GRAIN YIELD REDUCTION, GROWTH RETARDATION, AND VIRUS CONCENTRATION IN RICE PLANTS INFECTED WITH TUNGRO-ASSOCIATED VIRUSES

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ABSTRACT

Generally, rice plants infected with both rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) showed severe symptoms, a market reduction in grain yield and retardation in growth. RTBV-infected plants showed milder symptoms with a lower yield reduction and growth retardation. RTSV-infected plants showed no clear symptoms and the lowest yield reduction and growth retardation. Irrespective of virus species in plants, the infected plants of the cultivar Balimau Putih showed the lowest yield reduction and growth retardation, followed by plants of the Palasithari, Sigadis, and Utri Rajapan cultivars. Infected plants of the BW 272-6B, FK-135 and TN-1 cultivars displayed a greater yield reduction and growth retardation. Grain yield reduction in the BW 272-6B, FK-135 and TN1 plants infected with both RTBV and RTSV or RTBV alone was greater than 85 % in BW 272-6B, FK-135 and TN1, but less than 20 % in Balimau Putih. The reduction in grain yield in the RTSV-infected plants was as high as 40 % in IR36, about 20 % in IR54 and TN 1, and less than 5 % in Balimau Putih, Sigadis and Utri Rajapan. Generally, the yield reduction was higher in the plants infected at 3 weeks after soaking than in the plants infected at 1 to 5 weeks. The reduction was the lowest in the plants infected at 7 weeks. RTBV concentration in doubly infected plants was high in BW 272-6B, IR36 and TN1 but low in Balimau Putih, Jhingasail, Palasithari and Utri Rajapan. RTBV concentration was low in plants with milder symptoms. RTSV concentration was not significantly different among the plants with more severe and milder symptoms.

Introduction

Among the rice virus diseases occurring in South and South-East Asia, tungro is the most important one (Rivera and Ou, 1965; Ling, 1972). It is a composite disease caused by the rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (Hibino *et al.*, 1978,1979; Omura *et al.*, 1983). These viruses are transmitted in a semi-persistent manner by the green leafhopper (GLH) *Nephotettix virescens* (Distant) and some other leafhoppers (Hibino 1983a, 1983b; Hibino *et al.*, 1979). Interactions between the two viruses, and the vector leafhopper and rice plant are complex (Cabauatan and Hibino, 1985; Hibino, 1983b; Hibino *et al.*, 1978, 1987, 1988).

Tungro disease has been controlled mainly by the use of resistant cultivars. Most of the tungro-resistant high-yielding cultivars seem to be resistant only to the vector (Hibino *et al.*, 1987). After a few years of intensive cultivation, many of these resistant cultivars have become susceptible to tungro (Dahal *et al.*, 1988; Inoue and Ruay-Aree, 1977; Manwan *et al.*, 1985; Tantera, 1986). Although several cultivars with resistance to the virus agents have been identified (Daquioag *et al.*, 1984), the resistance genes have not been transferred to high-yielding cultivars. Cultivar Balimau Putih had a high level of tolerance (symptomatic resistance) to the tungro-associated viruses (Daquioag *et al.*, 1986).

To evaluate cultivars for tolerance to tungro and develop screening method for tungro tolerance, we compared the grain yield reduction, growth retardation, and virus concentration in plants of rice cultivars infected singly or doubly with RTBV and RTSV.

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Materials and methods

1 Viruses and insect

A tungro isolate originally collected at Laguna, Philippines was maintained on seedlings of the rice cultivar TN1 by successive transfers using GLH. A GLH colony collected at Laguna was maintained for many years on TN1 seedlings. The colony was found to be virus-free by testing from time to time the infectivity of the leafhoppers. Inoculated TN1 plants were indexed by ELISA. Plants infected with both RTBV and RTSV, or RTSV alone were used as virus sources at 45 or 60 days after inoculation. Newly emerged adults were confined for 4 days in a cage with a source plant. Immediately after the acquisition feeding, leafhoppers were used for the inoculation of test plants.

