from traditional hand-transplanting of rice seedlings into fields and toward direct-seeding (direct sowing) of fields, resulting in changes of the weed flora in favor of weedy and wild rice (Hussain et al. 2010).

Weedy rice infestations are now recognized as a major constraint in rice production worldwide, reducing harvests by up to 30 to 50% in the United States alone (Shivrain et al. 2009). Previous studies trying to better understand and infer the origin of weedy rice have revealed high morphological and genetic diversity and a complex pattern of multiple weed origins that vary, depending on the world region and ecotypes studied. Four biological processes are thought to be implicated in the origins of weedy rice: adaptation of wild rice varieties to cultivation; hybridization between cultivated rice

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and wild rice; hybridization between different cultivated rice varieties (including potentially between the *indica* and *japonica* subspecies); and de-domestication (escape and feralization) of cultivated rice to a weedy form (Londo and Schaal 2007; Zhang et al. 2012).

In Malaysia, the occurrence of a weedy form of rice was reported to occur sporadically in the southern part of the Muda area (Kedah state) in 1990 and has become a widespread problem in all Malaysian rice production areas since the 2000s (Azmi and Baki 2007; Karim et al. 2010). As with other Southeast Asian countries, one of the major factors contributing to the spread of weedy rice in Malaysia has been the change in rice establishment methods from transplanting of seedlings, which provides opportunities for hand-weeding of fields, to direct seeding. More than 90% of the rice areas in Peninsular Malaysia now practice at least some direct seeding (Azmi and Baki 2007). Together with farmers' limited knowledge about weedy rice, weak weed management practices, use of contaminated self-supplied seeds, and shared use of machinery and irrigation water, these agricultural changes have led to the rapid proliferation of weedy rice across Malaysian paddy fields (Karim et al. 2004; Watanabe et al. 2000).

Weedy rice is recognizably more difficult to control compared to other weeds in rice fields, because certain types are highly similar to commercially grown rice varieties. Studies of weedy rice across different countries have revealed high phenotypic diversity in weedy accessions, both within and among geographical regions (Karim et al. 2010; Shivrain et al. 2010; Song et al. 2014). Several morphological characteristics have been used in characterizing weedy accessions; these include hull color, seed characteristics, and other plant morphological traits (e.g., panicle length, flag leaf length, culm length/color) (Arrieta-Espinoza et al. 2005; Delouche et al. 2007; Shivrain et al. 2010; Fogliatto et al. 2012).

In Malaysia, limited periodical surveys and a few restricted, localized studies have been performed, mainly reporting on the detection, diversity, and degree of proliferation of weedy rice infestations (Hussain et al. 2010; Karim et al. 2010; Song et al. 2014). Similar to studies from other countries, many morphotypes of weedy rice have been identified in accessions collected from across Peninsular Malaysia (Bakar et al. 2000; Song et al. 2014). Our recent molecular study (Song et al. 2014) revealed complex origins of Malaysian weedy

rice with evidence of gene flow from elite *indica* cultivars, Malaysian *indica* landraces, and *O. rufipogon*. The extent of genetic heterogeneity among Malaysian weedy rice strains is much greater than their U.S. counterpart (Song et al. 2014). However, it is the phenotypic characteristics of the weeds that are most easily accessible to farmers in aiding in the identification of weed morphotypes in fields. Due to the diversity and complexity of weedy rice, a more comprehensive study comprising well-represented local sampling populations from all areas would be very useful to increase our understanding of weedy rice diversity, and for planning a more-informed control strategy that can help overcome this problematic weed. Therefore, our study aimed to characterize the diversity of weedy rice populations based on morphology, and explore the potential origins of different types of weedy rice in Peninsular Malaysia using morphological characters. We hope the morphological descriptors identified in our work will aid in furthering weedy rice management.

Materials and Methods

Plant Materials. A total of 193 weedy rice samples were collected from infested rice fields in 17 different locations, representing all rice cultivation areas (approximately 2,000 ha surveyed for seed collections) in Peninsular Malaysia. Selection of weedy rice was based on morphological characteristics that distinguish the weeds from domesticated rice. These granary areas are distributed across six states: Perlis, Kedah, Penang, Kelantan, Terengganu, and Selangor; for the first five states listed, they were sampled in September 2010 during main cropping season, whereas weedy rice seeds from Selangor were sampled in January and February of 2011 (Table 1). Field observations in the same rice planting localities across two planting seasons detected no differences in morphologies of weedy rice. For each individual plant, seeds from one panicle were collected and stored at 4 C cold room until used. In addition, five modern (elite) *indica* rice cultivars (MR211, MR219, MR249, and two MR220) and two local wild rice samples (*Oryza rufipogon*, designated MUSC201 and MUSC208, collected from Wakaf Bharu, Kelantan and Alor Setar, Kedah, respectively) were included in the study.

Field data such as plant height, panicle orientation, and awn presence were recorded during sampling at each location. Seed hull color, seed

Table 1. Population code, location, number of samples, range of coordinates of the weedy rice populations and other rice samples used.

State	Population code	Location	No. of samples	Longitude (E), latitude (N)
Perlis	PLPB	Kg. Padang Besar (FIDA), Perlis	13	100.120, 6.240
	PLGH	Kg. Guar Hujung Batu, Perlis	7	99.786, 5.984
Kedah	KDGC	Kg. Batu 20, Guar Cempedak (Yan), Kedah	13	100.266, 5.514
	KDHK	Taman Uda, Hutan Kampung, Kedah	14	100.230, 6.090
	KDKM	Kg. Masjid, Kedah	16	100.180, 6.080
	KDPD	Kg. Pida 4, Kedah	19	100.170, 6.210
	KDSL	Kg. Batu 17, Sg. Limau Dalam, Kedah	13	100.263, 5.534
Penang	SPBM	Bukit Mertajam, Seberang Perai	12	100.280, 5.220
	SPKB	Kepala Batas, Seberang Perai	7	100.250, 5.310
	SPSD	Sg. Dua, Seberang Perai	9	100.260, 5.270
Selangor	SLSB	Sungai Besar, Sabak Bernam, Selangor	15	101.592, 3.426
	SLSK	Sekinchan, Selangor	3	101.600, 3.300
Kelantan	KNBJ	Kg. Padang Melintang, Binjai, Kelantan	8	102.209, 6.048
	KNMA	Ladang Merdeka Ana I/II/III, Kelantan	7	102.080, 6.081
	KNML	Mulong, Kelantan	14	102.205, 6.048
	KNPB	Kg. Belian, Palekbang, Kelantan	13	102.128, 6.090
Terengganu	TR	Besut and Jerreh, Terengganu	10	102.329–102.350, 5.449–5.441
Total number of weedy rice sample			193	
Local modern cultivated varieties from Malaysia (CV)			5	
Local <i>O. rufipogon</i> accessions from Malaysia (OR)			2	

