

Y 1286 is a Balimau Putih-derived rice line resistant to rice tungro bacilliform and spherical viruses

(Y 1286 adalah titisan terbitan Balimau Putih yang rintang terhadap virus tungro basilifom dan sfera padi)

H. Habibuddin*, K. Hadzim**, O. Othman** and S. Azlan**

Key words: rice, resistance, tungro, RTBV, RTSV

Abstrak

Y 1286 ialah titisan padi yang rintang terhadap jangkitan tungro (PMV). Y 1286 terhasil daripada kacukan antara varieti keluaran tempatan MR 123 dengan Balimau Putih dari Indonesia. Balimau Putih ialah varieti yang rintang terhadap kedua-dua virus PMV (RTBV dan RTSV), tetapi peka terhadap fotokala, mudah rebah dan berhasil rendah, manakala MR 123 pula berhasil tinggi tetapi rentan terhadap jangkitan PMV. Y 1286 ialah hasil baka kacukan yang menunjukkan kerintangan terhadap multiplikasi RTBV. Ia turut rintang terhadap jangkitan RTSV. Y 1286 tidak menunjukkan pengurangan hasil yang ketara apabila diinokulat dengan virus PMV. Ia tidak peka terhadap fotokala, pokoknya rendah dan tidak mudah rebah. Hasilnya lebih rendah tetapi tidak ketara berbeza daripada MR 84. Y 1286 disyorkan untuk digunakan sebagai sumber kerintangan genetik dalam program biakbakaan untuk menghasilkan varieti padi berhasil tinggi yang rintang terhadap PMV. Benihnya disimpan dalam koleksi germplasma padi MARDI sebagai aksesori nombor 9344.

Abstract

Y 1286 is an improved tungro resistant rice line developed from a cross between a locally bred variety, MR 123 and an Indonesian variety, Balimau Putih. Balimau Putih is resistant to both tungro viruses (RTBV and RTSV), but is photoperiod sensitive, lodging prone and low yielding variety, while MR 123 is a high yielder but susceptible to tungro infection. The developed Y 1286 is an improved line, which showed resistance to RTBV multiplication. It is also resistant to RTSV infection. Its potential yield is not greatly affected after being inoculated with tungro viruses. Y 1286 line is non-photoperiod sensitive, shorter in height and resistant to lodging. Its yield is slightly lower but not significantly different from that of MR 84. Y 1286 is thus recommended to be used as a genetic resistance source in the breeding programme for the development of high yielding tungro resistant rice varieties. The seeds are deposited in the MARDI rice germplasm collection centre as accession number 9344.

*Strategic, Environment and Natural Resources Research Centre, MARDI Headquarters, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia

**MARDI Research Station, Seberang Perai, Locked Bag 203, 13200 Kepala Batas, Pulau Pinang, Malaysia

Authors' full names: Habibuddin Hashim, Hadzim Khalid, Othman Omar and Azlan Shaari

©Malaysian Agricultural Research and Development Institute 2000

Introduction

Rice tungro virus disease or locally known as Penyakit Merah Virus (PMV) is one of the most important viral diseases affecting rice production in several rice growing Asian countries (Hibino 1989). Hassanuddin et al. (1997) reported that between 1968 and 1994, the total tungro affected area in Indonesia was about 199 000 ha. In Malaysia, the estimated yield loss due to the disease in 1982 was about RM22 million (US\$8.8 million) (Department of Agriculture 1983).

Tungro disease is caused either by a single or mixed infection with the rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (Hibino et al. 1978). The yield of susceptible rice plant infected with RTSV-alone is not significantly affected, but plants with RTBV-alone or mixture with RTSV showed significant yield reduction (Hassanuddin and Hibino 1989). They reported that rice varieties TN1 and FK-135 infected by RTBV-alone, or mixed with RTSV suffered yield reduction of greater than 85%.