2 Test plants

In a preliminary test, 46 rice cultivars were evaluated for their reactions to tungro. Nine cultivars that differed in symptom severity were selected. Among the 9 cultivars, IR36, IR54, Palasithari and Sigadis were resistant to GLH, whereas Balimau Putih, Utri Rajapan, BW272-6B, FK135 and TN1 were susceptible. Some other cultivars were also used to study the correlation between the symptom severity and virus concentration.

Seedlings were grown in a screenhouse. For inoculation, seedlings of the selected cultivars at 1, 3, 5 or 7 weeks, after soaking were confined with virus-exposed GLH in test tubes or cages. Two to three weeks after inoculation, plants were indexed by the latex test or ELISA. Plants infected with both RTBV and RTSV, or RTBV alone were selected from those inoculated with a mixture of RTBV and RTSY. RTSV-infected plants were selected from those inoculated with RTSV alone.

3 Serology

1) Latex test

Latex particles (Difco Bacto-Latex 0.81) were sensitized with either anti-RTBV or -RTSV immunoglobulin (IgG), as described by Omura *et al.* (1984). Leaf samples about 15 cm long from the second or third youngest leaves of plants were separately homogenized in 0.05 M tris buffer (pH 7.2) in a combined leaf and bud press. Approximately, 30 μl of homogenate was mixed with 15 ul of sensitized latex suspension in each well of ELISA plate. The plate was shaken vigorously for 30–60 min. Clumping of latex particles as observed under a light microscope indicated the presence of the virus antigen.

2) ELISA

ELISA procedures essentially followed the methods described by Clark and Adams (1977) and Bajet *et al.* (1984). Leaf samples were homogenized in 0.1 M phosphate buffer containing 0.14 M NaCl and 0.05 % Tween 20 (PBS-T). For the quantitative analysis, whole plants excluding roots were homogenized with PBS-T. Polystyrene plate (Dynatech, Chantilly, VA, USA) was coated with IgG at 1 μg/ml for RTBV and 2 μg/ml for RTSV. IgG-alkaline phosphatase conjugate was diluted at 1/1000 for RTBV and 1/500 for RTSV. Two wells, one each for RTBV and RTSV, were used for each sample. Extracts of uninoculated plants served as a control. Virus concentration in extracts was indicated by absorbance at 405 nm. Samples with absorbance greater than 4 times the absorbance (means from 4 wells) of the uninoculated control were considered infected.

Results

1 Growth and yield in plants infected at seedling stage

Four seedlings of each cultivar, one each infected with both RTBV and RTSV together, RTBV alone, RTSV alone, and a healthy control, were transplanted in a 40 cm clay pot. A total of 45 pots, 5 per cultivar, was arranged randomly in a screenhouse. Plant height at 9 weeks after infection, panicle length, effective tiller number, filled grain number, unfilled grain number, 100-seed weight, filled grain weight, and days to first flowering were recorded for each plant. Two trials were made in

1986 and 1987. The results obtained in the two trials were comparable for each test cultivar (Figs. 1-9). In all the cultivars except IR36, the plants infected with both RTBV and RTSV showed the greatest reduction in plant height, effective tiller number, filled grain number, grain yield, and delay in days to flowering. (Figs. 11-12), followed by the plants infected with RTBV alone, and then by the plants infected with RTSV alone. In IR36, the RTSV-infected plants showed greater reduction in effective tiller number, filled grain number, and grain yield than the RTBV-infected plants (Fig. 4). Cultivars with a greater grain yield reduction showed a greater reduction in plant height, filled grain number, and effective tiller number. Irrespective of virus species in plants, Balimau Putih showed the lowest reduction in plant height, effective tiller number, filled grain number, and grain yield, followed by Palasithari , Sigadis and Utri Rajapan (Figs. 10 and 12). In the plants infected with both RTBV and RTSV, grain yield reduction was about 20 % in Balimau Putih, less than 40 % in Palasithari, Sigadis and Utri Rajapan, and greater than 90 % in BW 272-6B, FX-135 and TN1 (Fig. 12). The reduction in the plants infected with RTBV alone was also lower in Baliman Putih and greater in BW 272-6B, FK-135 and TN1. The reduction in the plants infected with RTSV alone was as high as 40 % in IR36, and about 20 % in IR 54 and TN1. In Balimau Putih, Palasithari and Sigadis had the delay in the number of days to flowering (Fig. 11) was the lowest.

