

# Inheritance of tolerance to rice tungro bacilliform virus (RTBV) in rice (*Oryza sativa* L.)\*

M. Shahjahan, B.S. Jalani, A.H. Zakri\*\*, T. Imbe<sup>1</sup> and O. Othman<sup>2</sup>

Department of Genetics, University of Kebangsaan Malaysia, Bangi

<sup>1</sup> Tropical Agriculture Research Center, Tsukuba, Japan

<sup>2</sup> Malaysian Agricultural Research and Development Institute, Bumbong Lima, Seberang Perai, Malaysia

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**Summary.** From a large number of rice varieties tested, no variety was identified as resistant to tungro bacilliform virus (RTBV). Only in Utri Merah was the RTBV multiplication restrictive, whereas other varieties such as Kataribhog and Pankhari 203 were identified as tolerant. These varieties were crossed with a susceptible variety, TN1, to study the inheritance of restrictive multiplication and tolerance to RTBV. After 3 weeks of inoculation with RTBV, F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> progenies were assessed by enzyme-linked immunosorbent assay (ELISA). The RTBV concentration in all F<sub>1</sub> populations was intermediate between parents. The frequency distribution of F<sub>2</sub> seedlings with various levels of RTBV concentration indicated that the RTBV tolerance is controlled by multiple genes. The RTBV concentrations in F<sub>1</sub> and F<sub>2</sub> progenies from the Utri Merah × TN1 cross revealed that restrictive multiplication of RTBV in Utri Merah is a polygenic character. The continuous variation observed in F<sub>2</sub> populations from crosses between tolerant varieties and Utri merah indicated no allelic relationships between tolerant and restrictive multiplication traits.

**Key words:** Rice – Tungro disease – RTBV – Tolerance – Inheritance

## Introduction

Tungro is one of the most widespread rice virus disease in Southeast Asia. In Malaysia nearly 18,000 ha of rice fields were infected in 1982, causing an estimated damage

of M\$ 21.55 million (Chang et al. 1985). In 1987 hundreds of hectares of rice fields were infected and damaged by the tungro virus disease in Rantau Panjang and Pulau Saya in Kedah State. In the Philippines, nearly 100,000 ha of rice fields were attacked by tungro virus in Luzon and neighboring islands in 1971 (Lande 1975). In Indonesia, the disease appeared in South Kalimantan, South Sumatra, and Lampung in 1969–70, where nearly 21,000 ha were infected. An estimated 660,000 ha were partially or heavily damaged during the 1966 growing season in Thailand. During the 1984–85 wet season in Chingleput, Tamil Nadu, India, the whole rice crop was destroyed by the tungro virus (Subiachandraselvan et al. 1986). The disease has also been found to be very common in Bangladesh (Nuque and Miah 1969).

The tungro disease is caused by joint infection by rice tungro bacilliform virus (RTBV) and RTSV (rice tungro spherical virus), where RTBV causes the disease and RTSV intensifies it (Hibino et al. 1978). Until recently, no information was available on the inheritance of resistance to RTBV and RTSV in rice. Identification of resistant sources and information about the genetics of resistance to the disease are important for breeding resistant cultivars. Very few varieties are known to be resistant to RTBV. The present study was undertaken to identify the sources of resistance or tolerance to RTBV and to study their mode of inheritance in rice.

## Materials and methods

Ten indica-type varieties reported to be resistant to RTV (rice tungro virus), i.e., Pankhari 203, Kataribhog, Utri Merah, Latisail, Peta, Bengawan, FB 24, Tjeremas, Gam Pai 15, and Remadja (Ling 1979), were used in this study. All these varieties were crossed with a highly susceptible variety, TN1, to study the inheritance of tolerance to RTBV. The seeds were obtained from

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\*\* To whom correspondence should be addressed

the germ plasm bank of the Malaysian Agricultural Research and Development Institute, Bumbong Lima. The green leafhopper (*Nephotettix virescens*), which had been reared on the TN1 variety for more than 100 generations, was used as a vector for the virus. Pankhari 203 biotype colony of the vector was used where Pankhari 203 was involved, in order to minimize the vector resistance factor. Crosses were made in the 1985 wet season; F<sub>1</sub> and F<sub>2</sub> progenies were raised in the following seasons.

