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Research Article

Morphological Variations of Weedy Rice (*Oryza sativa* ssp.) In Selected Rice Fields of Peninsular Malaysia

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ABSTRACT

Weedy rice or locally known as 'padi angin' has been infesting rice granary areas in Malaysia for the last 20 years. The outbreak of weedy rice in the country started during the late 1980s in Projek Barat Laut Selangor (PBLS) due to the adoption of direct-seeding practices. Weedy rice can be characterized by its early maturing and easy-shattering traits. The objective of this study was to determine the morphological variations of weedy rice in selected granary areas located in Peninsular Malaysia. A total of 110 weedy rice accessions were collected from nine locations in three granary areas in Peninsular Malaysia (the Western, Eastern, & Northern regions). The collected weedy rice accessions were planted under greenhouse conditions to study their morphological features. Results of the present study indicated that 73.6% of weedy rice accessions were awnless while the remaining 26.4% produced awn. Selected traits such as plant height, flag leaf length, culm length, number of tillers, number of panicles, and panicle length were included in the Principal Component Analysis (PCA). The weedy rice accessions were grouped into three different clusters mainly Cluster 1: cultivated rice and awnless populations, Cluster 2: brown and red pericarp awnless populations, and Cluster 3: awned populations. The findings of this study may be used as guidance for farmers and researchers for designing sustainable weedy rice management in the future.

Key words: Morphological variations, *Oryza sativa* management, weedy rice accessions

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the important crops worldwide and is well-known as a major diet for half of the human population across the globe. Over 40% of global food production consists of rice production which acts as the main staple food for several countries (Herman *et al*., 2015). The majority of countries in Asia depend on rice as their main source of food as well as provide income and employment for the inhabitants (Maclean *et al*., 2002; Makino, 2011). Furthermore, rice plays a vital role in certain countries' food security such as China and Malaysia (Zhang *et al*., 2005; Herman *et al*., 2015). According to Rajamoorthy *et al*., (2015), there will be an increasing demand for rice in Malaysia and a shortage of rice supply in the future.

Farmers in Malaysia have been battling with weedy rice for over several decades. The proliferation of weedy rice started in 1988 in Sekinchan located in the Northwest Selangor Project (PBLS) area (Wahab & Suhaimi 1991; Azmi *et al*., 2001). The infestation then spread to other areas such as Muda Irrigation Scheme in 1990 and was exacerbated further in 1993 (Azmi *et al*., 2001). Weedy rice (*Oryza sativa* L.) is locally known as 'the padi engine based on its easily shattering seeds characteristic (Sudianto *et al*., 2016; Mardiana-Jansar *et al*., 2019). Competition between weedy rice and cultivated rice occurred because they share similar characteristics, especially during early growth stages (Hussain *et al*., 2010; Sudianto *et al*., 2016). The shifting of the hand-transplanting method of rice seedlings to a direct-seeding approach appeared to be one of the main contributors to the weedy rice domination in rice granary areas (Azmi *et al*., 1994; Ziska *et al*., 2015; Sudianto *et al*., 2016). According to Karim *et al*., (2010), weedy rice has been a widespread problem nationwide since the 2000s. Therefore, integrated management practices are necessary to avoid productivity loss in rice fields and for efficient weed control (Nunes *et al*., 2018).

The usage of herbicides such as imazapic and imazapyr to eliminate weedy rice promoted residual activities in soil and water that can affect the environment (Bajrai *et al*., 2017). According to Ahmad *et al*., (2016), water resources quality at rice fields can be affected by the combination of land use activities and seasonal changes.