Tungro disease is transmitted in a semi-persistent manner by 6 leafhopper species, of which the rice green leafhopper (GLH) *Nephotettix virescens* (Dist.) (Hemiptera: cicadellidae) is the predominant vector (Ling 1972). GLH is capable of acquiring RTBV and RTSV on doubly infected plant in a relatively short access feeding period and able to transmit the viruses individually or as mixture of both immediately after feeding, and retained their infectivity for 3–4 days (Hibino et al. 1979; Cabauatan and Hibino 1985). While RTSV could be transmitted independently from plants infected with RTSV-alone or mixture with RTBV, the transmission of RTBV is RTSV-dependent. The successfulness of RTBV transmission required a preacquisition of a helper factor, probably a protein component produced only in the RTSV infected plant that might help in the absorption of RTBV particles to the wall of leafhopper stylet prior to the transmission

(Hibino et al. 1978; Cabauatan and Hibino 1985; Hibino 1989).

Generally, as in other semi-persistent viruses, the disease occurs in patches and spread rapidly through the fields. The percentage presence of viruliferous GLH population may indicate the extent of virus incidence in the field (Hibino 1989). However, application of insecticides against vector population is not very effective or successful in reducing tungro incidence in the field (Habibuddin and Saad 1986). This is probably due to one or combination of many factors such as the semi-persistent manner of the transmission, persistency of used insecticides, weather and others. The presence of staggered planting and relatively small farm size belonging to many farmers may hinder a simultaneous and blanket application of insecticide. As such, the use of resistant varieties is believed to be the most economical and appropriate approach of management strategy for virus diseases. This makes it an economically and environmentally acceptable tool in the formulation of an integrated disease management.

Resistance to tungro infection could be attained either through resistance to its vector population, types of tungro viruses or combination of them (Hibino 1989). Vector resistance has its limitation due to variation in the vector population, and its ability to develop new biotypes capable of adapting on resistant host varieties and thus causing resistance breakdown (Takita and Habibuddin 1986). On the contrary, resistance to the viruses is believed to be more durable (Koganezawa and Cabunagan 1997). Resistance to RTSV can suppress the potential of an epidemic while resistance to RTBV is capable of minimizing yield reduction. Hence, resistance of rice crop to both RTBV and RTSV will help to stabilize and sustain rice production.

There are many sources of RTSV resistance genes available in the world germplasm (Hibino et al. 1990). A few improved lines have also been developed in

Malaysia (Habibuddin et al. 1991; Othman et al. 1994). However, only a handful of RTBV resistant varieties was reportedly available among the rice germplasm evaluated so far at the International Rice Research Institute (IRRI) in the Philippines (Hibino et al. 1990; Koganezawa and Cabunagan 1997). These include Utri Merah, Utri Rajapan and Balimau Putih which also showed resistance to RTSV isolates in Malaysia (Habibuddin et al. 1994). Resistance to RTBV in those three varieties is classified as resistance to virus multiplication or tolerance but not of immunity type (Hassanuddin and Hibino 1989; Habibuddin et al. 1995). All are controlled by oligogenes (Habibuddin 1994). However, all the three varieties were foreign and of traditional characteristics. They are not suitable to be commercially grown in the Malaysian rice field due to their poor agronomic characteristics. Balimau Putih for example is tall, lodging-prone, low yielder and photoperiod sensitive. We have attempted to transfer RTBV resistance from these varieties into our modern domesticated varieties, either to be used directly or as candidate for genetic stock lines, which other breeders can use. We report here the development of a rice line Y 1286, derived from a cross between MR 123 and Balimau Putih, which is resistant to both RTBV and RTSV.

Materials and methods

Tungro source, green leafhopper and tungro evaluation

Tungro inoculum source originated and maintained in an insect proof glasshouse at MARDI Seberang Perai Station. The infectivity of the inoculum was maintained by serially transferring the viruses onto new host plants at various intervals or whenever necessary. This was done by inoculating one month-old rice variety Taichung Native One (TN1) plants by using viruliferous GLH. Infection of the inoculated plants with tungro viruses was confirmed by the double antibody sandwich enzyme-linked

immunosorbent assay (DAS-ELISA). Only plants doubly infected with both RTBV and RTSV were maintained and used as the inoculum source plants.

The GLH used for the transmission was originally collected from padi fields in Bumbong Lima, Pulau Pinang and reared on rice variety TN1. The GLH populations used in each experiment were about the same age and obtained by giving an oviposition access period of about 24 hours on the host plants. The vectors used were the newly emerged adults of about 2–4 days old. Viruliferous GLH were obtained after an acquisition access feeding on RTBV and RTSV mixed infected TN1 plants. Unless specified otherwise, the acquisition access feeding period was given for two days. Inoculation was done by introducing three viruliferous GLH per test seedling. Assessment of infection was done visually based on symptoms expression such as stunting of the newly emerged top leaves or leaf discoloration, or by using DAS-ELISA at 3 weeks after inoculation (WAI).

