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Morphological Study of the Relationships between Weedy Rice Accessions (*Oryza sativa* Complex) and Commercial Rice Varieties in Pulau Pinang Rice Granary Area

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Abstrak: Padi angin boleh ditemui dalam petak sawah yang mengamalkan kaedah tabur terus. Kebanyakan padi angin mempunyai ciri-ciri morfologi yang sama dengan varieti komersial, menyebabkan padi angin sukar dikawal. Satu persampelan aksesi-aksesi padi angin telah dijalankan di empat lokasi jelapang padi Pulau Pinang bertujuan untuk kajian perbandingan ciri-ciri morfologi dengan varieti komersial yang ditanam di lokasi tersebut. Sejumlah 36 varian berbeza telah dikenal pasti dari 4 lokasi melalui perbandingan 17 ciri morfometrik dan sifat penguguran biji dari tangkai serta kadar percambahan biji benih. Analisis Komponen Prinsipal (PCA) menunjukkan bahawa 45.88% taburan variasi aksesi padi angin dan varieti komersial terkandung dalam 3 paksi pertama. Aksesi padi angin seperti PB6, PP2 dan SGA5 mempunyai bilangan anak yang banyak, tangkai padi yang panjang, batang pokok yang tinggi dan daun yang lebih panjang berbanding varieti komersial. Panjang biji padi dan kandungan klorofil pada 60–70 DAS *(days after sowing)* pada varieti komersial adalah lebih tinggi berbanding aksesi padi angin. Hasil kajian berjaya menentukan kadar pertumbuhan pokok padi angin dan mungkin dapat digunakan dalam pengurusan rumpai di sawah dan amalan pertanian baik.

Kata kunci: Padi Angin, Morfologi Padi, *Oryza sativa*, Pulau Pinang, Analisis Kluster

Abstract: Weedy rice (WR) is found in many direct-seeded rice fields. WR possesses morphological characteristics that are similar to cultivated rice varieties in the early stage of growth, making them more difficult to control than other weeds. A comparative morphological study was conducted by collecting WR accessions from four sites within the Pulau Pinang rice growing areas. The objective of the study was to characterise WR accessions of the Pulau Pinang rice granary by comparing their morphological characteristics to those of commercially grown rice in the area. Their morphometric relations were established by comparing 17 morphological characteristics of the WR accessions and the commercial varieties. A total of 36 WR morphotypes were identified from these 4 sites based on 17 characteristics, which included grain shattering habit and germination rate. The Principal Component Analysis (PCA) showed that 45.88% of the variation observed among the WR accessions and commercial varieties were within the first 3 axes. PB6, PP2 and SGA5 WR accessions had a higher number of tillers and longer panicle lengths, culm heights and leaf lengths compared to the commercial rice. The grain sizes of the commercial varieties were slightly longer, and the chlorophyll contents at 60–70 days after sowing (DAS) were higher than those of the WR accessions. Results from

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this study are useful for predicting potential WR accession growth, which might improve WR management and agriculture practices that control WR in the future.

Keywords: Weedy Rice, Plant Morphology, *Oryza sativa*, Pulau Pinang, Cluster Analysis

INTRODUCTION

Presently, there are about 10,200 ha of rice fields in Pulau Pinang. They can be divided into northern, central, southern and south west sites. Direct seedling culture is the preferred method of crop establishment due to the high cost and limited supply of labour. Continuous practice of this culture has caused changes in the weed flora in favour of weedy rice (WR) and wild rice. WR, locally known as *padi angin,* became the most dominant and competitive weed. WR is the main competitor with rice, affecting both growth and yield, especially in regard to space and nutrient availability. There are many WR plants in direct-seeded rice fields, and their wide-spread infestation might be due to the use of machinery, contaminated seeds, irrigation water and animal dispersal (Labrada 2007).

