J. Trop. Agric. and Fd. Sc. 46(2)(2018): 127 – 136

Assessment of advanced Malaysian rice varieties for resistance to brown planthopper (*Nilaparvata lugens* Stal.)

[Penilaian kerintangan beberapa varieti padi Malaysia terhadap benah perang (*Nilaparvata lugens* Stal.)]

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Abstract

The resistance of several published resistant varieties and some advanced Malaysian rice varieties were evaluated against the brown planthopper (BPH), *Nilaparvata lugens*. The resistance of three varieties, namely Rathu Heenati, Babawee and Mudgo, controlled by major genes consistently showed strong resistance through the antibiosis, antixenosis, population growth and standard seed box screening (SSST) tests. Preliminary evaluation of MR varieties by SSST test indicated their variable levels of resistance to BPH. Results also showed that reactions of rice varieties MR 253 and MR 263 to multiple colonies of BPH tested ranged from moderately resistant to susceptible, albeit better than the universal susceptible check variety Taichung Native 1 (TN1). Their low level of resistance to BPH was assumed to be contributed by the unidentified and unexplained minor genes effect. Another released variety MRQ 76 was also found to be more susceptible to BPH than the currently popular rice variety MR 219.

Keywords: brown planthopper, rice varieties, evaluation for resistance, antixenosis, antibiosis

Introduction

The brown planthopper (BPH), *Nilaparvata lugens* Stal. (Hemiptera: Delphacide) is an economically important and wide-spread insect pest of rice (*Oryza sativa* L.). Incidence of BPH infestation in Malaysia has been drastically rising in the recent years and more hopperburn incidences had been reported. The damage caused to the rice plants could be in the form of direct and indirect damage. Direct damage is caused from the injury due to oviposition and from the excessive sucking of the rice plant sap (Catindig et al. 2009). Dehydration due to sap removal caused the leaves of heavily infested plants to turn yellow, wilt and die, a symptom known as hopperburn. Heavy infestation results in significant yield loss and deterioration of the quality of grains. Hopperburn could usually be observed on crops at the maximum tillering stage or around 80 days after sowing (DAS) which usually coincided with the emergence of the third generation of the invading population in the field (Habibuddin and Chang 1978). The indirect damage could result from the loss affected by incidences of rice ragged stunt and rice grassy stunt viruses transmitted by BPH.

Article history
Received: 15.5.2015
Accepted: 30.4.2018

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Timely application of insecticides could provide effective control against BPH. However, large scale chemical control is usually difficult and expensive. Repeated sprayings of broad spectrum insecticides could also affect the natural balance existing between the insect pest population and their natural enemies. It could also lead towards resistance development to a particular insecticide used (Kiritani 1972; Ku et al. 1976; Heinrichs et al. 1982; Cheng 1984). In addition, the indiscriminate and sub-optimal usage of certain groups of insecticides could also cause a phenomenon known as insect resurgence that instead of causing insect pest mortality, leads to the build-up of the target pest population due to various factors such as their mimic of hormone properties or destruction of natural enemies in paddy fields (Catindig et al. 2009)

Considering the undesirable effects of pesticides, a logical approach to BPH management would be to use biologically-based alternative such as the use of host plant resistance, which is also more in line with the integrated pest management programme concept. Host plant resistance has been recognised as one of the most economical and effective measures in controlling the brown planthopper population in the fields (Jairin et al. 2007). Therefore, utilisation of resistant rice varieties is a viable alternative to chemical control methods in managing BPH. MARDI has already initiated an organised resistance breeding programme towards BPH since 1977 (Habibuddin et al. 1980). At the beginning, the released and popular Malaysian rice varieties were evaluated for resistance assessment against BPH. This was later followed by screening of varieties obtained from the International Rice Research Institute (IRRI) of Philippines. Several identified resistant varieties were then utilised as donor parents in a local breeding programme.