Compared to other weeds, weedy rice is hard to control in the granary because of its similarities with cultivated rice (Chauhan 2013; Sudianto *et al*., 2016). According to Shrestha *et al*. (2020), as weed, weedy rice competes with cultivated rice for nutrients, space, and light. Several characteristics such as taller growth habit, higher tillering and the greater nutrient user make weedy rice more competitive. (Estorninos *et al*., 2002). Weedy rice is also known as 'red rice' due to its red pericarp color (Gealy *et al*., 2009; Nadir *et al*., 2017; Shrestha *et al*., 2020). The presence of the red-pigmented pericarp is because of the existence of anthocyanins, catechins, and cathecolic tannins (Baldi 1971). However, several weedy rice biotypes are possessing other pericarp colors such as white, light red, and light green (Prathepha 2009). On the other hand, some weedy rice possesses awn seeds and showed variability in the seed hull coloration (Fogliatto *et al*., 2012).

Morphological characteristics have been utilized to characterize weedy rice accessions and variably exhibit in weedy rice (Fogliatto *et al*., 2012; Sudianto *et al*., 2016). These morphological characteristics include hull coloration, seed characteristics, spikelet traits, panicle length, culm length, and flag leaf length (Arietta-Espinoza *et al*., 2005; Delouche *et al*., 2007; Shivrain *et al*., 2010; Fogliatto *et al*., 2012; Perera *et al*., 2012). According to Fogliatto *et al*. (2012), other plant and seed traits wide phenotypic variations are also discovered in weedy rice such as plant height, tillering, panicle characteristics, seed characteristics, and phenology. Thus, this study was conducted to determine the morphological diversity and variability of weedy rice in three different regions mainly the Integrated Agriculture Development Area (IADA) in the western region, the Muda Agricultural Development Authority (MADA) in the northern region, and the Kemubu Agricultural Development Authority (KADA) in the east coast region of Peninsular Malaysia and to study the distinctive morphological characteristics between cultivated rice and the weedy rice populations.

MATERIALS AND METHODS

Weedy rice collection

Nine locations with infested granary areas were selected from three different regions in Peninsular Malaysia. A total of 110 weedy rice samples were collected from the respective location and selection was made based on the distinctive morphological characteristics between the weedy rice and the cultivated rice – mostly the plant's height and shattering trait. The location includes five states (Selangor, Kelantan, Kedah, Perlis, & Perak) in Peninsular Malaysia. The states then were grouped into three based on their geographical regions namely West, East, and North (Table 1).

Weedy rice in the West region was sampled during the main season in December 2017 whilst weedy rice from the East and North regions was sampled during the main season in April and July 2018. The weedy rice sample was collected during the main season because no significant differences in weedy rice morphological characteristics were observed between the two planting seasons by the locals. Seeds from weedy rice sampled were collected from one panicle and refrigerated at 4°C for further use. In addition, three modern rice cultivars, MR220 CL2, MR263, and MR297 collected from Kuala Selangor, Selangor (West region), and Pasir Puteh, Kelantan (East region) were also included in this study. Morphological characteristics such as the plant height (PH), awn presence (AP), hull coloration (HC) pericarp coloration (PC), awn coloration (AC), and the morphotypes were determined based on the respective morphological characteristics.

Greenhouse experiment

Weedy rice seeds from 110 individuals sampled along with three Malaysian commercial cultivars (MR220 CL2, MR263, MR297) were grown in a controlled greenhouse located at the Green House Complex, Universiti Kebangsaan Malaysia, Bangi, Selangor. Approximately five seeds from a single panicle from the collected weedy rice were sown in small pots. The seeds were pre-germinated for two days to examine the seed viability. One seedling was transplanted into a bigger pail (15 cm diameter). Each accession was replicated thrice following a randomized complete block design (RCBD). Eighteen morphological characteristics were recorded following the International Rice Research Institute (IRRI)'s descriptors for wild and cultivated rice (Biodiversity International, IRRI, and WARDA 2007). The recorded morphological characteristics were seedling height (SH), leaf blade length (LBL), flag leaf length (FLL), culm

Table 1. Sampling location and a number of samples of weedy rice collected in a different region of Peninsular Malaysia