Ten-day-old seedlings were used for virus inoculation. After an acquisition access period of 24 h, two viruliferous vectors were caged with individual seedlings. After 24 h of inoculation, the vectors were removed from the test seedlings. After 3 weeks the inoculated entries were tested for the presence of RTBV by enzyme-linked immunosorbent assay (ELISA). An ELISA reader was used to determine the RTBV concentration in individual seedlings. For the convenience of data processing, the actual values from the ELISA reader were converted by dividing the individual values with the average of blank wells and then multiplying these by 100. Antiserum was kindly supplied by Dr. T Omura, Institute for Plant Virus Research, Tsukuba, Japan.

## Results

### *Reaction of parental varieties to RTBV*

Three weeks after virus inoculation, the varieties were assessed for the presence of RTBV by ELISA. As Table 1 shows, all varieties were infected with RTBV. However, in Utri Merah, RTBV multiplication was found to be restrictive (average virus concentration values was 10.8) compared to Kataribhog, Pankhari 203, Latisail, Peta, Remadja, Tjeremas, Gam Pai 15, and Bengawan, where the average concentration value of RTBV was found to be above 38.0. However, these varieties showed tolerance to the RTBV infection and recovered from early infection at later stages of development. The concentration observed in TN1 was the highest (average value = 88.9).

### *Mode of inheritance of restrictive multiplication to RTBV*

To determine the mode of inheritance of tolerance to RTBV and its restrictive multiplication, only F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> progenies from Kataribhog × TN1, Pankhari 203 × TN1, and Utri Merah × TN1 crosses were tested. Ten-day-old seedlings were used for inoculation and 3-week-old seedlings were assessed by ELISA for concentration of RTBV.

In the ELISA test, the RTBV concentration in F<sub>1</sub> progeny of the Utri Merah × TN1 cross was found to be approximately intermediate to that of the parents, i.e., with an average concentration value of 26.67 (Table 2 and Fig. 1), indicating that the restrictive multiplication of RTBV in Utri Merah may be due to an incompletely dominant gene or may be a polygenic trait. When the frequency of F<sub>2</sub> individuals with various levels of RTBV concentration was plotted in a histogram, a continuous variation was observed showing approximately a normal curve (Fig. 1), which is a characteristic of polygenic in-

**Table 1.** Concentration of RTBV in parental rice varieties

Varieties	RTBV concentration <sup>a</sup>
1. Kataribhog	64.5
2. Utri Merah	10.8
3. Pankhari 203	38.0
4. Latisail	59.1
5. Peta	79.5
6. Remadja	78.4
7. Tjeremas	47.5
8. Gam Pai 15	86.9
9. Bengawan	76.8
10. TN1	88.9

<sup>a</sup> Converted data from ELISA Reader

**Table 2.** RTBV concentration in parents and F<sub>1</sub> and F<sub>2</sub> progenies

Combinations		Mean	SD	Min	Max
1. Utri Merah × TN1	UM	10.83	9.16	1.58	39.07
	TN1	96.16	6.58	82.34	109.00
	F <sub>1</sub>	26.67	14.06	8.81	76.76
	F <sub>2</sub>	39.52	20.58	5.02	98.83
2. Kataribhog × TN1	Kat	64.52	6.52	54.03	75.36
	TN1	93.57	9.52	72.87	108.23
	F <sub>1</sub>	73.36	11.77	53.89	105.52
	F <sub>2</sub>	80.41	18.54	11.58	129.60
3. Pankhari 203 × TN1	Pan	38.03	23.00	7.20	85.10
	TN1	77.62	15.98	33.45	94.65
	F <sub>1</sub>	44.58	23.09	14.40	97.50
	F <sub>2</sub>	51.59	20.53	14.35	90.80

Min = Minimum, Max = Maximum, UM = Utri Mehra, Kat = Kataribhog, Pan = Pankhari 203

heritance. The same mode of segregation was also observed in F<sub>3</sub> progenies (Fig. 1), showing a normal curve when the frequency of individual families with various levels of RTBV concentration was plotted, and confirming the polygenic mode of inheritance for the restrictive multiplication of RTBV in the variety Utri Merah.