An attempt has also been taken to identify the resistance levels of Malaysian rice germplasm collection accessions against key insect pests. These accessions included both local traditional and foreign introductions as well as all breeding lines deposited in the Rice Germplasm Collection Center located at MARDI Seberang Perai Station. In addition, the entomologists at the Rice Research Centre (RI) at MARDI have consistently participated and led the process of screening and evaluating resistance levels against BPH using the breeding lines generated by breeders from multiple breeding programmes within MARDI. This paper provides information on the general status of BPH resistance among MARDI's developed or advanced lines with special emphasis on several newly released rice varieties.

Materials and methods

Rice varieties

The seeds of the test varieties were either obtained from the MARDI's Rice Germplasm Centre or from the rice breeders, particularly of the newly generated advanced breeding lines and varieties. These breeding lines were lines that had been promoted to Adaptability Trials (MR series) and several other standard check "control" varieties including Rathu Heenati and Taichung Native 1 (TN1), which are resistant and susceptible to BPH respectively.

Insect population

Unless specifically mentioned otherwise, the BPH *Nilaparvata lugens* used in the experiments was a laboratory-bred colony raised on the susceptible rice variety TN1, a colony originally collected from rice fields surrounding the MARDI Station at Seberang Perai. For each batch of the experiment, adult females were caged on healthy 30-day-old TN1 plants for a 24 h oviposition access period, before being removed in order to obtain insect population of relatively same age. The host plants with the eggs were then kept insect-free until the nymphs emerged. The plants were watered and replaced when necessary until the emerged nymphs were ready to be used in various screening tests that included the standard seedbox screening test (SSST), antixenosis, antibiosis and population buildup. All experiments were conducted at a temperature of 22 to 33 °C and relative humidity of 75 to 85%.

Standard seedbox screening test (SSST)
Resistance levels of the test varieties were evaluated using the SSST whereby experiments were arranged in a completely randomised design with three replications. Twenty five seeds of the test varieties were sown in rows, 5 cm apart, in 60 cm x 40 cm x 10 cm seed boxes. Rice varieties Rathu Heenati and TN1 were included as resistant and susceptible checks, respectively in each of the seed box. The outer rows in each seed box were planted with TN1 as guard rows to reduce insect escape. A single row across the centre of the box was also planted with TN1 to best increase insect pressure. At 5 days after seeding, the test varieties were thinned to about 20 seedlings per row. The seedlings were then infested with eight 2nd to 3rd instars BPH nymphs per seedling, by gently tapping the rearing pots to uniformly scatter a large number of BPH on the test bed. The nymphs would be able to kill seedlings of the susceptible check varieties in about 7 – 10 days. Scoring of seedlings response to infestation was taken when about 90% of the seedlings of the susceptible variety TN1 were killed (IRRI 1996). Plant damage was rated based on the standard scoring system (0 to 9) of which score 1 is for highly resistant plant and score 9 is for highly susceptible, usually done at about 7 – 10 days after infestation (*Table 1*).

Antixenosis to nymph of BPH in the rice seedlings
Antixenosis test was done according to the method described by Heinrichs et al. (1985). Germinated seeds of the test varieties together with the susceptible TN1 were sown as in the SSST with 25 seeds for each test variety and done in four replicates. Seedlings were thinned to 20 seedlings per row and then infested with the 2nd and 3rd instar nymphs at a rate of about eight insects per seedlings. The number of nymphs on the 10 seedlings per row was counted after 48 h infestation and the insect number was then expressed as the number of nymph per seedling basis.