Region	State	Sampling location	Code	No. of samples	Lat/Long
West	Selangor	Kg. Sawah Sempadan	SS	32	3.453°N 101.214°E
		Kg. Pasir Panjang	PP	21	3.567°N 101.067°E
East	Kelantan	Telok Mesira, Bachok.	TMK	13	6.143°N 102.343°E
		Jln. Tanjong, Tawang, Bachok	BCK	8	6.102°N 102.321°E
		Kg. Tok Ajam, Pasir Puteh	TAK	9	5.904°N 102.374°E
North	Kedah	Kg. Melele, Kodiang	KMM	2	6.347°N 100.346°E
	Perlis	Kg. Chermai, Arau (1)	ARA	14	6.400°N 100.271°E
		Kg. Chermai, Arau (2)	ARB	2	6.395°N 100.279°E
	Perak	Kg. Titi Serong, Parit Buntar	PBR	9	5.099°N 100.471°E

length (CL), awn length (AL), panicle length (PL), grain length (GL), grain width (GW), 100-grain weight (100GW), 10-grain weight (10GW), number of panicles (NOP), number of tillers (NOT), plant height (PH), awn color (AC), panicle attitude (PA), panicle thresh ability (PT), lemma and palea color (LPC) and pericarp color (PC) (Table 2). Each of the individual weedy rice accessions was designated as population, following Fogliatto *et al*. (2012). The weedy rice accessions were also grouped into six populations based on the presence of awn and pericarp color mainly brown (BNA), red (RNA), and white (WNA).

Statistical analysis

Morphological characteristics were analyzed using the analysis of variance (ANOVA) at $p \leq 0.05$ followed by Tukey's honestly significance different (HSD) post hoc test using the SPSS software version 22. The Principal Component Analysis (PCA) based on 18 morphological traits was also conducted using the Minitab software version 17. The agglomerative hierarchical clustering (AHC) analysis was also performed using the SPSS version 22. The clustering analysis was carried out following Ward's method and the Euclidian distance dissimilarities.

RESULTS AND DISCUSSION

Weedy rice grouping

Several morphological characteristics were selected to identify the weedy rice diversity such as hull coloration (Arrieta-Espinoza *et al*., 2005; Shivrain *et al*., 2010; Sudianto *et al*., 2016) and awn shape (Fogliatto *et al*., 2012). Thus, this study preferred to select pericarp coloration to group the weedy rice population because of its variability shown rather than the hull coloration through field observation. Weedy rice from awnless populations (72.7%) consists of 42.7% red pericarp (RNA) populations, 26.4% brown pericarp (BNA) populations whilst the remaining 3.64% possessed white pericarp (WNA) (Table 3). On the other hand, 27.3% of the awned populations consist of 16.4% red pericarp (RA) populations followed by brown pericarp (BA) (7.27%) and white pericarp (WA) populations (3.64%). The majority of RNA populations (50.9%) were distributed in the western region of Peninsular Malaysia while 40.7% of BNA were more abundant in the northern region. Awned populations were found to be more abundant in the western region of Peninsular Malaysia with 18.9% of the RA populations. According to Sudianto *et al*., (2016), most rice accessions in Peninsular Malaysia are awnless (67.9%).

Morphological traits of weedy rice

The plant height of cultivated rice varieties was significantly different as compared to the weedy rice accessions (Table 4). Cultivated rice varieties are shorter (88.4 ± 1.48 cm) than the weedy rice populations with the WA population having the highest plant height (136.7 ± 4.62 cm). A study by Rathore *et al*., (2016) exhibited that plant height is one of the important variables to characterize weedy rice variations and displayed an intermediate variation among weedy rice in India. This suggested that plant height characteristics can act as a benchmark for farmers during field observation. There is also a significant difference between the grain length of cultivated rice varieties and the weedy rice populations except for the WA populations. The grain of the cultivated rice varieties is found to be longer (10.32 ± 0.17 mm) than the weedy rice populations. BNA populations exhibited shorter grains with only 8.72 ± 0.10 cm long. Cultivated rice varieties were found to hold longer and slimmer seeds compared to weedy rice accessions in Pulau Pinang rice granary areas (Hussain *et al*., 2010). However, there is no significant difference in seedling height between the cultivated rice varieties and the weedy rice populations. The findings supported that weedy rice and cultivated rice varieties are not easily distinguished morphologically, especially during early growth (Ziska *et al*., 2015).