length, awn color, and seed pericarp color were also recorded for each sample (Supplemental Table 1; <http://dx.doi.org/10.1614/WS-D-15-00168.S1>). Morphotypes were then assigned to each weedy rice sample based on the abovementioned phenotypic characteristics (Supplemental Table 2; <http://dx.doi.org/10.1614/WS-D-15-00168.S2>).

Greenhouse Phenotyping Experiment. A total of 193 weedy rice samples, along with the five Malaysian *indica* cultivars (CV) and two Malaysian *O. rufipogon* (OR) accessions, were grown in controlled greenhouse conditions at Washington University (St. Louis, MO), with humidity in the range of 50 to 90%, and daytime and night-time temperatures set at 26 and 21 C, respectively. The photoperiod was set for 16 h, resembling the conditions in which the weeds were sampled. About 5 to 10 seeds, all derived from a single panicle of an individual collected accession, were germinated in 5-cm pots. One seedling per accession was transferred to 12-cm round pots after approximately 2 to 3 wk, and scored for morphological traits. The weedy rice accessions used in this study are a subset of the 207 used by Song et al. (2014); 14 accessions from that study were not used in the present study due to failure of seedlings to establish. Mature panicles from a single plant were designated as one sample. Twenty-two morphological characteristics were selected based on descriptors for wild and cultivated rice (*Oryza* spp.) (Bioversity International, IRRI, WARDA 2007): panicle type, degree of

seed shattering, panicle orientation, average panicle length, and number of panicles at maturity stage; presence or absence of awn, presence and distribution of awns along the panicle, length and color of awns; culm habit and number, culm anthocyanin coloration; grain length, grain width, grain shape class, hundred-grain weight, pericarp color, hull color; flag leaf attitude, flag leaf length; leaf blade attitude and surface roughness (Supplemental Table 3; <http://dx.doi.org/10.1614/WS-D-15-00168.S3>). These descriptors were selected because they can be easily measured by farmers and do not need specialized instruments for measurement. For quick identification, Malaysian weedy rice was classified based on hull coloration and awn presence into eight morphotypes. They include: strawhull (SH), intermediate strawhull (mSH), brownhull (BR), and blackhull (BH)—all without awns; and strawhull-awned (SHA), intermediate strawhull-awned (mSHA), brownhull-awned (BRA), and blackhull-awned (BHA). Intermediate strawhull refers to weedy rice samples that exhibited intermediate phenotypic forms between strawhull and brownhull with at least two other weedy traits (e.g., morphological combinations which included grain length, awn color, pericarp color, plant height, and panicle orientation).

Data Analysis. In order to determine phenotypic variability and identify morphological traits that contribute to the variability, phenotypic data

comprising 22 morphological variables were subjected to Principal Component Analysis (PCA) using XLSTAT Statistical Software version 2013.1.02 (Addinsoft, New York, NY). Test for appropriateness of factor analysis were performed by Kaiser–Meyer–Olkin (KMO) and Bartlett tests. Variables with low KMO and Bartlett tests values, low proportion of variance, and/or having multiple high correlation at more than one principal component were then excluded from further analysis. Our analysis yielded 17 morphological characters for 123 samples of weedy rice, along with five and two accessions of cultivated and wild rice respectively, totalling 130 accessions. The five excluded characters were grain width, culm anthocyanin coloration, leaf surface roughness, panicle attitude, and flag leaf length. Seventy samples had to be excluded from analysis because of incomplete data scoring. Data of the first two principal components for the weedy rice samples were plotted using the scatter plot visualization function of XLSTAT, and then grouped according to hull color.

To complement PCA analysis, agglomerative hierarchical cluster (AHC) analysis was performed based on the 22 scored weedy rice morphological traits (XLSTAT 2013.1.02). AHC helps to define relationship between each cluster in pairwise manner. Clustering was performed following Ward's agglomeration method and Euclidian distance for dissimilarities. The analysis was performed to characterize 120 weedy rice samples, along with five elite Malaysian rice cultivars and two *Oryza rufipogon* wild rice samples. Unlike PCA, AHC analysis was performed without factor analysis (e.g., KMO and Bartlett tests) of the data set; therefore, all 22 morphological traits were retained for the analysis. Nevertheless, incomplete data collection resulted in exclusion of 73 weedy rice accessions from this analysis. In addition, to analyze and compare the variations between eight weedy morphotypes, cultivars, and wild rice, all 22 morphological variables (18 nonparametric and four parametric) were subjected to analyses of variance. Steel–Dwass–Critchlow–Fligner multiple comparisons used with Kruskal–Wallis tests for nonparametric analysis, and Tukey's honestly significance difference (HSD) test used as a post hoc test for ANOVAs. The level of significance was set to $P < 0.05$.

Results and Discussion

Weedy Rice Sample Classification. Two key commonly used characters, namely hull color and

the absence or presence of awn, were found to be useful traits for distinguishing Malaysian weedy rice accessions (Figure 1). The majority of the weedy rice accessions collected in Peninsular Malaysia were awnless, consisting of the BR (32.6% of samples), followed by SH (20.7%) and mSH (11.9%) (Table 2). BH weedy rice was rarely encountered during our field sampling (2.6%); only one accession was collected from each of the states of Perlis, Penang, and Terengganu; two were collected from Kedah, and this form was absent altogether from Selangor and Kelantan samples collected in this study.