Oviposition preference
This experiment was conducted in a completely randomised design with four replicates. Germinated seeds of each test variety were sown separately. On the 35th day after sowing (DAS), each test variety was transferred into the four clay pots, each pot representing a replication. The dried or yellowing outer leaf sheaths were removed and their tillers were pruned into one tiller/ pot. All pots were set in a basin, enclosed with mylar film cages (45 cm height x 60 cm diameter) and then infested with 50 gravid females placed in a petri-dish at the

Table 1. Rating damage by BPH at the rice seedling stage

Damage symptom	Resistance scale	Resistance level[a]
No damage	0	I
Very slightly damaged	1	HR
1st and 2nd leaves of most plants partially yellowing	3	R
Pronounced yellowing and stunting or about 10 to 25% of the plants wilting	5	MR
More than half of the plants wilting or dead and remaining plants severely stunted or dying	7	S
All plants dead	9	HS

[a] I = immune; HR = highly resistant; R = resistant; MR = moderately resistant; S = susceptible; HS = highly susceptible

middle of the basin platform. The numbers of BPH on each plant were then counted after 24, 48 and 72 h following introduction. The leaf sheaths were dissected and the numbers of oviposited eggs on the rice stems were then counted at 72 h after insect introduction.

Nymphal survival

Antibiosis test was assessed based on the number of survived nymphs after being placed on the seedlings of the respective selected test varieties. Seedlings of each test variety were sown in six pots of 10 cm diameter at three seedlings per pot. The seedling on each pot was then caged with mylar cage (30 cm height x 9 cm diameter) at 30 DAS and then a total of 10 nymphs at the 1st instar stage were introduced through a slit or hole on the side of the mylar cage. The number of surviving nymphs on the respective plants was counted on the 20th day after the nymphs were introduced (DAI).

Population growth

Population growth experiment was conducted in a completely randomised design with three replications. Seedlings of each test variety sown in three pots represented replicates, at the rate of five seedlings per pot. The seedlings in each pot were then thinned at 5 DAS into three healthy seedlings per pot and caged with mylar film cage (20 cm diameter x 90 cm height). Plants at the age of 35 DAS were then infested with six gravid female adults (3-day-old adults) through a slit or hole on the side of the cage. Any dead hoppers were replaced one day after the first introduction. The number of developed BPH was counted at 25 DAI.

Statistical analysis

All summarising statistics were produced using Excel. SAS statistical package (SAS Institute 2000) was used for more detailed statistical analysis such as PROC ANOVA for the analysis of variance (ANOVA), meanwhile comparison of treatments means were conducted using Duncan's Multiple Range Test (DMRT). For correlation analysis, PROC GLM and PROC CORR were conducted on the antixenosis test to identify the relationship between parameters in this study.

Results and discussion

Preliminary assessment of resistance to BPH by SSST

Two hundred and forty MR accessions were evaluated for their resistance status to BPH population of the TN1 colony at the seedlings stage by using the SSST method. Based on overall distribution of the resistance-susceptibility spectrum, about 18.3% of the evaluated accessions scored less or equal to 3 which suggested that they were resistant to BPH (*Figure 1*). Among the varieties classified as resistant were MR 219, MR 251, MR 253, MR 263, MR 269 and MR 276 (*Table 2*). The other 75 accessions (31.3%) were moderately resistant which included MR 220, MR 232 and MRQ 74, while the remaining accessions were classified as susceptible (37.5%) or highly susceptible (12.9%).

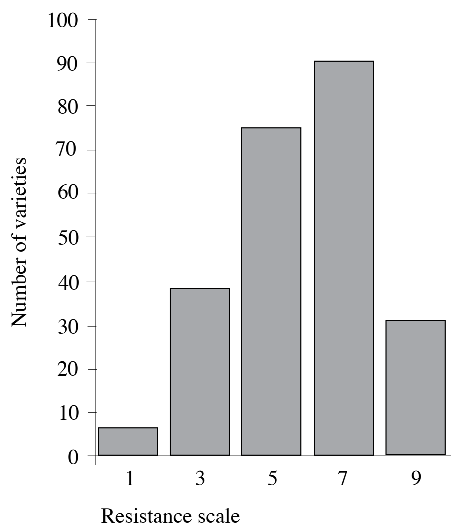

Figure 1. Distribution of BPH resistance scales in 240 MR accessions tested

Table 2. List of MR accessions for high resistance, resistance and moderate resistance to BPH