Principle component analysis (PCA)

About 32.7% of the variation was observed among the weedy rice populations and cultivated modern cultivars (CV) within the first two PCA components (Table 5). The first component accounted for 19.4% of weedy rice variation and was characterized by plant height, flag leaf length, culm height, and seedling height. The second component accounted for 13.3% of the variation and the weedy rice was characterized by the number of tillers, number of panicles, and panicle length. According to Fogliatto *et al*., (2012), Italian weedy rice was generally characterized by several morphological characteristics such as 1000 seed weight, plant height, flag leaf length, and whole seed length through PCA analysis. Besides that, Ratnasekara *et al*., (2014) reported that several morphological characteristics are important to characterize weedy rice variation such as plant height, seed weight, and both the number of tillers and panicles.

Agglomerative hierarchical clustering (AHC) analysis

The agglomerative dendrogram displayed in Figure 1 showed that the clustering of weedy rice collected in different Peninsular Malaysia region were clustered into three major groups: cluster 1 consists of the cultivated rice varieties and various awnless weedy rice populations (BNA, RNA, & WNA populations); cluster 2 consists majority of awnless populations with red and brown pericarps; and cluster 3 comprised with various awned weedy rice population (Table 6). Alongside cultivated rice varieties (CV), cluster 1 mainly consists of BNA (13) and RNA (26) populations with a total of 47 accessions while the rest of the BNA and RNA populations were grouped in cluster 2 (total of 38

Table 2. Morphological characteristics to determine the variation of weedy rice populations

Morphological characteristics	Stage of recording*	Description
Seedling height (cm)	30; late vegetative	Measured from the ground to the highest point of the seedling.
Culm height (cm)	60; 7 days after anthesis	Measured from ground level to the base of the panicle.
Plant height (cm)	90; after harvest	Measured from the ground level to the highest point of the plant.
Panicle – length (cm)	80-90; after harvest	Measured from the panicle base to the tip. Three panicles per accession
Panicle – attitude	60-70; near maturity	Represented by 1. erect, 3. semi-erect, 5. spreading, 7. horizontal
Panicle – thresh ability	90; after harvest	Represented by 1. difficult, 2. intermediate, 3. easy
Panicle – number	60-70; near maturity	Measured the number of panicles emerged
Tiller – number	90; after harvest	Measured the number of tillers
Foliage – flag leaf length (cm)	60; 7 days after anthesis	Measured from the ligule to the tip of the leaf
Foliage – leaf blade length (cm)	60; 7 days after anthesis	Measured the penultimate leaf (highest leaf below the flag leaf) from the ligule to the tip of the leaf
Seed – grain length (mm)	90; after harvest	Measured on 10 representative grains from the base to the tip of the grain (apiculus) using an electronic caliper
Seed – grain width (mm)	90; after harvest	Measured on 10 representative grains across the fertile lemma and palea using an electronic caliper
Seed – 10-grain weight (g)	90; after harvest	The measured weight of 10 well-developed, whole grains
Seed – 100-grain weight (g)	90; after harvest	The measured weight of 100 well-developed, whole grains
Seed – awn length (mm)	90; after harvest	Measured on 5-10 representative grains with awn
Seed – awn color	90; after harvest	Represented by 0. absent, 20. straw
Seed – lemma and palea coloration	90; after harvest	Represented by 10. white, 20. straw, 52. brown (tawny), 54. brown furrows
Seed – pericarp coloration	90; after harvest	Represented by 10. white, 51. light brown, 50. brown, 70. red

[*] Stage of recording according to IRRI's descriptors for wild and cultivated rice

Table 3. Distribution of weedy rice populations in three different regions of Peninsular Malaysia