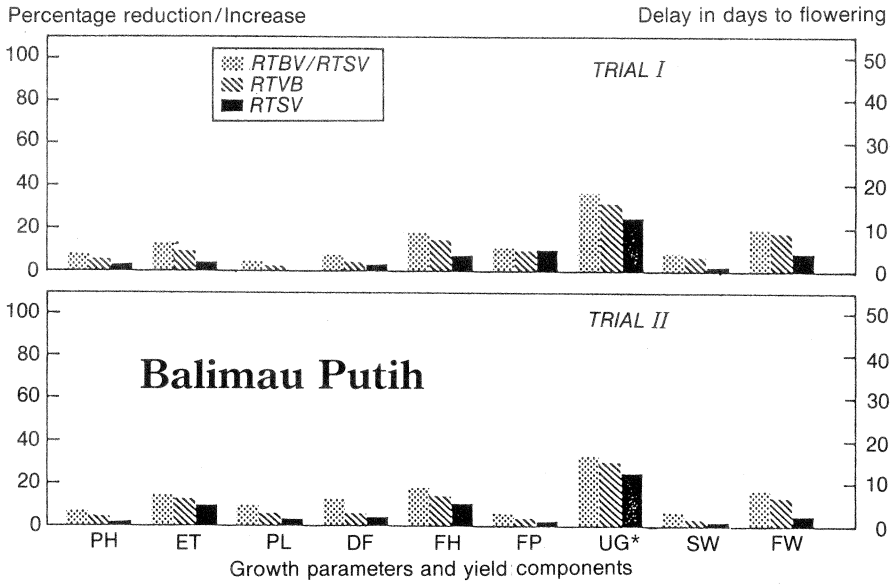

Fig. 1 Influence of tungro-associated viruses on growth parameters and yield components of Balimau Putih plants infected at 5-7 days after soaking. PH = plant height, ET = effective tiller number/hill, PL = panicle length, DF = days to flowering, FH = filled grain number/hill, EP = filled grain number/panicle, UG = percentage unfilled grains/hill, SW = 100-seed weight, FW = filled grain weight/hill.
* Percentage unfilled grains per hill.

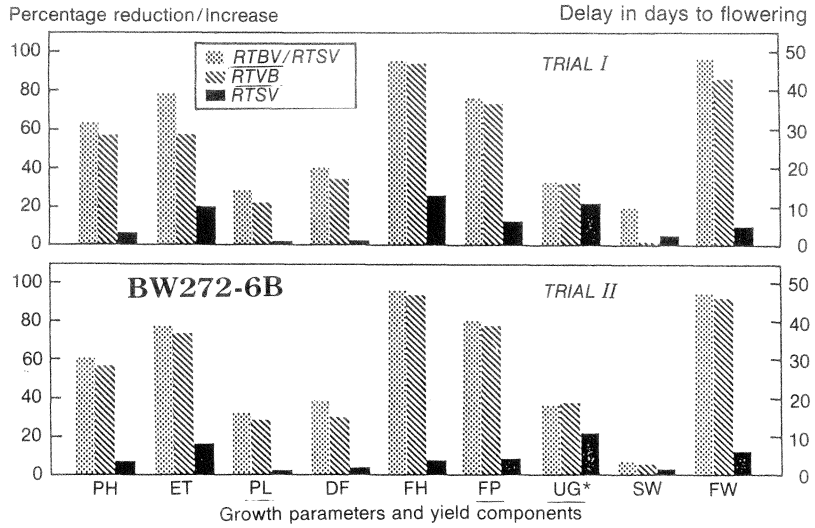

Fig. 2 Influence of tungro-associated viruses on growth parameters and yield components of BW272-6B plants infected at 5–7 days after soaking. PH = plant height, ET = effective tiller number/hill, PL = panicle length, DF = days to flowering, FH = filled grain number/hill, EP = filled grain number/panicle, UG = percentage unfilled grains/hill, SW = 100-seed weight, FW = filled grain weight/hill.
* Percentage unfilled grains per hill.