Resistance scale	Varieties
1*	MR 233, MR 237, MR 239, MR 251, MR 276 and MR 277
3	MR 77 (SEBERANG), MR 78 (Y 529), MR 90, MR 99, MR 105, MR 131, MR 159, MR 162, MR 181, MR 184, MR 211, MRQ 50, MR 219, MR 230, MR 231, MR 236, MR 240, MR 244, MR 250, MR 252, MR 253, MR 255, MR 257, MR 259, MR 260, MR 261, MR 262, MR 263, MR 264, MR 265, MR 266, MR 269, MR 270 and MR 271
5	MR 2, MR 14, MR 15, MR 16, MR 18, MR 19, MR 20, MR 21, MR 23, MR 24, MR 25, MR 26, MR 28, MR 29, MR 35, MR 54, MR 55, MR 59, MR 72 (Y 506), MR 79, MR 80, MR 81, MR 82, MR 85, MR 88, MR 98, MR 100, MR 101, MR 106, MR 111, MR 114, MR 115, MR 116, MR 118, MR 134, MR 143, MR 144, MR 148, MR 149, MR 151, MR 153, MR 161, MR 163, MR 166, MR 167, MR 168, MR 176, MR 177, MR 178, MR 179, MR 180, MR 183, MR 185, MR 186, MR 187, MR 189, MR 190, MR 191, MR 201, MR 202, MR 220, MR 232, MR 241, MR 242, MR 243, MR 245, MR 246, MR 247, MR 248, MRQ 74, MR 254, MR 256, MRQ 80, MR 268 and MR 275

*Score of 1 = highly resistant; score of 3 = resistant and score of 5 = moderately resistant

Resistance of selected MR varieties

Resistance levels of selected MR varieties namely, MR 219, MR 253, MR 263 and MRQ 76 were further assessed by using the antibiosis, antixenosis, oviposition and population growth tests. The antixenosis test measured insect preference to a particular host variety, measured as the number of nymphs landing and feeding on the test plants after they had been introduced at the seedling or matured plant stage. Results are shown in *Table 3*. This preference can result from the presence of attractant compounds released by the plants while repellent compounds would result in non-preference. Antixenosis test at the seedling stage showed that after 48 h exposure, the number of landed insects was lowest on the resistant variety Rathu Heenathi (1.4/seedling) and highest on the susceptible check rice variety TN1 (8.1). The number of nymphs observed on the seedling stems of MR 253, MR 263 and MR 219 were 5.0, 5.2 and 7.1 respectively, which indicated that varieties were moderately preferred. Since the number of nymphs on MR 253 and MR 263 were lower than that on MR 219, it was assumed that both varieties were less preferred than MR 219. In addition, the highest number of BPH nymphs among MR varieties was observed on MRQ 76 (7.2) which indicated that this variety was more preferred (*Table 3*). Correlation coefficient between resistance scale (damage grading) and population of nymphs revealed that the mean of resistance scale recorded positive correlation with nymphs population with a correlation coefficient (r = 0.803). Results on the preference test at the seedling stage was further confirmed and supported by the antixenosis and oviposition tests conducted at the vegetative stages of the rice varieties (*Table 4*). Adult BPH markedly preferred to settle on TN1 rather than on Rathu Heenathi. The number of eggs oviposited on the susceptible TN1 was 188.8 eggs per stem compared to only 16.0 eggs per stem of Rathu Heenathi, a resistant variety originating from Sri Lanka. The correlation coefficient value between