WR possesses similar morphological characteristics as the commercial varieties in the early stages of growth, making them more difficult to control than other weeds. Under moderate infestation of WR (15–20 panicles/m^2), yield loss is approximately 50%–60%; under high infestation (21–30 panicles/m^2), yield loss is 70%–80%, whereas under heavy infestation (more than 31 panicles/m^2), lodging of WR plants may occur, causing a total loss of yield (Azmi *et al.* 2004). Sometimes WR is taller than commercial rice and has easy and early grain shattering characteristics. WR accessions can also be distinguished based on their panicle structure, which can be either open or compact, on the pigmentation of the grain colour and on the presence or absence of awn. The morphological and stature characteristics of the plants, such as the shape and size of the grains and the structure of incidental features, are also important pieces of information aiding in the identification and classification of the plant species (Noda *et al.* 1985).

Studies of the characteristics of the WR complex, namely the morphological and physiological characteristics as well as the reproductive mechanisms and potential for hybridisation with commercial and wild rice varieties, are essential steps towards formulising a better WR control plan (Arrietta-Espinoza *et al.* 2005; Agostinetto *et al.* 2001; Noldin *et al.* 1999; Montealegre & Clavijo 1991). The current study was conducted by collecting samples of WR accessions from four different sites in the Pulau Pinang rice granary area. The main purpose of this study was to compare morphological characteristics among the accessions as well as between the accessions and cultivated rice, with the aim of identifying the number of represented WR morphotypes and the morphometric relationships among WR and commercial rice.

MATERIALS AND METHODS

WR accessions were identified and collected randomly from 4 sites within the rice fields of Pulau Pinang at the end of 2007. A total of 540 WR and 60 accessions of commercial varieties were collected from the 4 locations, and only 15 wild rice accessions were sampled from 3 locations. Sampling locations are shown in Figure 1. The WR seeds and three commercial rice seeds (MR84, MR219 and MR 185, which are widely planted in the selected sites) were used in this study for comparative purposes. Three hundred seeds from each accession were sown in small trays (48 x 30 x 10 cm) with three replicates, and seed germination was observed for a period of three months (January 2007 – March 2007). These trays were placed in the greenhouse for the study.

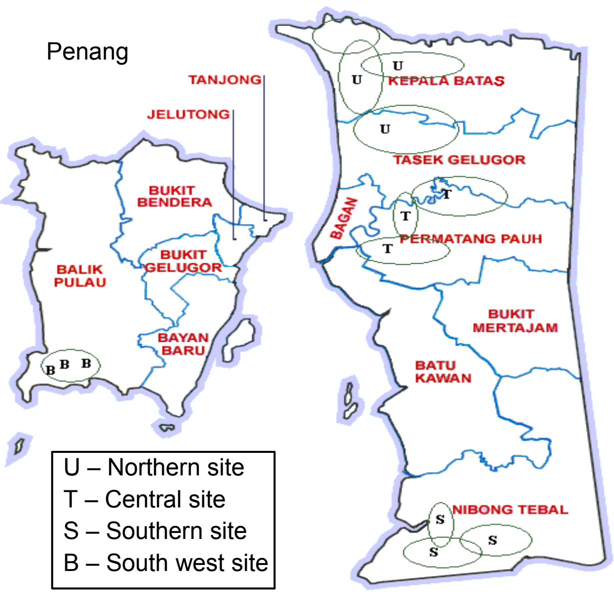

Figure 1: Map of sampling locations.

The same accessions were planted using a completely randomised design with three replicates in greenhouses at the Seberang Perai MARDI Station in the off seasons (April 2007 to July 2007) and the main season (September 2007 to December 2009) of 2007/2008. The seeds were soaked in water for 24 hours and air dried for 48 hours to germinate the seeds. Then, 45 seedlings from each accession were planted. The seeds were planted in rows leaving 20 cm between each seed and 25 cm between each row. Crop maintenance was done according to the *Manual penanaman padi berhasil tinggi* (Alias *et al.* 2002). The soil condition was loamy clay with a nutrient availability of 0.28% N, 0.18% P, 540.3 ppm K and 1.92% organic material. Fertilisers were applied 3 times at 14 days after sowing (DAS), 35 DAS and 65 DAS at a rate of

120 kg N ha^{-1}, 45 kg TSP ha^{-1} and 60 kg K_2O ha^{-1}, respectively. Cartap hydrochloride (Padan, Agricultural Chemicals Sdn. Bhd., Malaysia) was applied at the rate of 20 kg ha^{-1} to control important insect pests. The growth and developmental progress of all accessions was observed and recorded from the seedling stage until maturity using the Standard Evaluation System for Rice (SES) (International Rice Research Institute 1996) and the Manual for morpho-agronomic characteristics and performance traits of rice germplasm in the rice gene bank of MARDI (Abdullah *et al.* 1994).