Awned accessions comprised 32.1% of sampled weed accessions, with the following distribution across hull colors: 13.0% BRA, 7.3% mSHA, 6.7% SHA, and 5.2% BHA. The geographical distribution of awned weedy rice was more disparate than awnless accessions across the six sampled states. Awned accessions were found to be relatively more abundant in west coast states (Perlis, Penang, and Selangor, with 25% of SHA, 14.3% of mSHA, and 27.8% of BRA, respectively, across the three states), but infrequent in Kedah, Terengganu, and Kelantan. As with awnless accessions, blackhull forms of awned weedy rice were rarer than other hull colors. Only one and two samples of BHA were discovered in Penang and Kedah, respectively, with three samples in Selangor and four in Kelantan.

Our findings showed higher prevalence of SH and BR weedy morphotypes; this might be due to their close resemblance to cultivated rice (Figure 1), allowing them to escape detection in rice fields. In contrast, BHA weedy morphotypes are less common; they exhibit characteristics of wild rice that probably make them more prone to detection and removal by farmers' hand-roguing. Our recent genetic analyses (Song et al. 2014) postulated that the BHA weedy rice ecotype might have originated from recent weed–wild hybridization events, potentially coupled with crop-to-weed introgression associated with the weed's proliferation. Other weedy rice studies have reported high incidence of either strawhulls (Arrieta-Espinoza et al. 2005; Londo and Schaal 2007; Shivrain et al. 2010) or blackhulls (Londo and Schaal 2007). Brownhull weedy rice was reported as the least frequent weedy rice in the United States (Reagon et al. 2010; Shivrain et al. 2010). This difference in the phenotypic characteristics of weedy rice in various parts of the world could be largely due to the different forms of infestation and their origins.

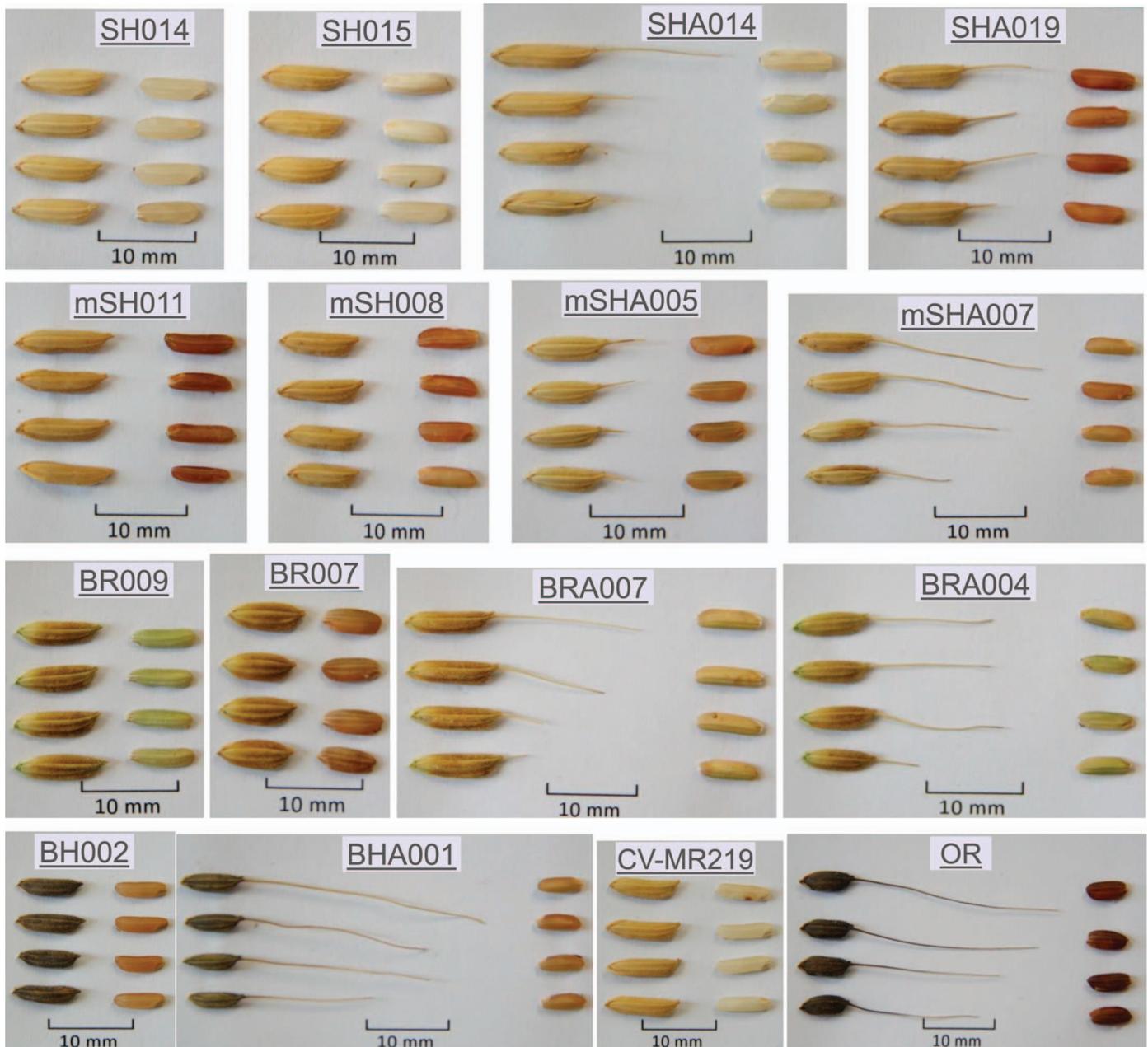


Figure 1. Morphotypes of weedy rice found in Peninsular Malaysia. (SH014, SH015) strawhulled, no awn; (SHA014, SHA019) strawhulled, with awn; (mSH011, mSH008) intermediate strawhulled, no awn; (mSHA005, mSHA007) intermediate strawhulled, with awn; (BR009, BR007) brownhulled, no awn; (BRA007, BRA004) brownhulled, with awn; (BH002) blackhulled, no awn; (BHA001) blackhulled, with awn; (CV-MR219) modern elite cultivar MR219; (OR) wild rice *Oryza rufipogon*. (Color for this figure is available in the online version of this article.)

