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Selection of rice line Y1036 resistant to the green leafhopper and tungro disease

(Pemilihan titisan padi Y1036 yang rintang terhadap lelompat daun hijau dan penyakit merah)

H. Habibuddin*, K. Hadzim*, O. Othman*, T. Imbe** and T. Omura***

Key words: rice line Y1036, selection, ELISA, resistance, tungro, RTSV, *Nephotettix virescens*, biotype

Abstrak

Suatu program kacukan dan pemilihan telah dijalankan bagi mempelbagai kerintangan padi terhadap virus penyakit merah (PMV) dan vektornya lelompat daun hijau (LDH) *Nephotettix virescens* Dist. (Homoptera: Cicadellidae). Varieti padi Pankhari 203 telah digunakan sebagai penderma kerintangan. Pankhari 203 dikacukkan dengan padi MR 67 dan F_1-nya telah dikacuk pula dengan varieti Muda. Pemilihan awal adalah berdasarkan gejala PMV setelah diinokulatkan dengan koloni-koloni LDH bervirus yang dipelihara pada varieti padi TN1 dan/ atau Pankhari 203. Pemilihan pada peringkat akhir dibuat dengan menggunakan cerakin virus dengan menggunakan teknik serologi ELISA. Pemilihan di ladang adalah berlandaskan sifat-sifat agronomi. Y1036 yang dihasilkan ialah titisan yang rintang terhadap virus PMV jenis rice tungro spherical virus (RTSV) dan vektornya LDH. Penghasilan dan sifat-sifat agronominya adalah memuaskan. Oleh itu, Y1036 dianggap sebagai induk yang lebih sesuai digunakan bagi program pembiakbakaan varieti rintang terhadap zarah RTSV dan vektornya. Saringan dengan menggunakan kaedah serologi dan koloni vektor yang berkenaan dicadang digunakan.

Abstract

A breeding programme was undertaken to diversify the resistance of rice varieties against tungro virus and its green leafhopper (GLH) vector *Nephotettix virescens* Dist. (Homoptera: Cicadellidae). Rice Pankhari 203 was used as a resistant donor. It was crossed with MR 67 and the F_1 was further crossed with Muda. Initial selections were based on symptoms after inoculation by viruliferous GLH colonies bred on rice variety TNI and/or Pankhari 203. Later selections were based on virus assays using the ELISA technique. Field selections were based on agronomic characters. Y1036 was selected as a line resistant to rice tungro spherical virus (RTSV) and its vector GLH. Yield and other agronomic characters of the line were acceptable. Therefore, Y1036 is recommended for use as a better parent for breeding varieties resistant to RTSV and the vector. Screening procedure using serological methods and relevant colony GLH is recommended.

*MARDI Rice Research Centre, Bumbong Lima, Kepala Batas, 13200 Seberang Prai, Malaysia
**Tropical Agriculture Research Centre, Ohwashi 1–2, Tsukuba, Ibaraki, 305 Japan
***National Agriculture Research Centre, Kannon-Dai, Tsukuba, 305 Japan
Authors' full names: Habibuddin Hashim, Hadzim Khalid, Othman Omar, Tokio Imbe and Toshihiro Omura

Introduction

Rice tungro disease, or locally known as penyakit merah (PMV), greatly decreased rice production in Peninsular Malaysia during the epidemic years of 1981–1983. A tungro resistant variety IR42 from IRRI was introduced to control the epidemic in 1983 (Habibuddin et al. 1987). The apparent resistance of this variety to tungro infection is due to its resistance to the vector, the green leafhopper (GLH), *Nephotettix virescens* Dist. (Homoptera: Cicadellidae) (Kobayashi et al. 1983). However, the appearence of a new vector biotype able to infect IR42 is expected because a colony adapted to IR42 was developed easily in the laboratory (Kobayashi et al. 1983). Recently, the appearence of such biotype was noticed in Muda Agricultural Development Aurhority Scheme (Anon. 1986).

The causal agents of rice tungro disease are rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). RTBV causes yellowing and stunting symptoms in rice plants and symptom severity is intensified in the presence of RTSV. On the other hand, RTSV causes no clear symptoms on indica varieties (Hibino et al. 1978). RTSV can be transmitted independently by GLH, whereas RTBV is transmitted concomitantly only when RTSV is acquired previously or simultaneously (Hibino et al. 1979). Therefore, RTSV plays an important role in the epidemiology of tungro disease. Accordingly, the introduction of the genes for RTSV resistance in addition to those conferring resistance to the vector and RTBV would increase the effectiveness and durability of disease management by resistant varieties.