Region	Frequency of weedy rice based on weedy rice populations*						TOTAL
	Awnless seed (*N*=80)			Awned seed (*N*=30)			
	WNA	BNA	RNA	WA	BA	RA	
West	1 (1.89)[a]	9 (17.0)	27 (50.9)	4 (7.55)	2 (3.77)	10 (18.9)	53
East	2 (6.67)	9 (30.0)	10 (33.3)	- (0)	6 (20.0)	3 (10.0)	30
North	1 (3.70)	11 (40.7)	10 (37.0)	- (0)	- (0)	5 (18.5)	27
TOTAL	4 (3.64)[b]	29 (26.4)	47 (42.7)	4 (3.64)	8 (7.27)	18 (16.4)	110

[*] Abbreviations for populations: BA; brown pericarp-awned, BNA; brown pericarp-awnless, RA; red pericarp-awned, RNA; red pericarp-awnless, WA; white pericarp-awned, WNA; white pericarp-awnless.
[a] Percentage of the total collection of weedy rice collected by region in Peninsular Malaysia.
[b] Percentage over a total collection of 110 weedy rice collected in different regions in Peninsular Malaysia.

Table 4. Quantitative morphological characteristics of weedy rice populations grouped according to the presence of awn and pericarp color

Morphological characteristics	Weedy rice populations*						
	CV	BNA	BA	RNA	RA	WNA	WA
Seedling height (cm)	45.7 ± 0.33 ab[a]	49.9 ± 0.93 ab	50.6 ± 1.70 ab	49.5 ± 0.77 ab	48.9 ± 1.12 ab	54.1 ± 4.91 b	43.5 ± 1.40 a
Culm height (cm)	56.3 ± 0.58 a	65.4 ± 1.07 ab	66.9 ± 1.38 b	65.6 ± 0.79 ab	65.8 ± 1.45 ab	68.2 ± 5.99 b	56.2 ± 1.50 a
Plant height (cm)	88.4 ± 1.48 a	131.5 ± 2.16 b	131.4 ± 3.84 b	129.9 ± 1.71 b	135.9 ± 2.58 b	131.8 ± 8.26 b	136.7 ± 4.62 b
Panicle – length (cm)	25.5 ± 0.83 a	25.2 ± 0.54 ab	24.9 ± 1.21 ab	26.1 ± 0.42 ab	27.1 ± 0.91 ab	25.7 ± 0.96 ab	29.2 ± 0.95 b
Panicle – number	11.11 ± 0.69 c	5.91 ± 3.11 ab	4.96 ± 0.43 ab	5.73 ± 0.28 ab	6.73 ± 0.51 b	3.33 ± 0.47 a	5.83 ± 0.27 ab
Tiller – number	12.22 ± 0.68 a	7.02 ± 0.39 b	5.29 ± 0.39 b	6.88 ± 0.31 b	7.55 ± 0.57 b	6.11 ± 0.74 b	6.25 ± 0.25 b
Foliage – flag leaf length (cm)	29.4 ± 1.16 a	39.4 ± 1.08 b	38.2 ± 2.40 ab	39.9 ± 0.86 b	39.1 ± 1.30 ab	41.2 ± 2.45 b	35.9 ± 1.31 ab
Foliage – leaf blade length (cm)	58.9 ± 0.76 b	53.5 ± 0.93 ab	47.7 ± 2.49 a	53.5 ± 0.80 ab	49.5 ± 1.26 a	54.6 ± 1.84 ab	52.7 ± 1.14 ab
Seed – grain length (mm)	10.32 ± 0.17 b	8.72 ± 0.10 a	9.25 ± 0.20 a	8.78 ± 0.08 a	9.54 ± 0.14 ab	9.28 ± 0.14 a	10.3 ± 0.22 b
Seed – grain width (mm)	2.69 ± 0.02 a	2.55 ± 0.02 a	2.63 ± 0.05 a	2.58 ± 0.02 a	2.54 ± 0.03 a	2.68 ± 0.03 a	2.49 ± 0.06 a
Seed – 10-grain weight (g)	0.2876 ± 0.003 a	0.2203 ± 0.027 a	0.226 ± 0.006 a	0.2386 ± 0.018 a	0.2677 ± 0.040 a	0.2452 ± 0.034 a	0.2060 ± 0.006 a
Seed – 100 grain weight (g)	2.9071 ± 0.045 c	1.9319 ± 0.043 a	2.2036 ± 0.054 ab	2.0746 ± 0.032 ab	2.3238 ± 0.053 ab	2.2523 ± 0.121 ab	2.0628 ± 0.068 ab
Seed – awn length (mm)	0.000 ± 0.000 a	0.000 ± 0.000 a	9.381 ± 1.350 b	0.000 ± 0.000 a	10.622 ± 0.636 b	0.000 ± 0.000 a	14.573 ± 1.299 c