DAS-ELISA was done according to the methods of Clark and Adams (1977). Immunoglobulin-G (IgG) was prepared from separate antisera to RTBV and RTSV (Omura et al. 1983). About 0.1 mg of the rice leaf blade from the tip of the second top most leaf was homogenized in 1.0 mL of 0.01 M phosphate buffer (PB) pH 7.4, using a combined leaf and bud press machine (Erich Polahne, FRG) to give a 10x fold dilution of sap sample. The detail steps was described previously (Habibuddin et al. 1991). Absorbance value of samples was measured colorimetrically by an ELISA Reader (EASY READER 400, SLT Labinstrument, Austria) at wavelength of 405 nm. Histograms of the absorbance values were constructed to help in the determination of negative-positive thresholds. Samples with an absorbance value of more than twice the value of the average absorbance of healthy sap are considered as positive (Hibino et al. 1990; Dahal et al. 1992). The threshold was

arbitrary chosen since the value was considered to produce the least number of false-negative samples (Sutula et al. 1986). This will help in ensuring the identification of true negative, uninfected or resistant individuals or lines.

Crossing and selection procedure

A crossing programme was initiated in 1991 involving an RTBV resistant variety Balimau Putih and a susceptible variety MR 123. The cross was coded as RU 5133. The F_1 plant was backcrossed to MR 123 (RU 5324) (Table 1). The B_1F_1 plants were

planted and their seeds were bulked as B_1F_2 seeds.

The B_1F_2 seeds were sown in the cement troughs in the greenhouse. Seedlings at 2 WAS were then mass inoculated with viruliferous GLH for a 3-day inoculation access feeding, at the rate of ca. three GLH per seedling. The GLH were regularly disturbed at various intervals to enhance their movement. Seedlings were then broadcasted with insecticide carbofuran to kill the introduced vector and their offspring, and the plants were allowed to flower and mature. Panicles from the

Table 1. Crossing and selection of Y 1286

Season	Generation
9100	MR 123/ Balimau Putih, coded as RU 5133
9192	RU 5133/ MR 123, coded as RU 5324
9200	B_1F_1 generation: production of B_1F_2 seeds
9293	B_1F_2 generation: bulk, inoculated against tungro and selection based on symptoms
9300	B_1F_3 generation: rebulk, inoculated and selection based on symptoms
9394	B_1F_4 generation: planted in the field as 250 pedigree lines selection based on agro-morphological characteristics
9400	B_1F_5 generation: planted in the field as 200 pedigree lines selection based on agro-morphological characteristics
9495	B_1F_6 generation: planted in the field as 13 progeny rows (7013-7027 PER) 2 plants per line were selected based on agro-morphological characteristics
9500	B_1F_7 generation: 26 PER (7144-7172) were evaluated in the greenhouse inoculated against tungro viruses, selection based on symptoms and ELISA a line -7166 was selected
9596	B_1F_8 generation: Line -7166 was evaluated in the greenhouse Further evaluation on homogeneity, harvested seeds were bulked
9600	B_1F_9 generation: Line -7166, virus concentration test Harvested seeds were bulked
9697	B_1F_{10} generation: Line -7166, Yield loss test under glasshouse experiments seeds were bulked
9700	B_1F_{11} generation: Line -7166, Preliminary agronomic trial seeds were harvested and bulked and coded as Y 1286

comparatively healthier plants were selected, harvested and their seeds were rebulked. These B₁F₃ seeds were further sown, inoculated and selected in a similar manner. Being an off-season crop, it also allowed us to select for non-photoperiodic sensitive individuals. A total of 250 plants were selected and their panicles were harvested.

The 250 panicles were sown and planted in the field as pedigree lines of B₁F₄ generation. Selection was made based on their morphological characteristics. A total of 40 lines were selected and five plants per line were chosen and harvested. Their panicles provided 200 pedigree lines of B₁F₅ generation, which were again planted in the field for observation. Thirteen plants from a single, better-performed line were selected.