### *Mode of inheritance of tolerance to RTBV*

The RTBV concentration in F<sub>1</sub> progenies of other crosses such as Kataribhog × TN1 and Pankhari 203 × TN1 was found to be nearly intermediate between parental values (Table 2 and Figs. 2 and 3), indicating that the tolerance to RTBV in Kataribhog and Pankhari 203 may also be due to an incompletely dominant gene or may be a polygenic character.

When the distribution of seedlings with various levels of RTBV concentration was plotted in histograms, the F<sub>2</sub> populations of these cross combinations showed continuous variation and approximately normal curves were

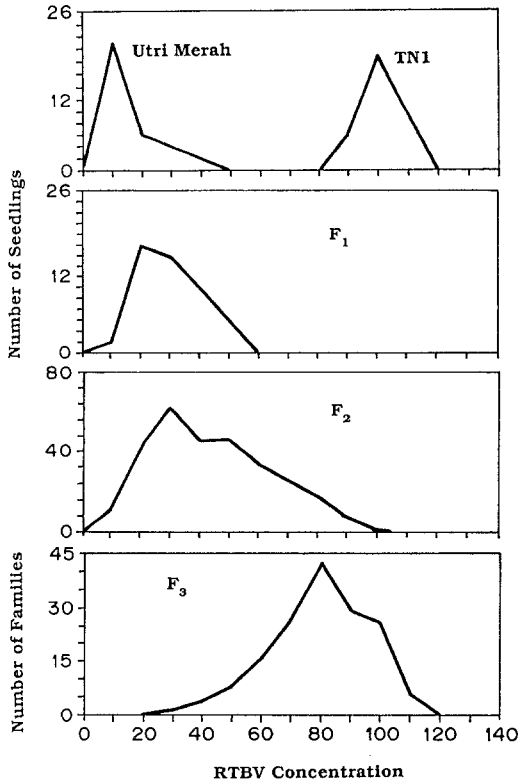


Fig. 1. Frequency distribution of seedlings with various levels of RTBV concentration from the cross Utri Merah  $\times$  TN1

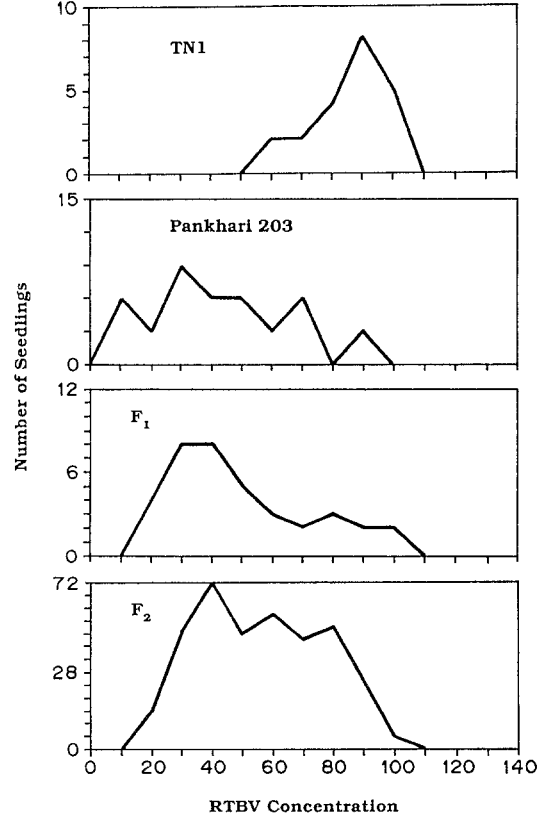


Fig. 3. Frequency distribution of seedlings with various levels of RTBV concentration from the cross Pankhari 203  $\times$  TN1