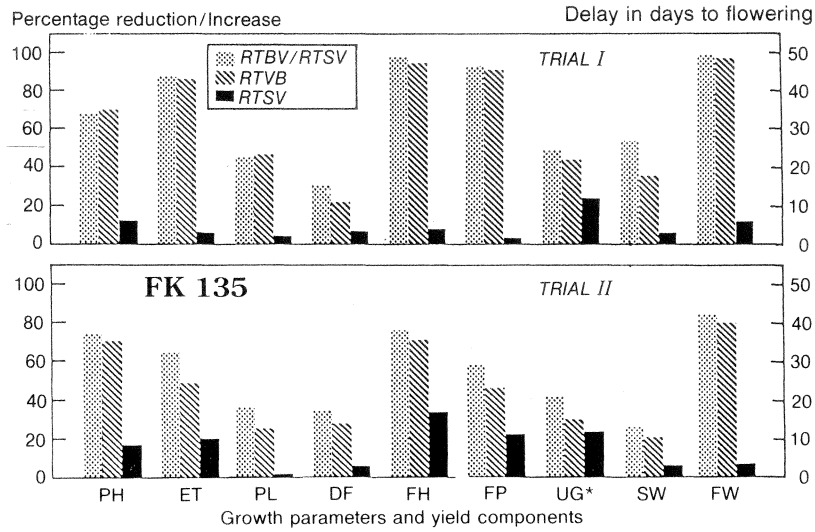

Fig. 3 Influence of tungro-associated viruses on growth parameters and yield components of FK 135 plants infected at 5–7 days after soaking. PH = plant height, ET = effective tiller number/hill, PL = panicle length, DF = days to flowering, FH = filled grain number/hill, EP = filled grain number/panicle, UG = percentage unfilled grains/hill, SW = 100-seed weight, FW = filled grain weight/hill.
* Percentage unfilled grains per hill.

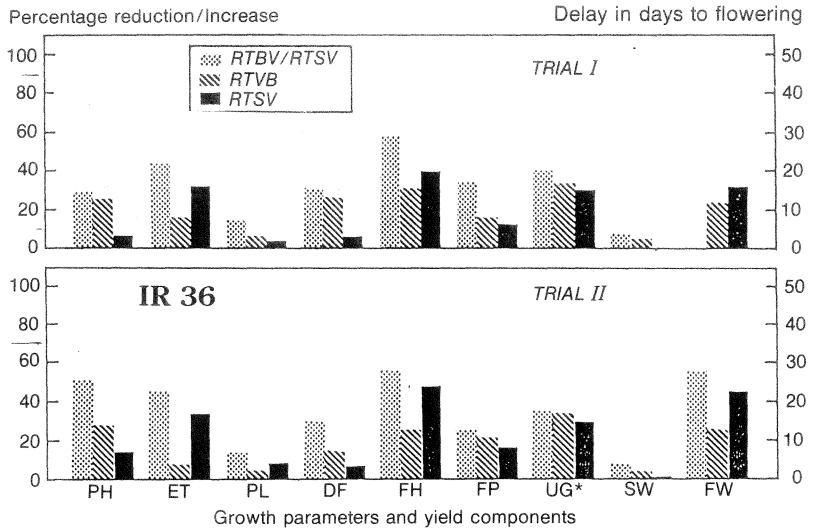

Fig. 4 Influence of tungro-associated viruses on growth parameters and yield components of IR 36 plants infected at 5–7 days after soaking. PH = plant height, ET = effective tiller number/hill, PL = panicle length, DF = days to flowering, FH = filled grain number/hill, EP = filled grain number/panicle, UG = percentage unfilled grains/hill, SW = 100-seed weight, FW = filled grain weight/hill.
* Percentage unfilled grains per hill.