Panicles from the 13 plants were planted in the field as 13 progeny ear rows (7013 to 7027) of B₁F₆ generation. Two plants per line were selected based on their agro-characteristics, which generated 26 ear rows (7144 to 7172) for tungro evaluation in the greenhouse. Selection was based on both the symptoms and ELISA results. A line, 7166 was selected. Line 7166 was further evaluated against tungro infection by ELISA at B₁F₈ generation for their homogeneity. The seeds were harvested and bulked. Further tests were conducted at later generations and the line was coded as Y 1286.

Incidence of tungro infection and virus concentration

Twenty-four plants from each line or variety of Y 1286, Balimau Putih, Utri Merah, MR 123, TN1 and MR 84 were inoculated individually with tungro viruses at 14 DAS by introducing three viruliferous GLH per plant for 2 days inoculation access feeding. Tungro infection was assessed at 3 WAI. Leaves from each test plant were homogenized at 10x-fold sap dilution. In addition, six plants per variety were randomly selected for the detection of their virus concentration in a dilution end point test. Their sap were serially diluted to give

the 20, 40, 80, 160, 320, 640, 1280 and 2560x fold sap dilutions which were used as samples in the ELISA.

Yield loss assessment

A greenhouse experiment was conducted to assess the potential yield loss of Y 1286 and other varieties tested following inoculation with tungro viruses. The experiment was carried out in a split plot design with varieties (Y 1286, MR 123, Balimau Putih, MR 84 and TN1) as the main factor and virus inoculation as the subfactor (inoculated and uninoculated). The experiment was done in 6 blocks.

Ten-day old seedlings were planted singly per point at a 25 cm spacing between seedlings. Fertilizer was given at the rate of 80 kg nitrogen (N), 40 kg phosphorus (P₂O₅) and 30 kg potassium (K₂O) per hectare. Half of N and all P and K were given as basal application. Second and third applications of N were given at 3 and 7 weeks after transplanting (WAT) at 1/4 rate, respectively. Hand weeding was done to eradicate weeds. Insecticide spraying was applied against insects when necessary.

Inoculation of tungro viruses on the inoculated subplot was done at 2 WAT. Each plant was caged within a plastic cylinder and inoculated with 3 viruliferous GLH for 24-h inoculation access feeding, followed by an application of insecticide carbofuran to kill the GLH. Plants were kept in the greenhouse until harvest. Data such as plant height, tiller number, panicle numbers and grain weight per hill were taken on individual plants. Grain weight was adjusted to 14% relative humidity.

Evaluation for yield and other agronomic characters

Rice varieties Y 1286, MR 123, Balimau Putih, MR 84 and TN1 were planted in the field during the off-season of 1997 in a randomized complete block design with four replications. The plot size was 3 m x 5 m. Single seedling, at 25 DAS was transplanted per hill at a 25-cm spacing. Fertilizer was

given at the rate of 80 kg N, 40 kg P₂O₅ and 30 kg K₂O/ha. Half of N and all P and K were given as basal. Second and third applications of N were given at the rate of 20 kg N at 3 and 7 WAT, respectively. Weedicide 2,4-D Amine was used for weed control. Insecticide butyl-phenyl-N-methyl-carbamate (BPMC) at the rate of 0.1% a.i. was applied to control leaf feeding and grain-sucking insects. Yield was harvested from an area of 2 m x 4 m, manually threshed and weighted. The weight was adjusted to 14% moisture content and converted to kg/ha.

Results

Development and selection of Y 1286

Steps beginning from the crossing stages to the identification and selection of a fixed line, resistant to both RTBV and RTSV are presented in *Table 1*. The selected line was designated as Y 1286.

Resistance to tungro infection and virus concentration

Symptoms appearance were not detected on seedlings of Y 1286 that were inoculated with mixtures of RTBV and RTSV, indicating the possibilities of their resistance or tolerance to tungro infection (*Table 2*). Similarly infection was not detected on its parent Balimau Putih and another RTBV tolerant check variety Utri Merah. However, a higher percentage of tungro infected plants were found on the current popular variety

MR 84, another susceptible parent MR 123 and the susceptible check TN1.