Visual observations of the panicle structure and other morphological characteristics were measured and recorded when plants were at 80 DAS. Seventeen characteristics were selected, including culm height, panicle length, panicle type, leaf length, leaf width, presence or absence of awn, panicle branches, number of tillers, grain length, grain width, total spikelet and total filled grains. The percentage of sterility for each accession was obtained by dividing the number of empty grains over the total number of grains per spikelet and multiplying by 100 (Table 1).

Table 1: Seventeen morphological traits of WR morphotypes and commercial varieties scored at the productive stage (after 60 DAS).

Variables	Character states used for each variable
Plant erectness intermediate (= 45º),	Culm angle at the growth: 1 = erect (<30º), 3 = intermediate (45º), > 5, 7 = open (60º).
Culm height	Measured from the ground level to the base of the panicle (cm)
Maturity	Measured the number of days from seedling to grain ripening (85% of grains on panicle are mature).
Panicle type	Panicles are classified according to their mode of the branching angle of the primary branches and the spikelet density: 1 = compact, 5 = intermediate, 9 = open.
Flag leaf erectness	Measured the angle of openness of the blade tip against the culm on the leaf below the flag leaf; 1 = erect, 3 = intermediate, 5 = horizontal, 7 = descending.
Leaf blade pubescence	Measured by ocular inspection, run fingers from the tips down on the leaf surface. Presence of hairs on the blade surface is classified: 1 = glabrous, 2 = intermediate, 3 = pubescent.
Flag leaf length	Measured from the topmost leaf blade (cm).
Chlorophyll content	Measured from the top leaf at 70 DAS using SPAD-502 meter (5 X reading).
Variables	Character states used for each variable
Plant height	Measured from the soil surface to the tip of the tallest panicle from the principal culm (cm).
Panicle length	Measured from the panicle base to the tip (cm).

(continued on next page)

Table 1: *(continued)*

Variables	Character states used for each variable
1000 grain weight (TGW)	Measured using 1000 mature grains.
Grain length	Measured as the distance from the base of the lowermost sterile lemma to the tip (apiculus) (mm).
Awn length	Measured on five seeds.
Lemna and palea colour	Measured on mature seed: 0 = straw, 1 = gold and gold furrows on straw background, 2 = brown spots on straw, 3 = brown furrow on straw, 4 = brown (tawny), 5 = reddish to light purple, 6 = purple spots on straw, 7 = purple furrows on straw, 8 = purple, 9 = black, 10 = white.
Total spikelet per panicle	Recorded as the total number of seed in one panicle.
Filled grain	Recorded as the total number of filled grain in one panicle.
Spikelet fertility	Identify the fertile spikelet by pressing the spikelets with the fingers and noting those that have no grains.

Five matured panicles of each accession were shattered using the hand grip technique. The grains that were detached from the panicle were counted as shattered. This characteristic has been used by several researchers to identify and characterise WR (Azmi *et al.* 2004; Arrietta-Espinoza *et al.* 2005). The percentage of shattering was calculated by dividing the number of shattered seed over the total number of seeds per spikelet and then multiplying by 100.

At maturity, five panicles per replicate for each accession were harvested for the analysis of the yield component. Relative chlorophyll content was measured on the same plant using a chlorophyll meter SPAD-502 (Minolta, Japan) at 60–70 DAS. Data were analysed for variance using ANOVA with means compared using Duncan's New Multiple Range test (p = 0.05) (SAS 1993, 2001). To analyse the morphometric relationships among the accessions, principal components analysis (PCA) was applied to the 17 morphological traits. Variable coefficients from the eigenvalues were used to calculate the principal components for the individual samples of the whole data set. These analyses were done using the computer program SPSS version 11.5 (SPSS 2002). Scatter plots of the most important PCA were used to graphically represent the relationships among the accessions.