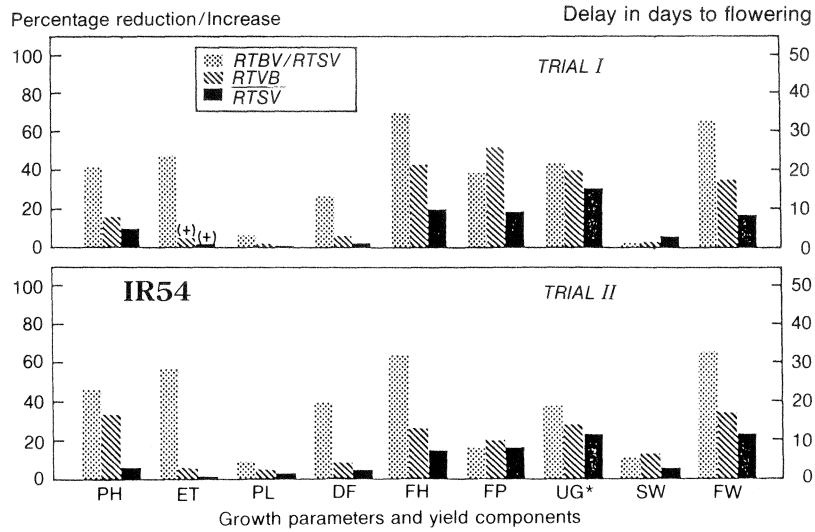

Fig. 5 Influence of tungro-associated viruses on growth parameters and yield components of IR54 plants infected at 5–7 days after soaking. PH = plant height, ET = effective tiller number/hill, PL = panicle length, DF = days to flowering, FH = filled grain number/hill, EP = filled grain number/panicle, UG = percentage unfilled grains/hill, SW = 100-seed weight, FW = filled grain weight/hill.
* Percentage unfilled grains per hill.

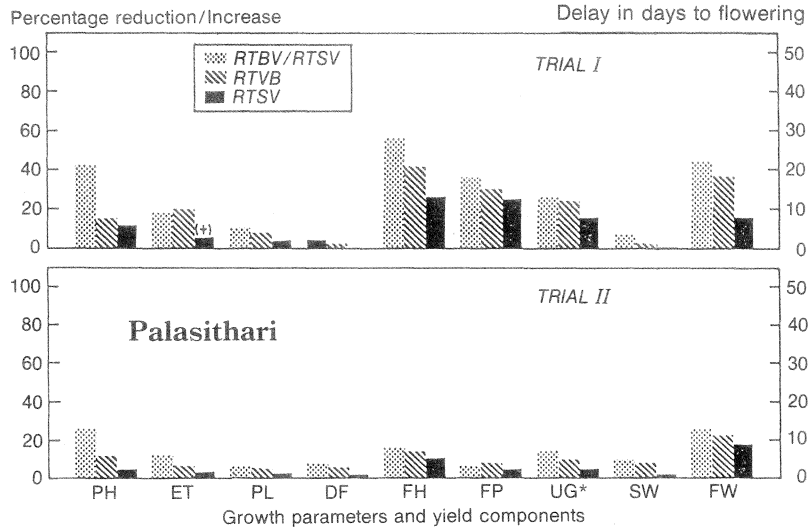

Fig. 6 Influence of tungro-associated viruses on growth parameters and yield components of Palasithari plants infected at 5–7 days after soaking. PH = plant height, ET = effective tiller number/hill, PL = panicle length, DF = days to flowering, FH = filled grain number/hill, EP = filled grain number/panicle, UG = percentage unfilled grains/hill, SW = 100-seed weight, FW = filled grain weight/hill.
* Percentage unfilled grains per hill.