The mean absorbance value for RTBV of Y 1286 was low (0.49). This value however exceeded the negative-positive threshold (0.16), which was arbitrarily set at 2x mean of healthy plant sap, and hence the sap of Y 1286 at 10x fold saps dilution was considered positively infected with RTBV. Serial dilution test on the sap of this line showed that positive detection of RTBV could only be detected up to its 40x sap dilution (*Table 2*). The absorbance values of Balimau Putih and Utri Merah were 0.57 and 0.49, respectively. These were followed by MR 84 and MR 123 in an ascending order. The absorbance value was highest on TN1 (1.83). The absorbance values of TN1 remained above the threshold limit and positive detection for the presence of RTBV could still be obtained at its 640x sap dilution. The results suggest that the multiplication of RTBV in Y 1286, Balimau Putih and Utri Merah were low, which indicate their resistance to RTBV multiplication. On the other hand, the highest absorbance value of TN1 and its positive detection at a higher sap dilution indicates its high susceptibility to rapid multiplication of RTBV.

The absorbance value for RTSV in the leaf sap of Y 1286 diluted at 10x fold was 0.16, which was about the negative-positive threshold and hence the sap could be classified as positive for the presence of

Table 2. Tungro incidence and concentration of viruses

Line	Symptom (%)	Mean ELISA absorbance at A405 nm			
		RTBV	Dilution end point	RTSV	Dilution end point
Y 1286	0	0.49 ⁺	40x	0.16 ⁺	10x
Balimau Putih	0	0.57	80x	0.34	40x
Utri Merah	0	0.49	40x	0.1	–
MR 123	87	1.33	320x	0.82	160x
MR 84	85	0.97	160x	0.51	80x
TN1	100	1.83	640x	1.28	320x

⁺Measured at 10x fold sap dilution, mean ELISA value of 24 samples. Means above two times means of healthy plant sap (0.16) were considered positive

RTSV in the sap. However, this value was lower than that of its donor parent Balimau Putih (0.34). While the absorbance of Y 1286 might only be positive at 10x sap dilution, the absorbance of Balimau Putih could still be positively detected at its 40x sap dilution. The absorbance value of Utri Merah was low, below the threshold and can be classified as negative and uninfected. These results indicate the low level of RTSV multiplication in the inoculated seedlings of Y 1286 and Balimau Putih and of complete immunity in Utri Merah. On the other hand, the absorbance values were high on MR 123 and TN1 (1.28). The presence of RTSV in TN1 sap could still be detected as positive at 320x sap dilution, indicating its high susceptibility to RTSV infection and multiplication (*Table 2*).

Yield loss assessment

Generally, plants inoculated with tungro viruses (mixed RTBV and RTSV) were shorter than those of the uninoculated. However, reduction in height was more severe on TN1 (45.1%), followed by MR 123 and MR 84 in descending order, respectively (*Table 3*). Height reduction was smaller in both Y 1286 (7.5%) and Balimau Putih (3.2%), indicating that their heights were least affected by infection and the possibility of their tolerance to infection.

Similarly, reduction in the panicle numbers per hill varied between varieties.

Some tillers in several affected varieties also produced panicles with incomplete emergence. Panicle exertion was highly reduced in TN1 (51.9%) and MR 84 (32.8%), but reduction was less in Y 1286 and Balimau Putih (about 10%). However, inoculated MR 123 showed an increase in the total number of tillers and panicles as compared to the uninoculated plants.

Most of the spikelets produced on TN1 were empty and half-filled, resulting in grain yield reduction of about 94.6%. The yield obtained from the inoculated MR 123 and MR 84 was reduced to 75.7% and 78.5%, respectively. On the contrary, Y 1286 showed a lower yield reduction of about 19.0%, which was slightly better than that of Balimau Putih (28.4%). These results indicate the tolerance nature of Y 1286 and Balimau Putih and the high susceptibility of TN1 to tungro infection when they were inoculated at seedling stage.