Among the traditional rice varieties identified to be resistant to tungro infection, Pankhari 203 of Indian origin was reported to be resistant to the virus as well as to the vector (Ling 1968, 1972). Recently, the resistance of this variety was identified more precisely as resistance to the vector and to RTSV (Habibuddin et al. 1990; Hibino et al. 1990). Although Pankhari 203 had the specific resistant gene(s) required, its agronomic characters are unacceptable. Therefore, there is a need to transfer the resistant gene(s) into improved varieties through conventional breeding procedures. The MARDI rice breeding programme in collaboration with the Tropical Agriculture Research Centre (TARC) of Japan had made some attempts in this respect. One of the relevent crosses was MR 67/Pankhari203// Muda.

The evaluation of tungro infection has until recently been based on symptomatology. Hence assaying the virus content of inoculated plants was not possible. However, the successful preparations of specific antisera to RTBV and RTSV (Omura et al. 1983) have made such assays possible. The application of serological techniques such as the enzyme-linked immunosorbent assay (ELISA) enabled the detection of infection and evaluation of multiplication of both RTBV and RTSV. Thus, a method for screening the resistance to RTBV and/or RTSV has been established. ELISA was found to be particularly useful for detecting RTSV which is not otherwise detectable in indica rices. Therefore, ELISA was used to screen resistance to RTSV besides evaluation of RTBV infection in the final stage of the breeding programme which resulted in the selection of Y1036.

Materials and methods

Green leafhopper

The TN1 colony of *N. virescens* was originated from padi fields at Bumbung Lima. It had been bred on a susceptible rice variety Taichung Native 1 (TN1) for more than 10 years. The P203 colony was selected from the TN1 colony and adapted to the GLH-resistant variety Pankhari 203 on which it has been bred for more than 4 years. The GLH populations used for each set of experiment were of the same age and obtained by giving adults an oviposition period of 24 h on their respective host plants.

Antibiosis test

Antibiosis tests were done using both TN1 and P203 colonies. Experiments were conducted separately for each colony in completely randomised design with five replications. Each replicate consisted of three seedlings in a clay pot and infested at 21 days after sowing (DAS). Twenty-five nymphs of second or third instar GLH was placed on the seedlings inside a plastic cage. The number of surviving nymphs were recorded 7 days after introduction.

Tungro evaluation with ELISA for breeding lines

Viruliferous GLH were obtained after an acquisition access period of 48 h on RTBV + RTSV-infected TN1 plants. Twenty-five seedlings planted in a clay pot were covered with a cage at 7 DAS and inoculated by releasing 25 viruliferous adult GLH for 24 h. All experiments were done in three replicates.

Serological evaluation of the virus infection by ELISA (Clark and Adams 1977) was made 3 weeks after inoculation (WAI). Immunoglobulin-G (IgG) was prepared from separate antisera to RTBV and RTSV (Omura et al. 1983). Each well of microplates (Nunc, Denmark) was filled with 0.2 mL of 0.05 M carbonate buffer, pH 9.6 containing 0.1 µg of IgG to RTBV or 1.0 µg of IgG to RTSV, and incubated for 4 h at 37 °C. Inoculated leaves were homogenised with 0.01 M phosphate-buffer, pH 7.4 using a combined leaf and bud press (Erich Pollahne, FRG) and 0.2 mL of the extract was applied to each well. After overnight incubation at 4 °C, the wells were washed and treated with IgG-alkaline phosphatase conjugated for 4 h at 37 °C. Reactions were evaluated at 405 nm using Easy Reader (SLT Labinstrument, Austria) after adding phosphatase substrate.

Crossing and selection procedure

The parental varieties in this study were MR 67, Pankhari 203 and Muda. Pankhari 203 has resistance to RTSV and the vector *N. virescens*, whereas Muda and MR 67 are susceptible to tungro but are agronomically acceptable. MR 67 was crossed with Pankhari 203 and the F_1 was then further crossed with Muda. The crossing and selection process are outlined in *Table 1*.

Mass screenings against tungro were carried out on bulk populations of F_2, F_3 and F_4. About 100 g (cu. 5 000 seeds) of F_2 seed was sown in the greenhouse seedbed and at 7 DAS the seedlings were mass inoculated with viruliferous TN1 colony of GLH, average of two GLH per seedling for 3 days. Seedlings which developed tungro symptoms within 14 days after inoculation were recorded and then discarded. Seedlings without apparent symptoms were allowed to grow until 30 DAS when they were transplanted to a padi field. Plants showing symptoms after transplating were further discarded. The F_3 seed from healthy F_2 plants was collected, rebulked and rescreened against tungro. The process was repeated until F_4. Selections of pedigree were carried out from F_5 onwards *(Table 1)*. Each pedigree line was duplicated for screening against tungro using TN1 and P203 colonies. At F_8 and F_9, lines were evaluated for tungro resistance by ELISA and also for yielding ability.