Principal Component Analysis and Clustering Analysis. First two PCA components explained approximately 46.14% of the variation observed (Figure 2; Supplemental Table 4; <http://dx.doi.org/10.1614/WS-D-15-00168.S4>). The first component (23.27%) has clearly separated weedy rice into cultivar-like (SH and SHA) and weed accessions that were associated more closely with wild rice, with strong factors in pericarp color, awn charac-

teristics, panicle shattering, and culm habit (Supplemental Table 4; <http://dx.doi.org/10.1614/WS-D-15-00168.S4>). Meanwhile, the second component (22.86%) distinguishes hull coloration among weedy rice samples. Some of the significant factors for second components are hull color, grain length, 100-grain weight, flag leaf attitude, and leaf blade coloration (Supplemental Table 4; <http://dx.doi.org/10.1614/WS-D-15-00168.S4>).

Table 2. Distribution frequency of weedy rice morphotypes in six states of Peninsular Malaysia.^a

Location	Frequency of weedy rice with morphotypes							
	Awnless				Awned			
	SH	mSH	BR	BH	SHA	mSHA	BRA	BHA
Perlis (N=20)	4 (20.0) ^b	2 (10.0)	5 (25.0)	1 (5.0)	5 (25.0)	— (0)	3 (15.0)	— (0)
Kedah (N=75)	16 (21.3)	8 (10.7)	27 (36.0)	2 (2.7)	4 (5.3)	6 (8.0)	10 (13.3)	2 (2.7)
Penang (N=28)	6 (21.4)	5 (17.9)	9 (32.1)	1 (3.6)	2 (7.1)	4 (14.3)	— (0)	1 (3.6)
Selangor (N=18)	4 (22.2)	2 (11.1)	4 (22.2)	— (0)	— (0)	— (0)	5 (27.8)	3 (16.7)
Terengganu (N=10)	2 (20.0)	1 (10.0)	5 (50.0)	1 (10.0)	— (0)	— (0)	1 (10.0)	— (0)
Kelantan (N=42)	8 (19.0)	5 (11.9)	13 (31.0)	— (0)	2 (4.8)	4 (9.5)	6 (14.3)	4 (9.5)
TOTAL	40 (20.7) ^c	23 (11.9)	63 (32.6)	5 (2.6)	13 (6.7)	14 (7.3)	25 (13.0)	10 (5.2)
		131 (67.9)				62 (32.1)		

^a Abbreviations: BH, blackhull; BHA, blackhull-awned; BR, brownhull; BRA, brownhull-awned; mSH, intermediate strawhull; mSHA, intermediate strawhull-awned; SH, strawhull; SHA, strawhull-awned.

^b Numbers in parentheses indicate percentage of the total collection in each state.

^c Numbers in parentheses indicate percentage of the total collection of 193 weedy rice across Peninsular Malaysia.

PCA scatter plot shows separation of cultivated rice (Malaysian elite *indica* cultivars MR220-06, MR220-07, MR211, MR219, and MR249), which formed a tight cluster, and the two wild rice samples, which are placed at some distance from each other. Although the Malaysian weedy rice samples were found to be dispersed across the plot (Figure 2), certain patterns were evident, such as the closer positioning of the mSH, SH, and SHA types near cultivated rice varieties, sharing common

characteristics with Malaysian *indica* rice cultivars, most importantly straw hull color. Four SH accessions (SH002-KNBJ01, SH007-KDKM01, SH007-PLPB01, and SH013-KDSL01) were tightly grouped with cultivated rice. In addition, most of the BRA and BHA weedy morphotypes were located closer to wild rice (*Oryza rufipogon*). The BRA, BHA, and mSHA types are mainly influenced by the first component traits, especially awn presence, awn distribution along the panicle, awn

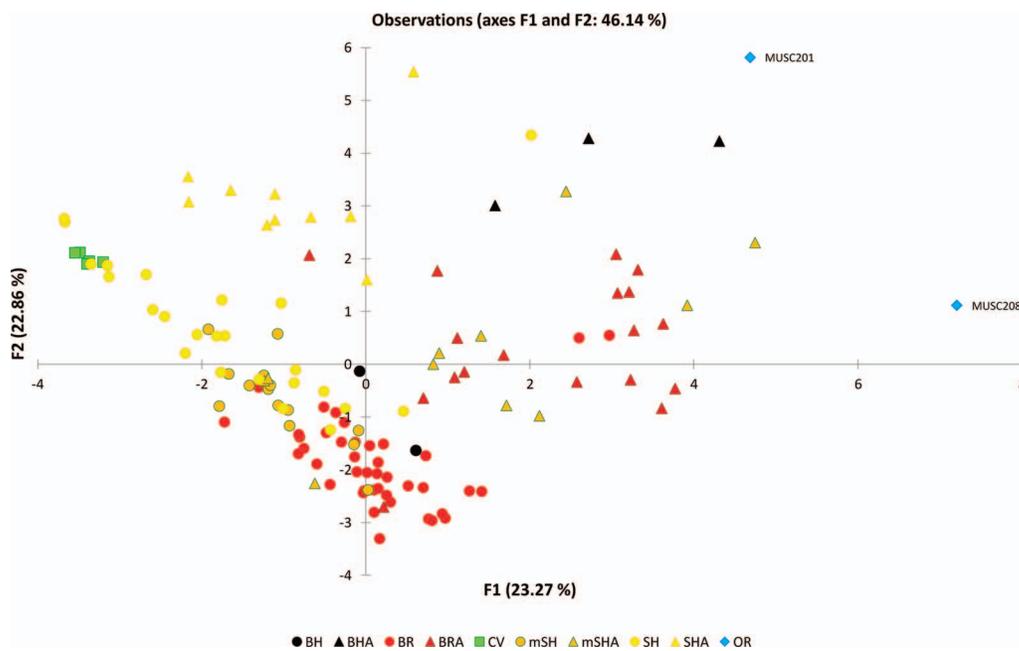


Figure 2. Scatter plot distribution of first and second components from 17 morphological characteristics of weedy rice populations collected from Peninsular Malaysia. Abbreviations: BH, blackhull awnless weedy rice; BHA, blackhull awned weedy rice; BR, brownhull, awnless weedy rice; BRA, brownhull, awned weedy rice; CV, Malaysian *O. sativa* elite cultivars; mSH, morphologically intermediate weed form between SH and BR; mSHA, morphologically intermediate weed form between SHA and BRA; SH, strawhull awnless weedy rice; SHA, strawhull awned weedy rice; OR, *O. rufipogon* accession. (Color for this figure is available in the online version of this article.)