Table 3. Preference of nymphs of BPH to varieties of rice seedlings

Variety	Feeding preference[a]		
	Nymphs (no./stem)	Seedling reaction[b]	Resistance scale
TN1 (susceptible check)	8.1a[c]	HS[d]	9.0a
MRQ 76	7.2ab	HS	9.0a
MR 219	7.1ab	MS	5.7b
IR 26	6.9ab	MR	4.3d
IR 36	6.7b	MR	4.3d
MR 263	5.2c	MR	5.0c
MR 253	5.0c	MR	5.0c
Babawee	2.2d	R	3.0e
Mudgo	2.1d	R	3.0e
Rathu Heenati (resistant check)	1.4d	HR	1.0f

[a]48 h exposure; [b]Rating damage following the standard evaluation system; [c]In a column, any two means followed by the same letter are not significantly different at the 5% level; [d]HS = highly susceptible; MR = moderately resistant; MS = moderately susceptible; R = resistant; HR = highly resistant

Table 4. Settling and preference of adult BPH on resistant and susceptible varieties of rice

Variety	Feeding and ovipositional preference[a]		
	Adults (no./stem)	Eggs (no./stem)	Seedling reaction
TN1	3.3a[b]	188.8 a	HS[c]
MRQ 76	2.0b	117.0b	HS
MR 219	1.9b	83.0bc	MR
IR 26	1.7bc	71.5c	MR
IR 36	1.0cd	57.0cd	MR
MR 263	0.8d	31.3de	MR
MR 253	0.7d	30.5de	MR
Mudgo	0.7d	28.3de	R
Babawee	0.5d	26.8de	R
Rathu Heenati	0.4d	16.0e	R

[a]24, 48 and 72 h exposure; [b]In a column any two means followed by the same letter are not significantly different at the 5% level; [c]HS = highly susceptible; MR = moderately resistant; R = resistant

resistance scale with adult population was $r = 0.711$ and with eggs was $r = 0.801$. Meanwhile, the correlation coefficient value between adult population with number of eggs was $r = 0.856$. The highly significant correlation coefficient seems to suggest that non-preference plays an important role in the resistance of the varieties to the brown planthopper in this experiment. However, there was some evidence that contradicted with this finding. Choi et al. (1976) conducted a laboratory experiment to evaluate the nature of resistance of Milyang 21 and Milyang 23 to BPH and found that the insect nymphs preferred to feed on the susceptible varieties Milyang 21, Milyang 23 and Taichung Native 1 rather than on the resistant Mudgo, but preferred to oviposit their eggs on Mudgo rather than on the susceptible varieties. Based on results of the preference and egg oviposition of adults on plants at the vegetative stage, it revealed that MRQ 76 was susceptible to BPH and the level of its susceptibility was severe than the varieties MR 219 or MR 253 and MR 263.

The moderate resistance of MR 253 and MR 263 was further confirmed by the antibiosis and population growth experiments. The antibiosis test was measured by the number of nymphs which survived on the introduced host plants that finally emerged as adults in the population growth test. The number of survived nymphs and emerged adults were moderate on both MR 253 and MR 263 (*Table 5*). The number of F_1 nymphs emerging after 25 days after the adult female had been introduced was higher on TN1 (285) followed by MRQ 76 (216), resulting in the emergence of more adults on these varieties, which was opposite to those introduced on Rathu Heenathi. Results indicated that MRQ 76 was susceptible to TN1 colony of BPH (*Figure 2*).

Table 5. Rate of adult emergence of BPH when 1st instar nymphs were infested on rice plants

Variety	Survival rate (%)	Sex ratio[a]
IR 26	46.7 (*n* = 60)	0.36
IR 36	51.7 (*n* = 60)	0.45
Mudgo	53.3 (*n* = 60)	0.53
Babawee	56.3 (*n* = 64)	0.44
Rathu Heenati	61.9 (*n* = 63)	0.46
MR 219	68.9 (*n* = 61)	0.36
MR 253	71.7 (*n* = 60)	0.56
MR 263	73.3 (*n* = 60)	0.43
MRQ 76	87.1 (*n* = 62)	0.63
TN1	96.7 (*n* = 61)	0.49

[a]Female/(female+male)