Evaluation for yield and other agronomic characters

Rice varieties Y1036, Muda, MR 67 and Pankhari 203 were planted in the field in randomised complete block design with four replications. The plot size was 5 m x 5 m. Single seedlings of 25 DAS were transplanted per hill at 25 cm spacing. Fertilizer was given at the rate of 80 kg nitrogen (N), 40 kg phosphorous (P) and 30 kg potassium (K) per hectare. Half of N, and all P and K were given as basal application. Second and third application of 0.25 of N were given at 3 and 7 weeks after transplanting (WAT) respectively. Weedicide was used to control weeds. Insecticides (BPMC) at the rate of 0.1% a.i. was applied to control leaf feeding insects

Table 1. Crossing and selection procedure of Y1036

Season	Generation	Selection procedure
Main season 1982	MR 67 x Pankhari 203	
	↓	
Off-season 1983	F_1 x Muda	
	↓	
Main season 1983	F_1	
	↓	
Off-season 1984	F_2	Bulk population, screened against tungro with TN1 colony of GLH
	↓	
Main season 1984	F_3	"
	↓	
Off-season 1985	F_4	"
	↓	
Main season 1985	F_5	Line selection. Simultaneous screening sets with TN1 and P203 colonies
	↓	
Main season 1986	F_6	Selection based on agronomic characters
	↓	
Off-season 1987	F_7	"
	↓	
Main season 1987	F_8	Evaluation of tungro resistance by ELISA technique using TN1 and P203 colonies and preliminary yield test
	↓	
Off-season 1988	F_9	ELISA, other tests including yield test
	↓	
	Y1036	

when the need arise and against grain-sucking insects during the milky stage.

Results

Selection of Y1036

Lines that were resistant to tungro infection when inoculated with the TN1 colony but were susceptible with P203 colony are assumed to be vector resistant. By contrast, lines with little or no tungro infection by using both the colonies were assumed to be either resistant to virus or to both. Based on this critirion, 65 (56.5%) of the 115 F_5 lines were resistant only to the vector and 14 (12.2%) lines were either resistant to the virus or to the vector and virus. Only two of the F_6 lines were confirmed to be highly resistant to both vector and virus. One of these two lines was selected and designated as RU2252-43-3. Further selection for resistance between lines within the family and agronomically amongst individual plant within the line was persued. Eighteen sister lines at F_8 were evaluated for tungro

resistance by ELISA. Since reactions to RTSV infection within the families were observed to be still segregating, six lines with the lowest RTSV infection were selected and re-evaluated for tungro infection at F_9. Thus, fixation within families was much improved. RU2252-43-3-3-3-1 was finally selected for further evaluation of performance and yield. This line was coded as Y1036.

Antibiosis test

Few nymphs of the TN1 colony survived on Y1036 demonstrating that the line is resistant to GLH. The resistance level of Y1036 was similar to that of the donor parent Pankhari 203 and the GLH-resistant check, rice variety IR42. Of the other parents, Muda was moderately resistant and MR 67 was slightly less susceptible than TN1. However, when the P203 colony was used in the antibiosis test, only IR42 had a similar level of resistance as that shown to the TN1 colony and all other varieties were much more susceptible. Pankhari 203 was susceptible to P203 colony while Y1036 was moderately resistant *(Table 2)*.

Tungro evaluation and ELISA test for breeding lines

When inoculated with TN1 colony, varieties Y1036 and Pankhari 203 were highly resistant to RTSV and was followed by IR42 while TN1 was the most susceptible *(Table 3)*. By contrast, Y1036 was highly resistant to RTBV infection and Pankhari 203 showed partial resistance similar to IR42. When inoculated with the P203 colony, neither Y1036 nor Pankhari 203 was infected with RTSV. However, RTBV infection on Pankhari 203 was comparable with that of TN1 and significantly higher than Y1036.

Since Y1036 showed a level of resistance to RTBV infection greater than its resistant parent Pankhari 203 *(Table 3)*, MR 67 and Muda were included in further evaluation of virus infection using the P203 colony. Y1036 was found to be less infected by RTBV and the infection rate was significantly lower than the parents *(Table 4)*.

Table 2. Survival of *N. virescens* nymphs on different rice varieties at 7 days after introduction

Variety	Nymph survival (%)	
	TN1 colony	P203 colony
Y1036	18d	75c
Pankhari 203	15d	94a
MR 67	81b	91ab
Muda	39c	88b
IR42	12d	20d
TN1	90a	86b

Analysis was done on the Arcsin-transformed data

All means within a column with the same letter are not significantly different at $p = 0.05$ by DMRT

Yield and other trait evaluations

The culm height of Y1036 was shorter than the parents *(Table 5)*. The line matured in similar period as MR 67 and Muda, and it is classified as medium maturing type. The total number of panicles per hill was comparable with that of Pankhari 203 and MR 67. The yield was not significantly different from that of MR 67 or Muda, but higher than that of Pankhari 203. Y1036 was more tolerant to lodging than Pankhari 203 and Muda. The wing-glumes of Pankhari 203 were absent in Y1036.