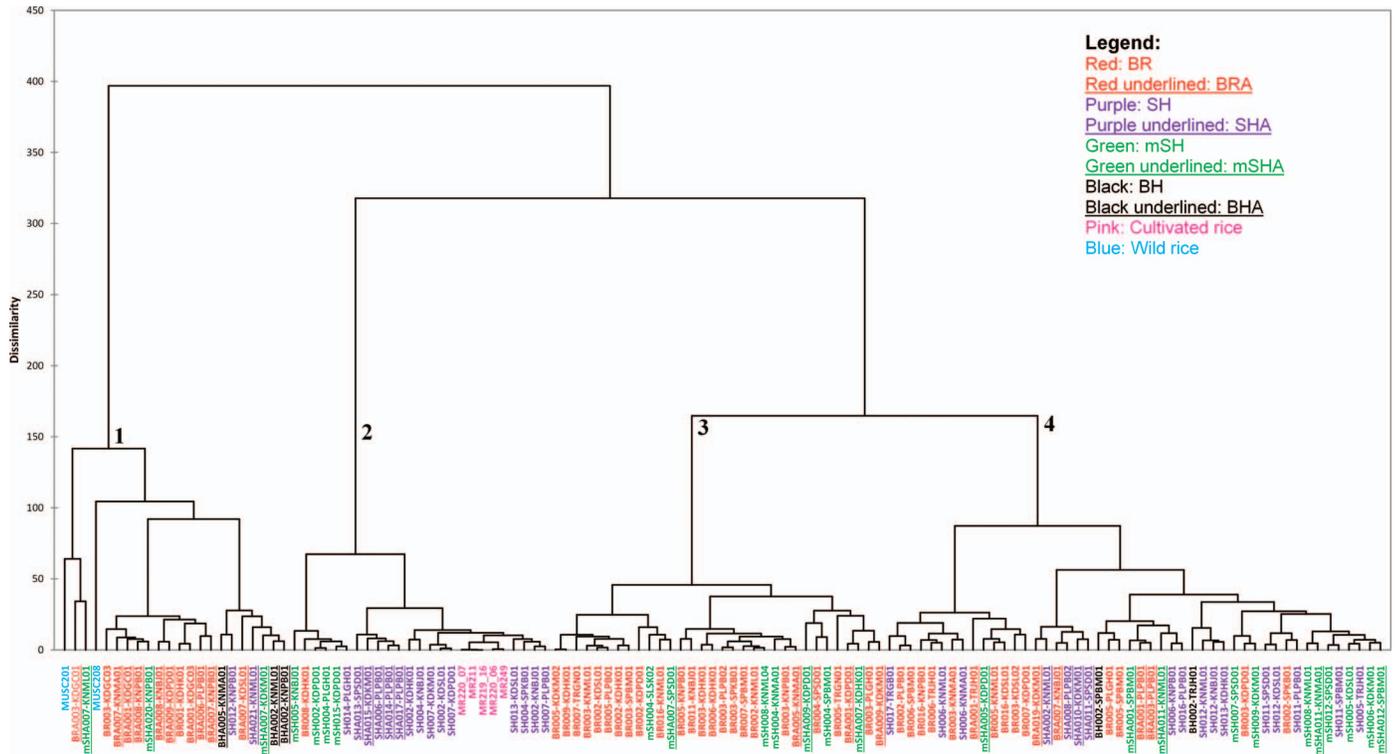


Figure 3. Agglomerative hierarchical clustering of Peninsular Malaysian weedy rice based on 22 morphological traits by using Ward's agglomeration method and Euclidean distance dissimilarities. (Color for this figure is available in the online version of this article.)

length, awn color, panicle shattering, and culm habit, loosely sharing certain characteristics with *Oryza rufipogon*.

Besides the six easily observed morphological traits, other traits measured in the present study were also useful for morphological characterizations. PCA analysis further indicates the importance of a combination of the 17 characteristics in distinguishing among Malaysian weedy rice morphotypes. Some of these traits, such as 100-grain weight, awn presence, and awn length, have been previously described as important traits in characterizing variation among weedy rice populations (e.g., Fogliatto et al. 2012; Suh et al. 1997). Another important indicator of wild rice, flag leaf

length, which is thought to reflect relative leaf sizes of the plant (and thus indirectly influencing its photosynthesis capacity and shading potential; see Shivrain et al. 2010), was observed in our study to be greater in all the weedy morphotypes collected (and in wild rice) than in cultivated rice, perhaps making them more competitive in the field.

The dendrogram of AHC analysis (Figure 3) revealed clustering of Malaysian weedy rice into four major groups: the majority of BHA, and BRA types together with *Oryza rufipogon* (cluster 1); the majority of SH and SHA types together with cultivated rice (cluster 2); the majority of BR (cluster 3); and a mixture of all weedy morphotypes in cluster 4 (Table 3). In the first cluster, which

Table 3. Distribution of 127 Peninsular Malaysian rice accessions^a based on Agglomerative Hierarchical Clustering of 22 morphological traits.