Yield and other agronomic characteristics

The height of Y 1286 was 88.5 cm, almost comparable to that of MR 123 and MR 84 (*Table 4*). Its height was much improved towards the desired height as compared to that of the tall Balimau Putih (156 cm). Similarly, the tiller and panicle numbers of Y 1286 were comparable to that of the other released varieties (about 17). The low number of panicles on Balimau Putih (3.5/hill) was probably due to its

Table 3. Effects of tungro infection on the growth and yield of Y 1286 and other rice varieties, measured as percentage reduction of the inoculated against the uninoculated pairs

Lines	Percentage reduction			
	Height	Tiller	Panicle	Yield
Y 1286	7.5 ⁺⁺	12.9 ^{ns}	9.2 ^{ns}	19.0 ⁺
Balimau Putih	3.2 ⁺	18.9 ⁺	10.2 ^{ns}	28.4 ⁺
MR 123	33.3 ⁺⁺	(17.9) ⁺⁺	(4.2) ^{ns}	75.7 ⁺⁺
MR 84	30.9 ⁺⁺	29.9 ⁺⁺	32.8 ⁺⁺	78.5 ⁺⁺
TN1	45.1 ⁺⁺	8.1 ^{ns}	51.9 ⁺⁺	94.6 ⁺⁺

() values in parentheses indicate an increment

⁺⁺, ⁺, ^{ns} significantly different between the inoculated and uninoculated plants by LSD at 5% level and 1%; and not significant, respectively

Table 4. Agronomic characteristics of rice line Y1286 and other varieties evaluated in the off-season 1997

Lines	Culm height (cm)	Tiller (number)	Panicle (number)	Maturation (days)	Yield (kg/ha)
Y 1286	88.5c	17.6a	16.2a	135a	3586b
Balimau Putih	156a	9.4b	3.5c	photoperiod	300d
MR 123	94.6b	17.5a	15.8a	135a	4390a
MR 84	98.3b	16.8a	15.4ab	137a	3948ab
TN1	83.7d	16.7a	13.9a	110b	2001c

All means within a column followed by a common letter are not significantly different at the 5% probability level by DMRT

photoperiod sensitive nature during the off-season when the experiment was conducted.

The obtained yield between varieties was varied and the difference was highly significant. The lowest yield was recorded on Balimau Putih. Its low yield might be correlated with the low number of panicles produced on it, which again might be associated with its photoperiod sensitivity during the experiment. The second lowest yield was recorded by another foreign japonica variety, TN1. The yield differences between the other three locally produced varieties were significant, but their differences were not very wide. The obtained yield of Y 1286 was 3 586 kg/ha, slightly lower than that of MR 84, albeit the difference between the two was not significant. Its yield was significantly lower than that of its susceptible parent, MR 123. However, the yield of Y 1286 was significantly higher than that of its resistant parent, Balimau Putih.

Discussion

The use of resistant varieties is a component in the management of rice tungro virus disease in Malaysia. Resistance to RTBV would enable the prevention of potential crop loss in the event of possible tungro epidemic. Currently there is no improved rice variety that is resistant to RTBV infection (Koganezawa and Cabunagan 1997), although Hibino et al. (1990) demonstrated the presence of some potential donor parents among the germplasm collections evaluated at IRRI. However, all

the identified resistant donors are foreign traditional varieties, which are agronomically unacceptable under Malaysian conditions. Balimau Putih for example is a photoperiod sensitive variety that cannot be commercially grown in a double cropping system in Malaysia. In addition, it is also lodging prone and a low yielder. In view of the above, a breeding programme was undertaken with an objective to incorporate tungro resistance gene(s) into the local varieties, and as a result we have successfully developed an improved RTBV resistant line designated as Y 1286.

The rate of RTBV multiplication in the inoculated Y 1286 was lower than that of its susceptible parent MR 123, or to that of MR 84, the popularly grown rice variety in Malaysia at present, which implies the successfulness in transferring RTBV resistant gene(s) from Balimau Putih into Y 1286. The absorbance value of the ELISA reader of Y 1286 at 10x sap dilution (0.49) was comparable to that of its resistant parent Balimau Putih (0.57). These values were about 27% of the absorbance value of the most susceptible variety TN1 (1.83). The low concentration of RTBV in Y 1286 was further confirmed by results of serial dilution test. The absorbance of Y 1286 at 10x sap dilution is almost comparable to the absorbance of TN1 when the sap of the latter was diluted 320 times before ELISA. In addition, while RTBV could still be positively detected at 640x sap dilution of TN1, positive detection of Y 1286 and

Balimau Putih were only up to 40x and 80x, respectively. Shahjahan et al. (1990) who investigated on another tolerant variety namely Utri Merah also observed that RTBV concentration in the variety is about one-tenth of that of TN1 at 10x dilution, but its actual concentration is about one-thirtieth of TN1 by serial dilution test.