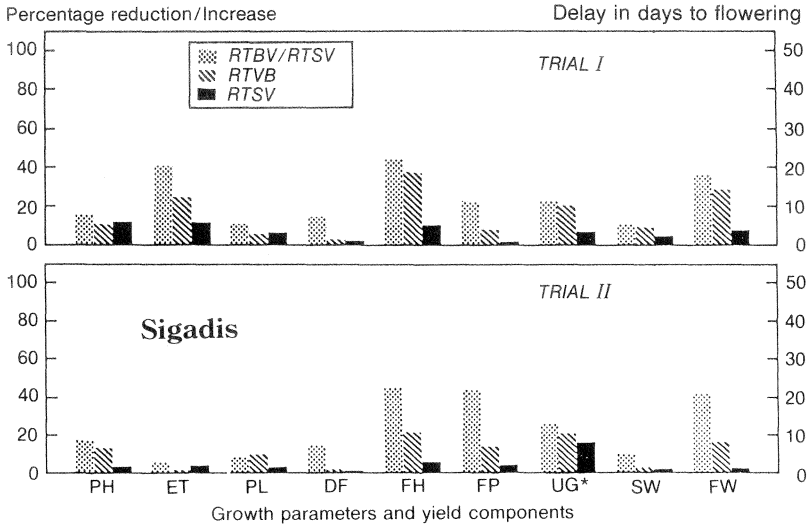

Fig. 7 Influence of tungro-associated viruses on growth parameters and yield components of Sigadis plants infected at 5–7 days after soaking. PH = plant height, ET = effective tiller number/hill, PL = panicle length, DF = days to flowering, FH = filled grain number/hill, EP = filled grain number/panicle, UG = percentage unfilled grains/hill, SW = 100-seed weight, FW = filled grain weight/hill.
* Percentage unfilled grains per hill.

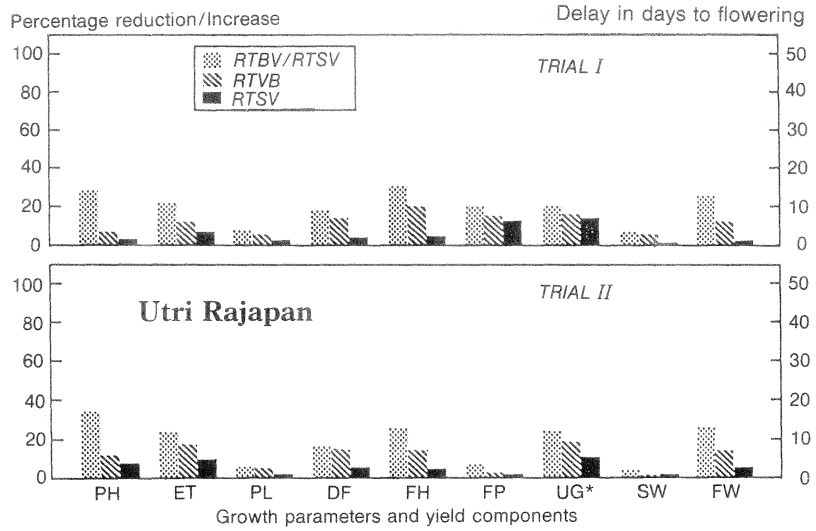

Fig. 8 Influence of tungro-associated viruses on growth parameters and yield components of Utri Rajapan plants infected at 5–7 days after soaking. PH = plant height, ET = effective tiller number/hill, PL = panicle length, DF = days to flowering, FH = filled grain number/hill, EP = filled grain number/panicle, UG = percentage unfilled grains/hill, SW = 100-seed weight, FW = filled grain weight/hill.
* Percentage unfilled grains per hill.