RESULTS

Identification of Weedy Rice Morphotypes

Thirty six WR morphotypes were identified based on plant erectness, culm height, days until maturity and panicle types. This morphotypes comprised nine different accessions from each site, namely PB1 to PB9 from the northern site, PP1 to PP9 from the central site, SGA1 to SGA9 from southern site and SGN1 to SGN9 from the south-western site. Eighteen accessions have an erect plant type similar to the three commercial varieties. These accessions include four from the northern site (PB1, PB2, PB5, PB9), five accessions from the central site (PP2, PP3, PP4, PP5, PP8), four accessions from the south-western site (SGN3, SGN4, SGN7, SGN8) and five accessions from the southern site (SGA1, SGA3, SGA4, SGA5, SGA6). Additionally, other accessions have intermediate and open plant types.

WR had variable maturity periods among themselves and in comparison to the commercial varieties. All WR accessions reached maturity 2 to 20 days earlier than the commercial varieties. SGA1, SGA7, SGN1 and SGN2 reached 85% maturity within 80 DAS, whereas PB6 and SGA9 took 99 DAS to reach 85% maturity. In comparison, commercial varieties needed more than 100 DAS to reach 85% maturity.

WR accessions from all sites produced either pubescent or intermediate glabrous leaves, whereas commercial varieties only possessed pubescent leaves. 28% of WR accessions produced intermediate leaves, for which only the middle of the leaf had a glabrous character, while both sides of the leaf and the end tips had pubescent characteristics. Flag leaf length varied among all accessions, including the commercial varieties, and ranged from 28 to 55 cm in length. PB9 produced the longest flag leaf at 55.8 cm, whereas SGA7 produced the shortest flag leaf at 28.3 cm.

The WR accessions were typically 12 to 57 cm taller than the 3 commercial varieties with the exception of SGA8 (83.5 cm), which was about the same height as MR185, and the shortest plant, SGA1 (73 cm). The panicle lengths of the commercial varieties ranged from 20.0 to 28.3 cm, whereas the panicle lengths of WR ranged between 21.9 and 36.1 cm. In general, WR produced more primary and secondary branches in the panicles compared to the commercial rice.

Seed Shattering and Seed Germination

Eleven WR accessions (28.2%) showed higher grain shattering, whereas 20 WR accessions (51.2%) showed moderate to high grain shattering. A few accessions, such as PP2, SGN4, SGN6, SGA6 and PB6, showed moderate grain shattering (Table 2). Even though these accessions were grouped together with commercial varieties that had 5%–11% detached seeds, they produced more grains that were detached from the panicle, with percentages of detached seed ranging from 17%–25%.

The seed germination traits varied between the WR accessions and the commercial varieties. WR accessions such as PB7, PP8, PP9, SGA6 and SGA8 had germination rates of 70% within 30 days that increased to more than 80% after 90 days, which are similar to commercial varieties (90% after 90 days). Eleven accessions, PB1, PB2, PP3, PP7, SGN3, SGN4, SGN7, SGN9, SGA4, SGA5 and SGA9, had a low germination rate of 30% within 30 days that only increased to less than 40% after 90 days, whereas the others had germination rates between 40%–80% (Table 2).

Table 2: Panicle shattering and germination rates of WR variants and commercial varieties.