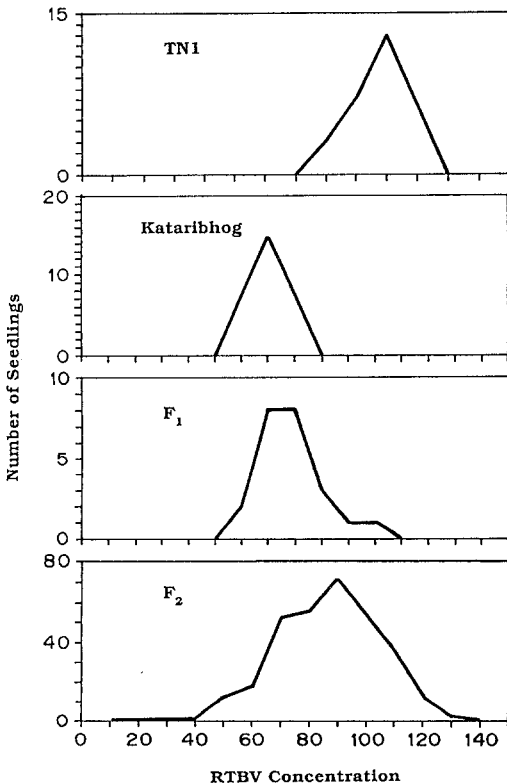


Fig. 2. Frequency distribution of seedlings with various levels of RTBV concentration from the cross Kataribhog  $\times$  TN1

observed, indicating that the tolerance of RTBV in Kataribhog and Pankhari 203 is also under polygenic control (Figs. 2 and 3).

*Allelic relationships*

The allelic relationships between the genes for RTBV restrictive multiplication (in Utri Merah) and tolerance (in Kataribhog and Pankhari 203) were studied, and the data are presented in Figs. 4 and 5. The RTBV concentration in  $F_1$  populations from Utri Merah  $\times$  Kataribhog and Utri Merah  $\times$  Pankhari 203 crosses were nearly intermediate between the parents. The RTBV concentration in the seedlings of  $F_2$  populations and  $F_3$  families of these crosses showed a unimodal distribution, suggesting that nonallelic genes govern restrictive multiplication of RTBV in Utri Merah and tolerance to RTBV in Kataribhog and Pankhari 203.

**Discussion**

Tungro virus disease is a complex of two viruses (RTBV and RTSV), where RTBV produces the disease and RTSV intensifies the degree of infection (Hibino et al. 1978). Recent studies suggest that there is no serological

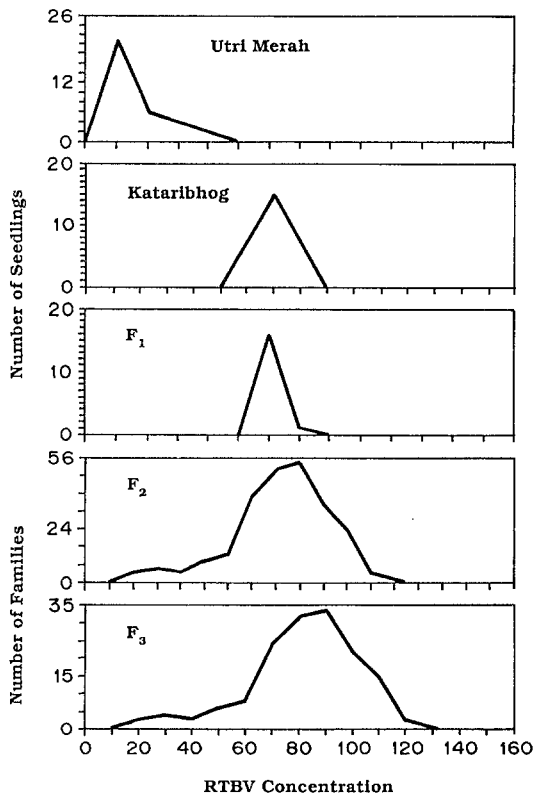


Fig. 4. Frequency distribution of seedlings with various levels of RTBV concentration from the cross Utri Merah  $\times$  Kataribhog