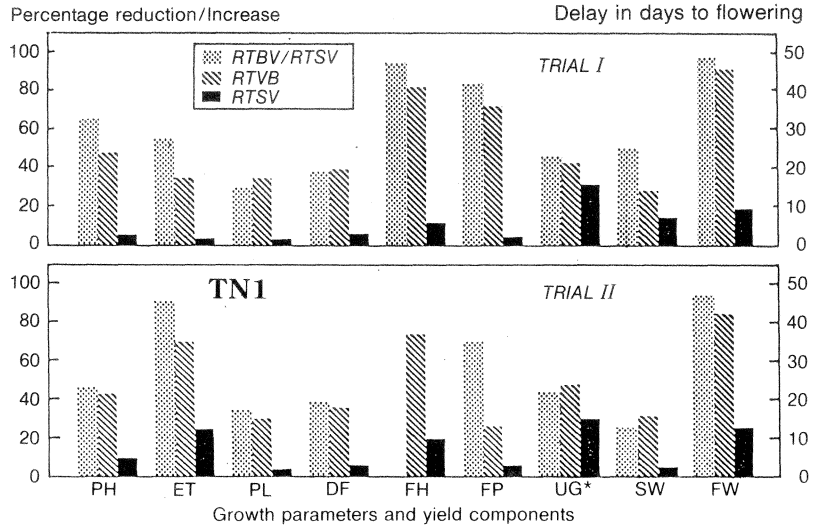

Fig. 9 Influence of tungro-associated viruses on growth parameters and yield components of TN1 plants infected at 5–7 days after soaking. PH = plant height, ET = effective tiller number/hill, PL = panicle length, DF = days to flowering, FH = filled grain number/hill, EP = filled grain number/panicle, UG = percentage unfilled grains/hill, SW = 100-seed weight, FW = filled grain weight/hill.
* Percentage unfilled grains per hill.

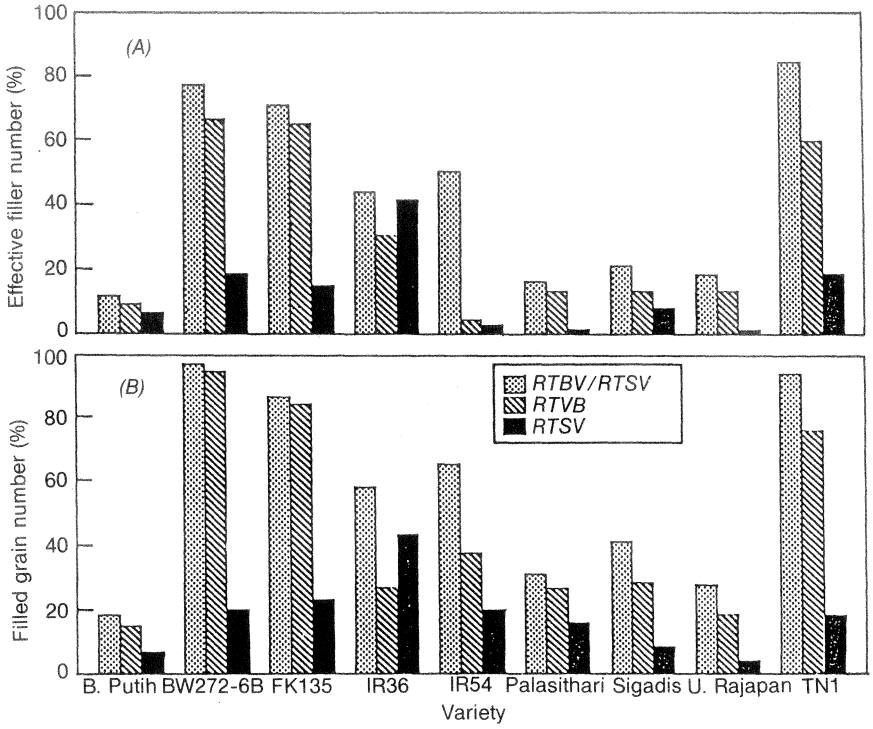

Fig. 10 Reduction in effective tiller number/hill (A) and filled grain number/hill (B) in plants of nine selected infected with RTBV and/or RTSV at 5–7 days after soaking (means of two trials).