Group	Variants	Pash* (%)	Seed germination rate (%)	
			30 days	90 days
North Pulau Pinang	PB1	9	32	34
	PB2	7	33	38
	PB3	9	43	61
	PB4	7	40	68
	PB5	7	54	71
	PB6	5	46	65
	PB7	5	70	88
	PB8	5	54	70
	PB9	5	46	64
Central Pulau Pinang	PP1	7	60	72
	PP2	5	64	72
	PP3	7	34	38
	PP4	7	58	63
	PP5	7	54	61
	PP6	9	42	51
	PP7	9	34	37
	PP8	7	84	89
	PP9	7	74	81
South West Pulau Pinang	SGN1	9	48	57
	SGN2	9	34	49
	SGN3	7	30	35
	SGN4	5	32	36
	SGN5	7	34	65
	SGN6	5	54	77
	SGN7	7	24	37
	SGN8	7	42	63
	SGN9	7	35	28
South Pulau Pinang	SGA1	7	58	71
	SGA 2	9	64	72
	SGA 3	7	52	67
	SGA 4	7	30	34
	SGA 5	9	36	39
	SGA 6	5	84	89
	SGA 7	9	74	79
	SGA 8	9	76	85
	SGA 9	5	33	37
Control variety	MR219	5	88	91
	MR185	5	90	92
	MR84	5	84	90

Notes: Panicle shattering (Pash*). Five classes, determined as the total seeds shattered from the panicle: 1 = very low (less than 1%), 3 = low (1%–5%), 5 = moderate (6%–25%), 7 = moderate high (26%–50%), 9 = high (more than 50%).

Morphometric Relations among the Weedy Rice Morphotypes

The eigenvalues of the morphological characters for each component are shown in Table 3. The PCA analyses showed that 45.88% of the variation observed among the WR accessions and commercial varieties were within the first 3 axes. The first axis accounted for 20.63% of the variation and was characterised by 1000 grain-weight (TGW), number of filled grains, number of empty grains, leaf width, plant erectness and leaf pubescence.

Table 3: The eigenvalues of the correlation matrix for 17 morphological characters of WR accessions and commercial varieties.

Variable	Principal components *(eigenvalue; proportion of explained variance)		
	1st *(3.51; 20.63%)	2nd *(2.64; 15.54%)	3rd *(1.65; 9.71%)
TGW	**0.975**	0.319	0.145
Grain width	**0.714**	0.180	0.180
Culm height	**–0.656**	**–0.770**	0.309
Grain length	**0.642**	0.409	0.087
Plant type	**0.505**	0.041	0.120
Leaf width	**0.499**	0.313	0.004
Leaf length	–0.439	0.386	0.131
Total spikelet by panicle	–0.176	**0.878**	–0.234
Filled grain by panicle	0.200	**0.673**	–0.121
No. of rachis	–0.276	**0.549**	0.135
Panicle length	–0.417	**0.513**	0.385
Apical panicle sterility	–0.446	**0.510**	–0.197
No. of tiller per hill	0.058	–0.017	**–0.673**
Leaf pubescence	–0.214	0.132	**–0.609**
Lemna and palea colour	0.135	0.047	0.351
Panicle type	0.001	–0.055	0.384
Leaf erectness	–0.486	0.137	0.307

Note: *Coefficients with absolute values greater than, or equal to 0.498, are shown in bold.

The second axis accounted for 15.54% of the variation and was characterised by morphological characters such as culm height, number of empty grains, total number of spikelets, leaf width and number of tillers per hill. Finally, the third axis, which accounted for 9.71% of the variation, was defined by grain length, culm height, number of primary branches (panicles), lemma and palea colour, leaf pubescence and number of tillers per hill. The distribution of the WR and commercial varieties according to panicle type as shown in Figure 2 indicated that all the commercial varieties were grouped together but separately from the WR accessions**.**

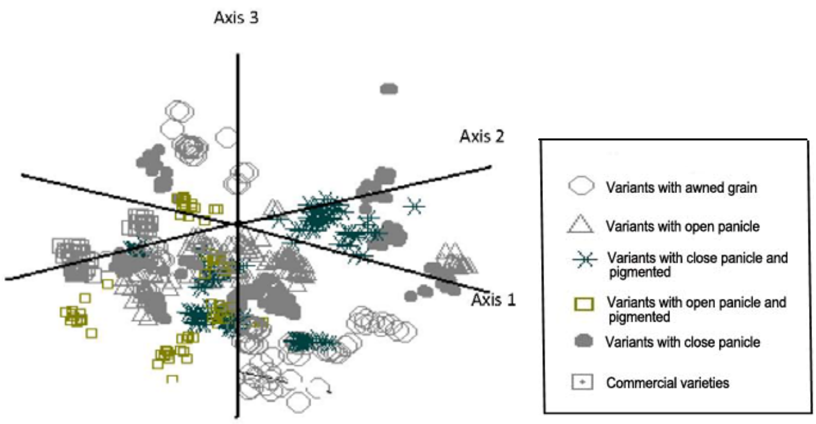

Figure 2: Distribution of WR and commercial varieties according to panicle type from PCA.