*Abbreviations for populations: BA; brown pericarp-awned, BNA; brown pericarp-awnless, RA; red pericarp-awned, RNA; red pericarp-awnless, WA; white pericarp-awned, WNA; white pericarp-awnless, CV; cultivated varieties.

Table 5. First two principal components from 18 morphological characteristics of weedy rice accessions

Morphological traits	1st Component (19.4%)*	2nd Component (13.3%)
Seedling height (cm)	0.325	0.298
Culm height (cm)	0.326	0.331
Plant height (cm)	0.380	-0.004
Panicle – length (cm)	0.152	-0.384
Panicle – attitude	0.016	0.003
Panicle – thresh ability	0.279	0.173
Panicle – number	-0.208	0.441
Tiller – number	-0.209	0.452
Foliage – flag leaf length (cm)	0.359	-0.203
Foliage – leaf blade length (cm)	0.240	-0.130
Seed – grain length (mm)	-0.267	-0.061
Seed – grain width (mm)	-0.008	0.262
Seed – 10-grain weight (g)	-0.141	0.061
Seed – 100-grain weight (g)	-0.283	-0.009
Seed – awn length (mm)	-0.190	-0.172
Seed – awn color	-0.156	-0.181
Seed – lemma and palea coloration	0.127	0.162
Seed – pericarp coloration	0.148	0.045

*Values in brackets depict the percentage of the variances accounted for by each component.

Table 6. Distribution of weedy rice morphotypes and cultivated varieties clustered according to agglomerative hierarchical clustering (AHC) of 18 morphological characteristics

Cluster	Weedy rice populations (*f*)						
	CV	BNA	BA	RNA	RA	WNA	WA
1 (N = 47)*	3	13	0	26	1	4	0
2 (N = 38)	0	15	0	23	1	0	0
3 (N = 28)	0	0	8	0	16	0	4

*Total number of weedy rice populations in each respective cluster.
*Abbreviations for populations: BA; brown pericarp-awned, BNA; brown pericarp-awnless, RA; red pericarp-awned, RNA; red pericarp-awnless, WA; white pericarp-awned, WNA; white pericarp-awnless, CV; cultivated varieties.

accessions). A total of 28 weedy rice accessions were clustered together in cluster 3 which derived from awned populations: BA (8), RA (16), and WA (4) (Table 6). A study by Hussain *et al*. (2010) displayed a similar pattern where the commercial rice varieties were grouped closely together with separation from weedy rice accessions in Pulau Pinang. Malaysian elite *indica* cultivars namely MR220-06, MR220-07, MR211, MR219, and MR249 were clustered tightly together and parted with weedy rice accessions with different hull colorations (Sudianto *et al*., 2016). Straw hull-colored weedy rice shared common characteristics with some modern Malaysian *indica* rice cultivars (Sudianto *et al*., 2016).