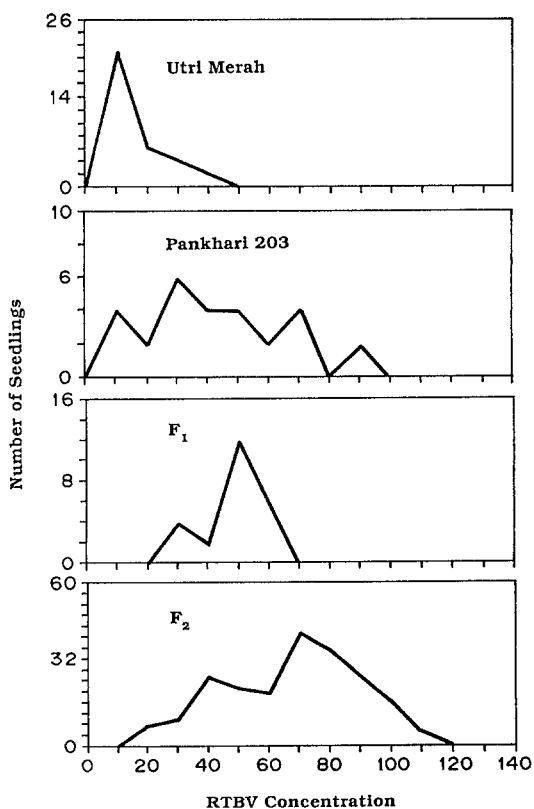


Fig. 5. Frequency distribution of seedlings with various levels of RTBV concentration from the cross Utri Merah  $\times$  Pankhari 203

relationship between RTBV and RTSV (Omura et al. 1983). Therefore, studies on inheritance of resistance to RTBV and RTSV should be undertaken separately for each virus, because RTSV can operate independently of RTBV (Aguiero et al. 1986).

Previous studies on inheritance of resistance to RTV disease that were based on visual assessment showed resistance to be a dominant character controlled by two pairs of genes (IRRI 1966; Shastry et al. 1972; Lande 1975; Seetharaman et al. 1976). Most of the sources of resistance used by these workers were resistant to the vector. Visual assessment alone is not reliable because it is not possible to differentiate between seedlings infected by one virus or both.

Until recently, no variety was reported to be completely resistant to RTBV. In the present study, Utri Merah has been found to be restrictive to multiplication of RTBV. The concentration of RTBV in Utri Merah and TN1 was found to be 10.8 and 88.9, respectively (Table 1). The RTBV concentrations in Kataribhog, Pankhari 203, Peta, Remadja, Tjeremas, and Latisail were higher than in Utri Merah but lower than in TN1 (Table 1). These varieties have been regarded as tolerant to RTBV because of their ability to recover from early infection, although the virus concentration remains nearly the same until maturity.

The RTBV concentration in  $F_1$  progenies of Utri Merah  $\times$  TN1 was nearly intermediate between the two parents (Table 2), and the distribution of  $F_2$  seedlings with various levels of RTBV concentration suggested that the restrictive multiplication of RTBV in Utri Merah is a polygenic character (Figs. 1 and 2). The normal distribution of the  $F_2$  seedlings with various levels of RTBV concentration in Kataribhog  $\times$  TN1 and Pankhari 203  $\times$  TN1 crosses also implies the polygenic nature of the trait (Figs. 2 and 3).

Although Utri Merah, Kataribhog, and Pankhari 203 were infected by RTBV, no conspicuous symptom was observed, except for mild stunting of the seedlings at the early stage of inoculation. This is probably due to a very low concentration of RTBV in these varieties. For example, in Utri Merah RTBV concentration was nearly one-tenth of that in TN1, but a serial dilution test showed that the actual concentration was nearly one-thirtieth. In other words, the ELISA reader gave a similar value (as found in Utri Merah, Table 1) of RTBV concentration for TN1 when its extracted juice was diluted 30 times before ELISA.

These same three varieties were also completely resistant to RTSV, and studies on the inheritance of resistance to RTSV revealed that a single recessive gene in Utri Merah and three complementary recessive genes in Kataribhog and Pankhari 203 conveyed the resistance (Shahjahan 1989). Utri Merah is an ideal source of resistance for RTSV and for restrictive multiplication of

RTBV, Pankhari 203 and Kataribhog may also be used in breeding programs to transfer the tolerance character.

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