Cluster	No. of accessions	Frequency of morphotypes									
		SH	SHA	BH	BHA	BR	BRA	mSH	mSHA	Wild rice	CV
1	22	1	1	0	3	2	10	0	3	2	0
2	25	10	5	0	0	1	0	4	0	0	5
3	32	0	0	0	0	22	3	4	3	0	0
4	48	13	4	2	0	13	5	6	5	0	0

^a Abbreviations: BH, blackhull; BHA, blackhull-awned; BR, brownhull; BRA, brownhull-awned; CV, Malaysian *O. sativa* elite cultivars; mSH, intermediate strawhull; mSHA, intermediate strawhull-awned; SH, strawhull; SHA, strawhull-awned.

contains BHA and BRA weedy morphotypes similar to wild rice, the two *Oryza rufipogon* samples are intermingled with the weedy rice accessions. Five strawhull (three mSHA, one SHA, one SH), and three awnless (one SH, two BR) samples also resolved within this group, indicating close morphologies to wild rice. A second distinct cluster is represented by the strawhull types of weedy rice (both SH and SHA), which show similarities with the Malaysian elite *indica* cultivars. Four mSH samples and one BR sample also resolved within this group. The remaining weedy accessions (80 accessions) grouped into two subclusters, clusters 3 and 4. The former comprises most BR type samples (22), BRA (3), and mSH/mSHA types (7); and the latter is composed of SH (13), SHA (4), BR (13), BRA (5), mSH (6), mSHA (5), and BH (2) types. No differentiation of weedy rice populations based on localities was observed, because all four main clusters contained weedy rice accessions collected from different granaries.

In this study, PCA was used to capture as much data variation as possible in the first two dimensions. It provides insights about how genetically diverse a population is, with low diversity samples usually grouped tightly (as observed in the SH and BR groups) and highly diverse individuals tending to form looser clusters (as observed in the SHA and BRA groups) in the scatter plot (Figure 2). Meanwhile, AHC was used to build a hierarchy of clusters and disclose the relationships between different accessions. For instance, we can infer a close relationship of SH group (cluster 2) to those of mixed morphotypes group (cluster 3 and 4). The BHA and BRA group (cluster 1) is distinctly related to the other three clusters due to their higher dissimilarity score (Figure 3). Both the PCA and AHC analyses revealed higher morphological diversity among intermediate strawhulls (both with and without awns) as compared to other groups (Figures 2 and 3), potentially due to ongoing introgression among weedy rice accessions that can promote genetic diversity. Our analysis of Malaysian weedy rice using simple sequence repeat (SSR) markers has revealed that intermediate strawhulls (both awned and awnless) are likely genetic admixtures of strawhull and brownhull populations (Song et al. 2014). In the United States, the brownhull population was predicted to be from possible hybridizations between *indica* and *aus* groups, SH and BHA weedy rice groups, *indica* and BHA, or *aus* and SH (Reagon et al. 2010).

Quantitative and Qualitative Morphological Traits. The Tukey and Steel–Dwass–Critchlow–Fligner multiple comparison tests revealed four quantitative and 18 qualitative traits descriptors, respectively (Supplemental Table 5; <http://dx.doi.org/10.1614/WS-D-15-00168.S5>; Supplemental Table 6; <http://dx.doi.org/10.1614/WS-D-15-00168.S6>). Among the four quantitative traits, grain width was found to be relatively more useful in discriminating the morphotypes. Wild rice had the widest grain (2.53 ± 0.43 mm), with mSHA having the narrowest grain (2.09 ± 0.13 mm). This provides a convenient indicator for farmers to separate the wild rice from weedy rice types. A rice grain size chart displaying a range of grain shapes and sizes could be developed to achieve this purpose. Groupings based on Tukey's test showed that wild rice can be easily distinguished from mSH, mSHA, SH, and cultivated rice. However, no weedy rice accessions other than BR could be significantly distinguished from cultivated rice using grain width. In terms of the other three traits, there were overlaps for many of the morphotypes, indicating that these types cannot be easily differentiated from each other. For panicle length, cultivated rice, which has undergone artificial selection for increased yield, has the longest panicle length (25.08 ± 0.08). For the nonparametric data, groupings based on the Steel–Dwass–Critchlow–Fligner test showed unexpected overlaps between most of the morphotypes. However, some of the qualitative traits, such as panicle shattering and pericarp color, are good separators for differentiating BR–BRA–BH–BHA groups from CV–SH–SHA morphotypes. Likewise, using the awn distribution trait, awned accessions can also be easily differentiated from awnless accessions based on Steel–Dwass–Critchlow–Fligner groupings (Supplemental Table 6; <http://dx.doi.org/10.1614/WS-D-15-00168.S6>).

Based on the number of morphotype pairs showing significant differentiation ($P < 0.05$) and Steel–Dwass–Critchlow–Fligner groupings, a combination of six morphological traits, namely hull color, pericarp color, grain length class, grain shape class, awn distribution, and panicle shattering, have been identified for effectively discriminating weed accessions in field. It is worth noting that the six descriptors can be easily observed and measured by nonexperts. For each of these six selected traits, phenotypic distributions among weedy rice groups are presented in Figure 4. One of these characters, panicle shattering, is also an important trait for distinguishing weedy rice samples from cultivated