The combination of the first axis and second axis explained 36.1% of the total variation covered by all accessions' panicle type. Open panicle and pigmented grain accessions were located near axis 1, whereas closed panicle accessions, closed panicle and pigmented grain accessions were near axis 2. The distribution of these accessions in the axis was due to the type of panicle and differences in lemma and palea colour. Six WR accessions, namely PB1, PB9, SGN3, SGN5, SGN7 and SGA7, produced reddish to light purple pigmented grains. Some of the WR accessions also produced different grain pigments, such as brown furrows on straw (PP2, SGA5), purple spots on straw (PP7) and black straw (SGA6).

However, about 9.7% of the variation was explained by the third axis, and all of the commercial varieties were found near this axis. Grain length, flag leaf character, chlorophyll content and TGW were the main indicators differentiating them from the WR accessions. Leaf colour is either green, light green or dark-green in all the WR accessions, but commercial varieties only have dark-green leaves. Commercial varieties have higher chlorophyll contents compared to all WR accessions; PB3 showed highest chlorophyll content among the WR accessions. The commercial varieties generally produced long-slender grain with straw-coloured husk that was more than 9.4 mm length. However, WR accessions produced shorter grain, intermediate between medium and long grain types, and the grain also varied in colour. The grain length of WR varied between 6.8 to 9.85 mm, and its TGW ranged from 9.45 to 24.85 g .In comparison, the TGW of commercial varieties was between 24.7 and 27.2 g. SGA7 had the shortest grain (6.8 mm), and its TGW was only 9.45 g.

WR accessions with the awned grain type were scattered on axis 1 and axis 2. Accessions that have grains with awns were clearly separated from those accessions without awns. Five WR accessions, namely PB3, PB4, PP3, PP6 and SGA2, produced awn, with PB4 having the longest awn. These accessions have some characters similar to the commercial varieties, such as panicle type, leaf character and plant type that explain their proximity in the scatter plot.

The differences between WR and commercial varieties based on the areas of collection are shown in the PCA (Fig. 3). The first 3 axis components explained 47.5% of the variation including all types of panicle accessions. The first and second axis explained 25.2% of the variation and showed that accessions from the northern site and a few accessions from the southern site were scattered near axis 2, closer to the commercial varieties. In addition, axis 3 explained 20.7% of the variation and included the majority of accessions from the south-west area, the southern site and the central site. This result indicated that the separation of the accessions was influenced by the WR morphotypes without being affected by their locations.

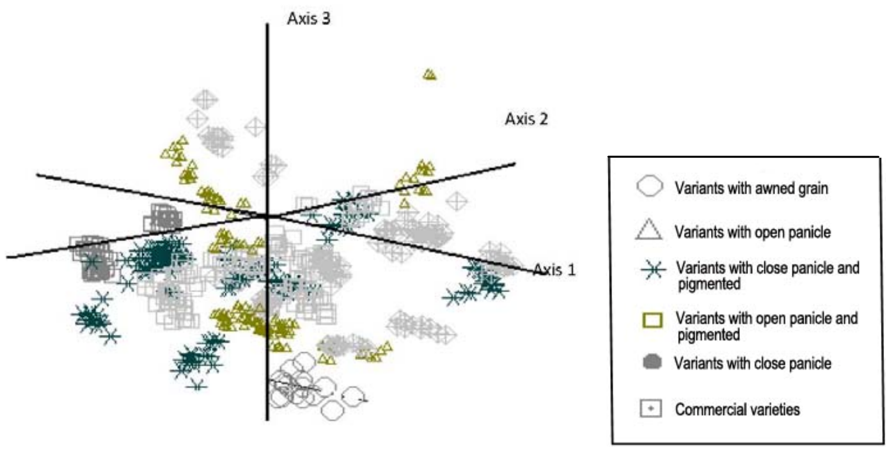

Figure 3: Distribution of WR and commercial varieties according to locations from PCA.