Resistance of MR 253 and MR 263 to field colonies

Performance of MR 253, MR 263 and MRQ 76 were also evaluated in the SSST test by using the recently collected BPH colonies from Tumpat, Seberang Perak, Tanjung Karang, Sabak Bernam and Alor Star. Results showed that there were some degree of variations between the results obtained from this test and results generated by using the TN1 colony of BPH (*Table 6*). It was shown that aside from the colony of BPH collected from Tumpat, the rice varieties MR 253 and MR 263 were more susceptible to these colonies compared to MR 219. This result had great implication, particularly on the protocol of the testing and screening of breeding lines in the process of identifying candidate resistant breeding lines. While using multiple colonies is not practical during the process of conducting a routine large scale screening of breeding lines, the results demonstrated the need of using multiple colonies of BPH recently collected from different regions for conducting resistance evaluation for BPH resistance

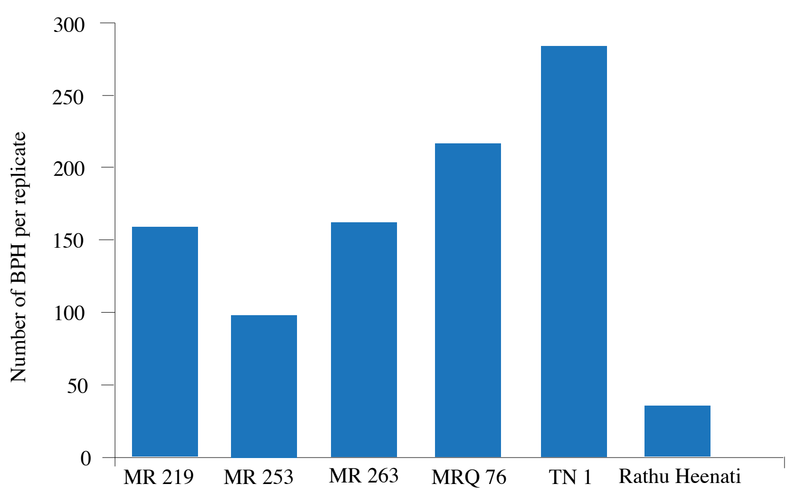

Figure 2. Number of F1 generation emergence from the initial source of infestation

Table 6. Reaction of tested MR varieties on different field populations

Variety	Tumpat	Seb. Perak	Tg. Karang	Sabak Bernam	Alor Star
MRQ 76	MS	HS	S	HS	HS
MR 219	MR	MS	MR	MR	S
MR 253	R	S	S	MR	S
MR 263	MR	HS	S	HS	HS
TN1	HS	HS	HS	HS	HS
Rathu Heenati	HR	HR	HR	HR	HR

HR = highly resistant; R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible; HS = highly susceptible

among advance breeding prior to their commercial release.

Conclusion

Past experience with BPH in Malaysia showed that *N. lugens* is a key pest in rice cultivation. In recent years, potential resistant varieties have received greater attention due to growing awareness on the shortcomings of pesticides. Rathu Heenathi with *bph3* gene is one of the rice varieties that consistently showed resistance to multiple biotypes of BPH from different rice growing regions and countries that needs to be utilised (Jairin et al. 2010). Results from the present study indicated that two of the released varieties, MR 253 and MR 263, were only moderately resistant and susceptible respectively, against BPH populations while MRQ 76 was susceptible. These results were as expected since none of their immediate parents were known to have strong resistance to BPH (*Figures 3* and *4*). PTB 33 which is highly resistant to BPH was used as the donor parent in a backcrossing programme to produce MR 253 (*Figure 3*), but it is suspected that the lines selected and promoted for further generation advancement were not BPH resistant lines. Moderate resistance of these varieties to BPH could probably be contributed by minor, unidentified and unexplained gene mechanisms. The authors opine that if the targeted breeding objective is to generate high yielding varieties possessing strong resistance to BPH, then the appropriate