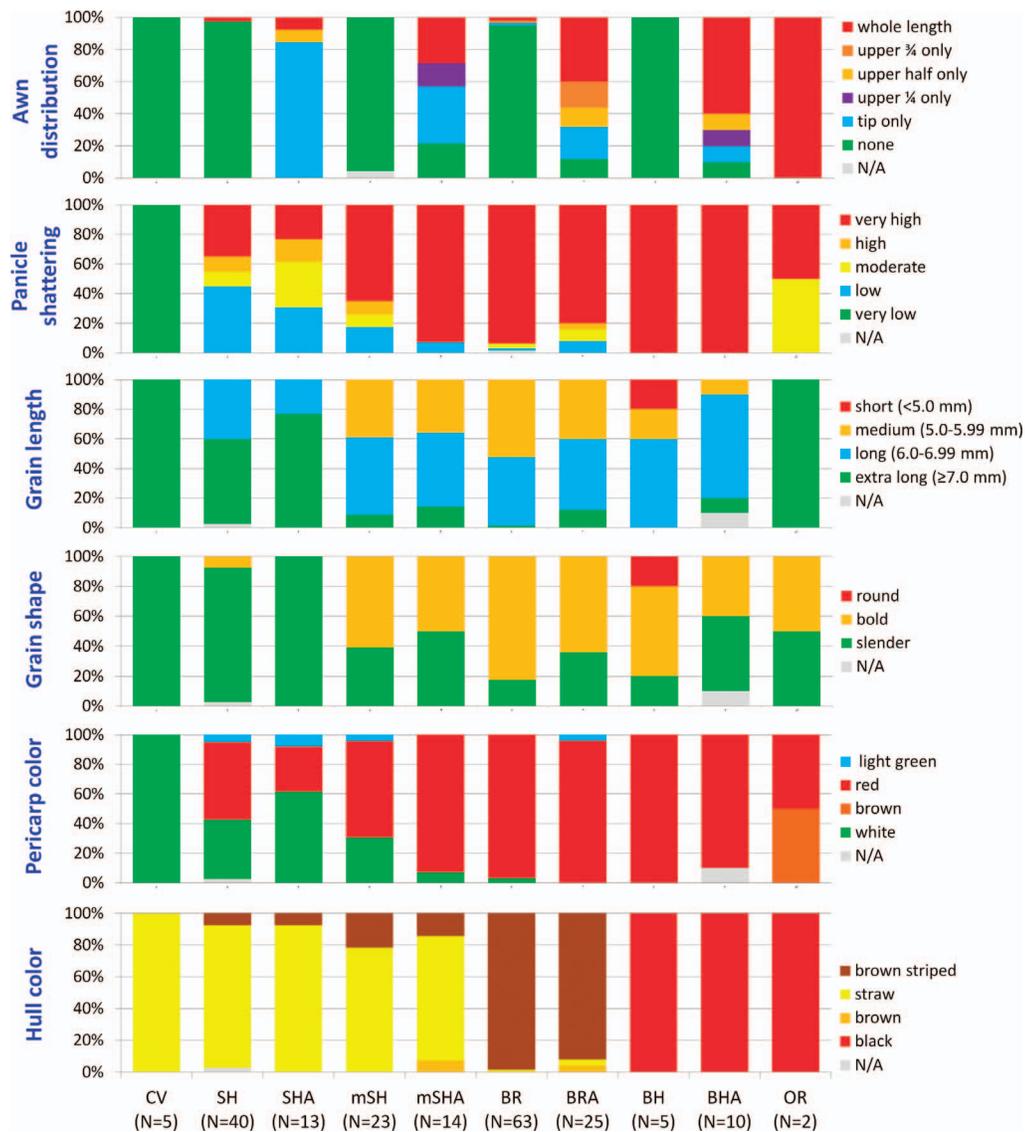


Figure 4. Percentage of rice individuals in each of rice group possessing various phenotypic categories of the six most easily observed morphological traits (hull color, pericarp color, grain length class, grain shape class, awn distribution and panicle shattering). Abbreviations: BR, brownhull, awnless weedy rice; BRA, brownhull, awned weedy rice; BH, blackhull awnless weedy rice; BHA, blackhull awned weedy rice; CV, Malaysian *O. sativa* elite cultivars; mSH, morphologically intermediate weed form between SH and BR; mSHA, morphologically intermediate weed form between SHA and BRA; SH, strawhull awnless weedy rice; SHA, strawhull awned weedy rice; OR, *O. rufipogon* wild rice accession. (Color for this figure is available in the online version of this article.)

rice. In line with our findings, morphometric analysis performed by Bakar et al. (2000) found grain characteristics (awn presence, length, and pericarp color) to be useful diagnostic characters for weedy rice. Suites of phenotypic combinations, as observed in Figure 4, could help in weed control management in rice planting areas.

As part of our initiatives to address a long-term objective of weedy rice control, our population-scale phenotypic data collected in this study, coupled with its PCA and AHC diversity analyses, can be fed into a mobile application (app), allowing farmers to effectively undertake weed control management

activities in the fields. This makes identification of weedy rice easier by defining weedy baseline in rice groups. Additionally, automated comparison between the photograph snapped on-site and pre-installed photograph database would potentially help farmers to quickly identify problematic crop, thus leading towards a more rapid and effective weedy rice control. To the best of our knowledge, such similar mobile-based apps are currently available only for winter crops such as wheat and barley (e.g., Weed ID, <https://www.grdc.com.au/Resources/Apps>).

Diversity of Peninsular Malaysian Weedy Rice Morphology. Weedy rice populations in Peninsular Malaysia were found to be highly diverse morphologically. Four major clusters, which can be subdivided into 10 smaller subclusters (at Euclidian distance 50; Figure 3), are characterized in this study. Interestingly, a previous study by Azmi et al. (1994) did not observe significant morphological variation among weedy rice from the Muda area in Kedah (measuring panicle length, number of spikelets, number of unfilled grains, and 1,000-grain weight). In contrast, our analyses revealed weedy rice from the Muda area to be scattered across all four major clusters (Figure 3), indicating a significant morphological variation in the sampling site. This could indicate a relatively recent origin of the morphological diversity in this region, perhaps due to the rapid spread of weedy rice across rice-planting areas of Peninsular Malaysia since the early 2000s (Song et al. 2014). More recently, Ahmed et al. (2012) conducted a study of 14 weedy rice morphotypes from Perak using three vegetative and five reproductive descriptors, and also reported much greater heterogeneity in weedy rice compared to the four cultivated rice samples tested.

Origin of Weedy Rice in Peninsular Malaysia. Previous morphology-based studies examining the origin(s) of weedy rice have suggested that weedy accessions from different world regions have originated from cultivated and/or wild rice through multiple complex mechanisms (Fogliatto et al. 2012; Shivrain et al. 2010). For Peninsular Malaysia, both PCA and AHC clustering analyses showed a small group of mainly awned, brownhull and black hull weedy rice (10 BRA and three BHA accessions) that is very closely grouped with *Oryza rufipogon* (within the same cluster; see Figures 2 and 3), apart from other weedy and cultivated rice. Given that the observation is also supported by molecular data (Song et al. 2014), it stands to corroborate the notion that these weedy rice were originating from locally occurring wild rice populations. Two processes could contribute to this close relationship between wild rice and weedy rice: (1) evolution of weedy forms from selection and adaptation of wild rice to disturbed (cultivated) conditions and/or (2) hybridization between cultivated and wild rice (Londo and Schaal 2007). Although different flowering times of wild rice and cultivars would tend to reduce the likelihood of crop–wild hybridization as the main mechanism for the occurrence of weedy rice (Bakar et al. 2000), recent molecular evidence of introgression between

wild and cultivated rice suggests that crop–wild hybridization might be a primary mechanism for the origin of Malaysian weedy rice (Song et al. 2014).