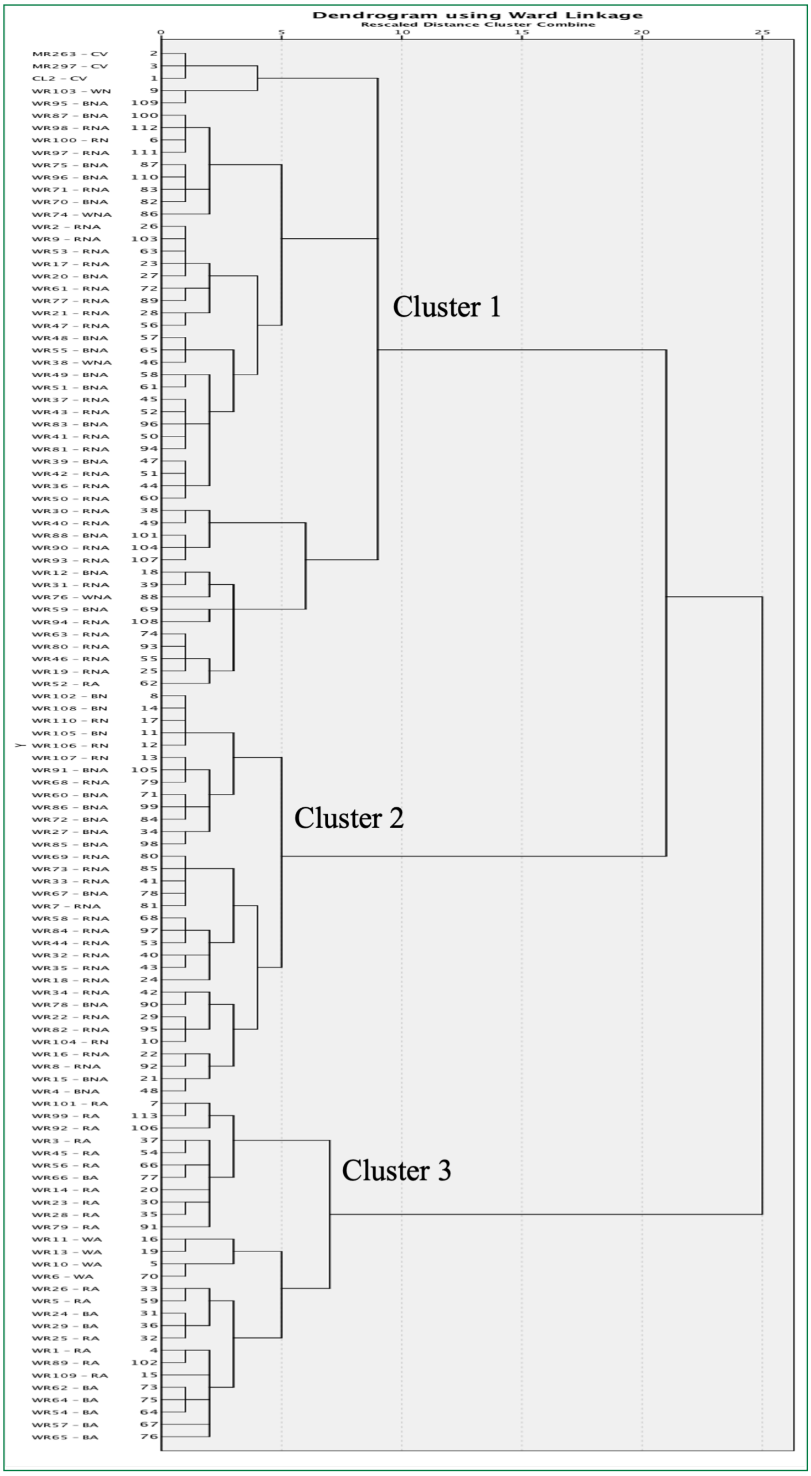

Fig.1. Hierarchical clustering of weedy rice collected from different regions in Peninsular Malaysia by 18 morphological characteristics using Ward's agglomerative method and Euclidian distance dissimilarities projected by SPSS software version.

CONCLUSIONS

The weedy rice collected was varied based on selected morphological characteristics that influence the variation among the populations. This study suggested that certain morphological characteristics such as plant height, flag leaf length, and culm height are important to categorize weedy rice populations in Peninsular Malaysia. The findings from this study will guide for farmers to distinguish the weedy rice population from the cultivated rice varieties especially during manual weeding to control weedy rice emergence. Generally, this study was conducted to assist researchers and farmers to construct a new sustainable weedy rice management system in the future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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