However, resistance to RTBV in Y 1286 is not of complete immunity, but resistance to virus multiplication or tolerance (Russell 1978). The nature of the presence and multiplication of the virus in its resistant parent, Balimau Putih, might explain resistance to RTBV multiplication and the low level of RTBV concentration in Y1286. Sta Cruz et al. (1993) demonstrated that RTBV was present in both the xylem and phloem of the infected plants. However, the number of infected cells and the amount of RTBV within the infected cells were far fewer in Balimau Putih than that of TN1. Hence, they suggested that the low concentration of RTBV in the variety be due to both the fewer number of susceptible cells and the low multiplication of RTBV within the infected cells of that variety.

Resistance to RTBV multiplication has resulted in Y 1286 to manifest its tolerance to tungro infection. It was found that its plant height was not reduced greatly and the yield was not affected severely after being inoculated by both viruses. Such manifestation of tolerance was also observed in its parent Balimau Putih and other varieties such as Utri Rajapan and Utri Merah (Hassanuddin and Hibino 1989). These types of varieties are recommended to be grown in an endemic area to reduce the potential of severe yield loss and ensuring yield sustainability. Another advantage of Y 1286 is that it is also resistant to RTSV multiplication, which it inherited from Balimau Putih. The low concentration of RTSV in Balimau Putih was attributed to a smaller number of susceptible phloem cells or limited virus movement within the infected plants and not due to the inhibition of its multiplication (Sta Cruz et al. 1993).

The low concentration of RTBV and RTSV in the parent of Y 1286, Balimau Putih was found to contribute to its lesser efficiency as source of virus inoculum for the transmission of the disease (Habibuddin et al. 1995). Hence, large scale planting of this type of variety will reduce the presence of RTSV inoculum source in the field. As such, Y 1286 is anticipated to be able to help in suppressing the potential spread or epidemic of tungro disease which is RTSV-dependent (Hibino et al. 1978).

The only limitation for Y 1286 acceptability among the farmers is its potential yield, which is slightly lower than that of the currently popular variety grown in Malaysia, MR 84. Hence, further improvement on Y 1286 needs to be done. The incorporation of RTBV resistant genes may not be really difficult since its heritability estimate was moderate (Habibuddin 1994). Probably, by delaying the selection procedure to the F₄ or F₅ generations, coupled with large population size for selection, may increase the chance of obtaining more resistant segregants, through increasing homozygosity and the reduction in the non-additive effects (Muhammad and Jones 1990). Y 1286 with its improved morphological traits is a much more convenient parent to be used as donor for tungro resistance in normal rice breeding. Its seeds was deposited in the MARDI rice germplasm as accession number 9344.

Acknowledgements

The authours wish to thank the Director General of MARDI for permitting this research to be undertaken and to En. Zakaria Abdullah, Pn. Rodziah Ishak and En. Lourdusamy s/o Soosay for their various technical helps.

References

- Cabauatan, P. Q. and Hibino, H. (1985). Transmission of rice tungro bacilliform and spherical viruses by *Nephotettix virescens* Distant. *Phil. Phytopathol.* **21**: 103-9