Both morphological PCA and hierarchical clustering indicate that the SH and SHA types are very closely related to Malaysian elite cultivar varieties, *Oryza sativa* ssp. *indica*. Results of the Tukey's tests (based on grain width, 100-grain weight, panicle length, and flag leaf length) showed no significant difference because both straw-colored morphotypes and cultivated rice were placed in the same group (Supplemental Table 6 <http://dx.doi.org/10.1614/WS-D-15-00168.S6>). The 100-grain weight of the SHA type showed significantly higher value than all the other weedy morphotypes. Weedy rice showing close morphological resemblance to cultivated rice in the United States, Central America, and China (e.g., with white pericarps) has been taken to be indicative of genetic introgression between crop and weed (Delouche et al. 2007; Shivrain et al. 2010). Therefore, the similarities detected between straw-colored morphotypes and cultivated rice in this study might reflect successful crop–weed introgressions in the Malaysian rice fields. This is also supported by our SSR dataset, which revealed evolution of the Malaysian weedy rice population through crop–weed introgressions (Song et al. 2014).

The recent molecular study supports the notion that modern elite cultivars play an important role in the evolution of SH-like Malaysian weedy rice (Song et al. 2014). Based on a panel of 24 polymorphic SSR markers and principal coordinate analysis (PCoA), Malaysian SH morphotypes are found closely grouped with elite cultivars (Song et al. 2014). It was thus proposed that some weedy rice in Malaysia evolved from easy-shattering feral crop forms, i.e., reversion of crop varieties to a weedy form. The high morphological similarity shown between these weedy rice samples and cultivated rice in our study supports this earlier hypothesis of dedomestication from local cultivated rice. Zhang et al. (2012) concluded that in the Guangdong and Liaoning provinces of China, dedomestication of weedy rice from local cultivated rice has frequently occurred in rice-growing regions, based on their findings of *japonica*-like and *indica*-like weedy rice ecotypes using SSR markers. Other studies that also reported the possibility of weedy rice originating from cultivated rice include weedy morphotypes in Bhutan (Ishikawa et al. 2005) and in the Mediterranean region (Delouche et al. 2007). In short, both molecular (Song et al. 2014) and the present

morphological study collectively suggest that wild *Oryza* and modern-bred elite cultivars genetically contribute to the complexity of Malaysian weedy rice. Both studies, including some of the overlapping grouping results as shown in Supplemental Table 6 (<http://dx.doi.org/10.1614/WS-D-15-00168.S6>), suggest that ongoing gene flow among different morphotypes could have potentially resulted in new morphotypes of weedy rice in planting areas.

Implications for the Management of Weedy Rice.

Results of our study show that weedy morphotypes are highly diverse but not structured geographically (by region), such that different types of weedy rice can be found in one area (and even one field). Growers and farmers must be alerted to the types of weedy rice in their fields and be advised accordingly, because different impacts on yield have been documented in the interference capability of red rice ecotypes (Shivrain et al. 2009). Our study provides a combination of six key morphological descriptors (hull color, pericarp color, grain length class, grain shape class, awn distribution, and panicle shattering; Figure 4) for each of the major type of weedy rice. This update can be helpful for farmers to use during periodic manual weeding to remove existing weedy rice from the field during the planting season, so as to not contribute to the build-up of the seed bank in the fields. Results of our study noted a group of weedy rice strains closely related to cultivated rice (group 2), which might also prove to mimic cultivated rice by showing later seedling emergence, similar hull color, and grain shape. Furthermore, given the large number of BR–BRA samples in clusters 3 and 4 (representing 77% of those clusters; Table 3, Figure 3), and considering that these forms possess high grain-shattering ability (represented by bar colored in red, Figure 4), these morphotypes could be among the most competitive and invasive weedy morphotypes in the field.

Increased occurrence of weedy rice has been partly associated with the adoption of a specific rice cultivation technique, i.e., direct seeding and/or volunteer seeding (Watanabe et al. 2000), which seems to create an environment suitable for the survival and propagation of weedy rice (Zhang et al. 2012). Therefore, special efforts should be taken to create or maintain field environments that prevent the growth of weedy rice or rice mutants. Examples of such field management include mechanical transplanting, crop rotation, frequent tillage, and better water management (Azmi and Abdullah 1998). However, although it was reported that

these practices—especially the reversion from direct seeding to transplanting rice seedlings or wet seeding—managed to significantly reduce the weedy rice problem even in heavily infested fields (Azmi and Abdullah 1998), they have not been widely adopted, most probably due to higher labor demands and associated costs.

The finding of highly diversified weedy rice populations in Peninsular Malaysia stresses the importance of discriminating the weedy rice morphotypes in the field. Coupled with the combination of morphological traits (Figures 2 and 3) and the six key descriptors identified here (Figure 4), these results are hence potentially helpful to ease the difficulties in managing weedy rice due to clear distinction of traits between weedy rice and cultivated rice. Additionally, various datasets, such as measurements of wide variety of weedy phenotypic traits, photos of categorized weedy rice morphotypes, lifecycle information, genetic data analysis, environmental data (e.g., temperature, humidity, water supply, soil type), and genotypic data (e.g., single nucleotide polymorphisms, SSR), can potentially be integrated into a smartphone-based weedy rice app. Some of the resources were already obtained in the present study, and genotypic datasets will be compiled through various genomic-related projects undertaken in our laboratories. Development of this easy-to-use app would help to pave the way toward more effective weed control in paddy fields. Our future study would be to extend the plant phenotyping and apps development to other weed species in rice planting areas.

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