Discussion

Line Y1036 which was selected from the cross involving Pankhari 203, was resistant to RTSV infection and less infected with RTBV particles in addition to having vector resistance similar to that of Pankhari 203 (Habibuddin et al. 1990). These results indicate that the GLH and RTSV genes of Pankhari 203 were transferred to an improved line Y1036.

In the earlier screening procedure for tungro resistance, GLH that were bred on the susceptible TN1 variety were used (Ling 1974; Khush 1978). According to present results and other studies (Habibuddin et al.

Table 3. Rice seedlings infected with RTBV and RTSV when inoculated with TN1and P203 colony of *N. virescens*

Variety	Rice seedlings (%) infected with virus					
	TN1 colony			P203 colony		
	RTBV+ RTSV	Total RTBV	Total RTSV	RTBV+ RTSV	Total RTBV	Total RTSV
Y1036	0b	3c	0c	0c	23b	0c
Pankhari 203	0b	42ab	0c	0c	90a	0c
IR42	15b	31b	21b	17b	36b	22b
TN1	69a	73a	75a	90a	92a	92a

Analysis was done on the Arcsin-transformed data
All means within a column with the same letter are not significantly different at $p = 0.05$ by DMRT

Table 4. Infection with tungro viruses in Y1036 and other varieties inoculated with P203 colony of *N. virescens*

Variety	Infection (%)		
	RTBV + RTSV	Total RTBV	Total RTSV
Y1036	0b	30bc	0d
Pankhari 203	3b	60a	3cd
MR 67	22a	49ab	22ab
Muda	37a	60a	37a
IR42	3b	13c	7bc
TN1	43a	70a	43a

Analysis was done on the Arcsin-transformed data
All means within a column with the same letter are not significantly different at $p = 0.05$ by DMRT

Table 5. Agronomic characters and yield of line Y1036 and its parents, Bumbong Lima (1988)

Variety	Panicle per m^2	Culm height (cm)	Maturation (days)	Yield (kg/ha)	1 000-grain weight (g)
Y1036	304a	62b	138a	4 708a	18.8b
Pankhari 203	304a	80a	110c	2 501b	19.2b
MR 67	304a	81a	135b	4 797a	17.8b
Muda	224b	80a	135b	4 758a	24.8a

All means within a column with the same letter are not significantly different at $p = 0.05$ by DMRT

1990), such procedures are inappropriate for screening both vector and virus resistance because the resistance of Pankhari 203 to RTSV infection could not be differentiated using TN1 colony. In selecting Y1036, screening was carried out using the TN1 colony to identify vector resistance and the P203 colony for virus resistance. Furthermore, ELISA was employed to assess virus resistance because RTSV causes no clear symptoms on indica varieties.

Pankhari 203 and TN1 were infected similarly with RTBV when the P203 colony was used as the vector *(Table 3* and *Table 4)*. The results demonstrate that the apparent RTBV resistance of Pankhari 203 was due to its vector resistance when TN1 colony was used as the vector. The incidence of RTBV in Y1036 was significantly less than in Pankhari 203. However, Y1036 was also more resistant to P203 colony than Pankhari 203 based on the antibiosis test *(Table 2)*.

So whether the lower infection of RTBV in Y1036 was due to the vector resistance or really resistance to RTBV infection was not fully determined. Further experiments using Y1036 colony might explain the reason for low RTBV infection in this line. The above, however, suggests that Y1036 may have inherited the vector resistant gene Glh1 from Pankhari 203 (Athwal et al. 1971) as well as other minor or modifier genes derived from Muda or MR 67 which might have resulted in the lower RTBV infection in Y1036. The higher level of GLH resistance of Y1036 to the P203 colony and the lower infection of RTBV in Y1036 as compared with its parents are unexpected advantages of the new line, and the possible occurrence of other minor or modifier genes should be studied further.

Although Y1036 is generally observed to be slightly inferior than MR 67 and Muda in terms of yield and other agronomic characters, the line is suitable for use under epidemic situations. Nevertheless, the line can be further improved to increase yield and regional adaptation. In view of the current over-reliance on IR42 in the breeding programme, Y1036 is recommended for used by the regional breeders as an alternative source of tungro resistance. Its gene for vector resistance is different from that of IR42 (Takita and Habibuddin 1985) and it is also resistant to RTSV.

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