DISCUSSION

Thirty six WR morphotypes were identified based on plant height, awned apiculus, colour of the lemma and palea, and grain shattering by the hand grip technique. A higher number of easy shattering grains were counted with the hand grip technique for all of the WR accessions compared to the commercial varieties. Easy shattering is one of the WR characteristics (Azmi *et al.* 2004). A PCA to identify morphometric differences between WR and commercial varieties showed that all of the commercial varieties formed a group that was separated from the WR accessions. Most of the WR accessions scatter in groups according to their panicle types even though they were found in different locations.

Some of the WR accessions differ in plant types, such as the erect plant type and the open plant type. The distribution of the culm angle ranging from erect to open suggested that the erect plants' growth habit is recessive to that of a spreading or procumbent growth habit (Adair & Jodon 1973). The presence of erect plant types could be due to back-crossing between accessions or with the commercial rice cultivar (Gealy *et al.* 2006). The differences in the heights of the WR accessions were possibly due to competition for nutrients and sunlight or as a result of heterosis in some WR accessions.

Differences in the colours of leaves and stems are due to variations in the chlorophyll contents in the rice plant (Gealy 2005) and the decline of the chlorophyll content during grain filling (Yang *et al.* 2002). Another factor affecting the colour variation is the plant's senescence, which is the final stage of growth and development in monocarpic plants such as rice (Okatan *et al.* 1981; Nooden 1998). Early senescence reduced photosynthetic rates and shortened the grain-filling period, also reducing grain weight (Brown *et al.* 1991; Palta *et al.* 1994).

From our observations, WR accessions with awned grains were present in locations where wild rice (*Oryza rufipogon*) was present nearby. In the south-western site, where awned grains were absent, wild rice was not detected near the area. Out-crossing might have occurred between the wild rice and commercial rice or local rice accessions that may have been present in the site, resulting in WR accessions with awns. Crosses derived from awned rice parents produced F_1 plants with awns, indicating that awn production is a dominant trait (Adair & Jodon 1973). Most WR accessions produced more primary and secondary branches, which increased the number of grains per panicle.

There are a few WR accessions that had more branches per panicle and a higher number of grains but also a high percentage of sterility due to a large number of unfilled grains. Compared to the primary branch grains, the grains on secondary branches were mostly empty, and this may affect the grains setting ability of the plant and may also result in differences in distribution patterns (Xu *et al.* 2006; Tsnoda & Takahashi 1984). Filled grain is the fertile grain that will germinate and grow in suitable conditions and environments. The result indicated that some of the WR accessions produced a lot of fertile and productive grain, causing an increase in the number of those accessions and their coverage of the rice fields. The performance of the seedlings and the early growth pattern of WR accessions indicated that they are well adapted to the environment (Wahab &

Suhaimi 1991; Vaughan *et al.* 1995). WR can be considered the most troublesome weed in the rice fields, especially in the direct-seeded rice fields of Malaysia (Watanabe *et al.* 1996; Azmi & Abdullah 2002).

CONCLUSION

There are many similar characteristics among WR accessions and commercial varieties. However, there are also several characteristics such as grain shattering, culm height and awned grain that may be used to distinguish WR accessions from commercial rice. In our study, nine WR morphotypes were identified from each location. From every observation site, there was at least one accession with awned grain except in the south-western location where awned grain was absent. Information from this study is useful to predict the potential growth and maturation period of a WR accession in Pulau Pinang granary areas. This information will help to determine a suitable time to carry out the rouging of the panicle before maturity, to apply good agricultural practices and to develop an appropriate weed management practice to control WR in the future. However, more in-depth studies on morphological and physiological characters and their genetic relationships are needed to clearly define and compare all of the WR accessions to find the best system to control WR distribution. Currently, molecular techniques are being used to determine the distinct characteristics of WR and commercial rice.

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