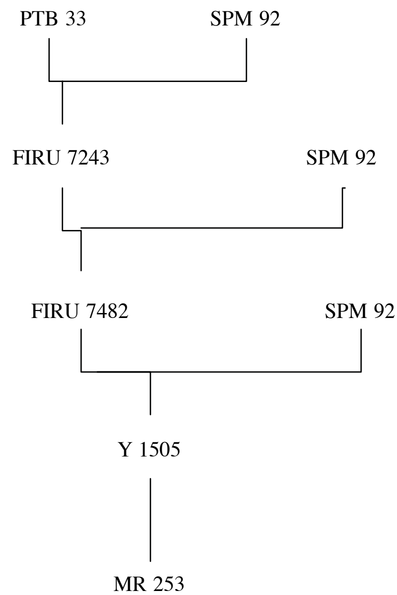

Figure 3. Parentage of MR 253

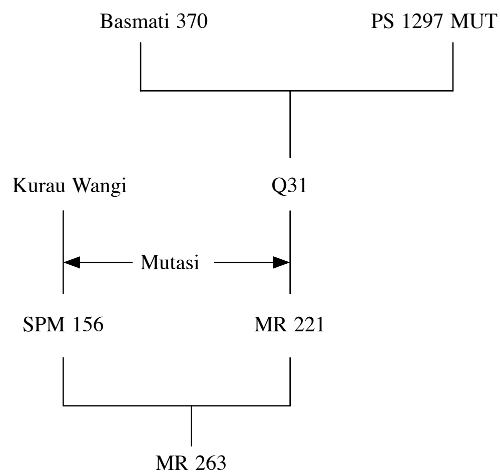

Figure 4. Parentage of MR 263

breeding strategies to be applied should be by an organised backcrossing breeding strategy with greater participation from entomologists, and probably with the greater application of molecular markers as an additional tool in the process. Otherwise, other breeding strategies will only generate moderately resistant varieties contributed by unexplained minor genes at best, and that was co-incidentally identified but not by properly planned breeding strategies, as shown through the case of MR 263, MR 219 and a few other released varieties.

Acknowledgement
The authors wish to thank Mrs Zamilah Abdullah, Mr Abd. Razak Hashim, Mr Abd. Rahim Majid, Mr Zakaria Abdullah and Mr Ahmad Sabri Che Man for helping in the experiments, and MARDI rice breeders for providing some MR varieties. This study was supported by Development Grants from Malaysian Agricultural Research and Development Institute (2100300140001).

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Abstrak
Kerintangan beberapa jenis varieti rintang yang telah dikenal pasti dan beberapa varieti padi Malaysia dinilai terhadap benah perang, *Nilaparvata lugens*. Tiga varieti rintang iaitu Rathu Heenati, Babawee dan Mudgo yang mana ciri-ciri kerintangannya dikawal oleh gen-gen yang utama secara konsisten menunjukkan tahap kerintangan yang kuat melalui ujian antibiosis, antixenosis, pertumbuhan populasi dan kaedah ujian kotak saringan (SSST). Penilaian awal varieti MR yang diuji melalui ujian SSST menunjukkan tahap pemboleh ubah kerintangan varieti-varieti ini terhadap benah perang. Keputusan yang diperoleh menunjukkan bahawa tindak balas varieti padi MR 253 dan MR 263 terhadap koloni dari populasi berbeza berada dalam lingkungan sederhana rintang kepada rentan. Walau bagaimanapun, kedua-dua varieti ini lebih baik jika dibandingkan dengan varieti rentan TN1. Tahap kerintangan yang rendah terhadap benah perang diandaikan disumbangkan oleh gen yang tidak diketahui dan dari gen yang minor dan tidak dapat dijelaskan. Varieti lain yang telah diisytiharkan iaitu MRQ 76 juga telah dikenal pasti sebagai lebih rentan terhadap benah perang berbanding dengan varieti popular MR 219.