- Clark, M. F. and Adams, A. N. (1977). Characteristics of microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. *J. Gen. Virol.* **34**: 475–83
- Dahal, G., Dasgupta, I., Lee, G. and Hull, R. (1992). Comparative transmission of, and varietal reaction to, three isolates of rice tungro virus disease. *Ann. Appl. Biol.* **120**: 287–300
- Department of Agriculture Malaysia (1983). Laporan kawalan penyakit merah di Semenanjung Malaysia. Laporan suku tahun, Cawangan Pemeliharaan Tanaman. Kuala Lumpur: Kementerian Pertanian
- Habibuddin, H. (1994). Mechanism and inheritance of tungro resistance in rices. Ph.D. Thesis, Universiti Kebangsaan Malaysia, 263 p.
- Habibuddin, H., Ahmad, I. B., Mahir, A. M., Jalani, B. S., Imbe, T. and Omura, T. (1994). Differentiation of vector and virus resistance in several rice varieties to tungro disease. *MARDI Res. J.* **22(1)**: 157–67
- Habibuddin, H., Ahmad, I. B., Mahir, A. M., Jalani, B. S. and Omura, T. (1995). Resistance in rice to the multiplication of the two tungro viruses. *MARDI Res. J.* **23(1)**: 27–36
- Habibuddin, H., Hadzim, K., Othman, O., Imbe, T. and Omura, T. (1991). Selection of rice line Y 1036 resistant to the green leafhopper and tungro disease. *MARDI Res. J.* **19(2)**: 169–75
- Habibuddin, H. and Saad, A. (1986). Kesan cara penggunaan racun serangga carbofuran terhadap kejadian penyakit merah virus dan penghasilan tiga varieti padi di kawasan MUDA, Kedah. *Tekno. Padi MARDI 2(1)*: 35–9
- Hassanuddin, A. and Hibino, H. (1989). Grain yield reduction, growth retardation and virus concentration in rice plants infected with tungro-associated viruses. *Trop. Agric. Res. Ser* **22**: 56–73
- Hassanuddin, A., Koesnang and Baco, D. (1997). Rice tungro virus in Indonesia. Present status and current management strategy. In *Epidemiology and management of rice tungro disease* (Chancellor, T. C. B. and Thresh, J. M., ed.) p. 94–102. Chatham, U. K.: National Resources Institute
- Hibino, H. (1989). Insect-borne viruses of rice. *Advances in Disease Vector Research* **6**: 209–41
- Hibino, H., Daquioag, R. D., Mesina, E. A. and Aquiero, V. M. (1990). Resistance in rice to tungro-viruses. *Plant Dis.* **74**: 923–6
- Hibino, H., Roechan, M. and Sudarisman, S. (1978). Association of two types of virus particles with penyakit habang (tungro disease) of rice in Indonesia. *Phytopathology* **68**: 1412–6
- Hibino, H., Saleh, N. and Roechan, S. (1979). Transmission of two kinds of rice tungro-associated viruses by insect vectors. *Phytopathology* **69**: 1266–8
- Koganezawa, H. and Cabunagan, R. C. (1997). Resistance to rice tungro virus disease. In *Epidemiology and management of rice tungro disease* (Chancellor, T. C. B. and Thresh, J. M., ed.) p. 54–9. Chatham, U.K.: National Resources Institute
- Ling, K. C. (1972). *Rice virus diseases*. p. 142. Los Banos, Philippines: International Rice Research Institute
- Muhammad, N. and Jones, J. E. (1990). Genetics of resistance to reniform nematode in upland cotton. *Crop science* **30**: 13–6
- Omura, T., Saito, Y., Usugi, T. and Hibino, H. (1983). Purification and serology of rice tungro spherical and rice tungro bacilliform viruses. *Ann. Phytopathol. Soc. Japan* **49**: 73–6
- Othman, O., Habibuddin, H., Hadzim, K. and Chen, Y. H. (1994). Genetics and development of rice varieties resistant to penyakit merah virus. *Proc. of the first national congress on genetics* (Koh, C. L., ed.). Kuala Lumpur: Genet. Soc. of Malaysia
- Russell, G. E. (1978). *Plant breeding for pest and disease resistance*. 485 p. London: Butterworth
- Shahjahan, M., Jalani, B. S., Zakri, A. H., Imbe, T. and Othman, O. (1990). Inheritance of tolerance to rice tungro bacilliform virus (RTBV) in rice (*Oryza sativa* L.). *Theo. Appl. Genet.* **80**: 513–7
- Sta Cruz, F. C., Koganezawa, H. and Hibino, H. (1993). Comparative cytology of rice tungro viruses in selected rice cultivars. *J. Phytopathology* **138**: 274–82
- Sutula, C. L., Gillett, J. M., Morrissey, S. M. and Ramsdell, D. E. (1986). Interpreting ELISA data and establishing the positive-negative threshold. *Plant Dis.* **70**: 722–6
- Takita, T. and Habibuddin, H. (1986). Utilization of vector resistance to control tungro virus disease in rice and breeding strategy to overcome outbreaks of new biotypes in Malaysia. *Trop. Agr. Res. Ser.* **19**: 229–35