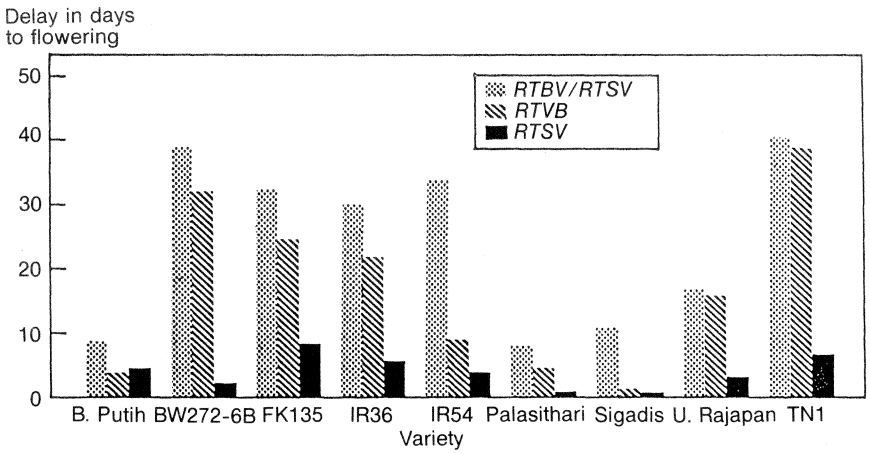

Fig. 11 Delay in days to flowering in plants of nine selected cultivars infected with RTBV and/or RTSV at 5–7 days after soaking (means of two trials).

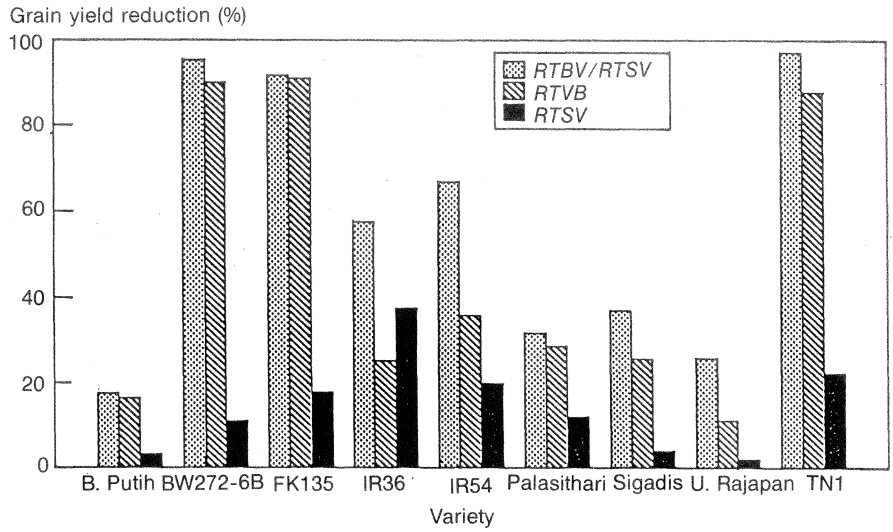

Fig. 12 Reduction in grain yield in plants of nine selected cultivars infected with RTBV and/or RTSV at 5-7 days after soaking (means of two trials).

2 Effect of plant age when infected

One week old seedings of Balimau Putih, IR54, Sigadis and TN1 inoculated with a mixture of RTBV and RTSV, or RTSV, alone were separately transplanted in 30 m pots. Also, one week old seedlings were transplanted in pots and then inoculated with the mixture or RTSV alone at 2, 4 or 6 weeks after transplanting. Two weeks after inoculation, the seedlings were indexed by the latex test. Five seedlings each either infected with both viruses, RTBV alone, RTSV alone, or a healthy control were selected for each combination of cultivars and ages. The yield components and growth parameters were recorded for each plant.

Regardless of plant age when infected and virus species present, Balimau Putih showed the lowest reduction in grain yield, effective plant number, filled grain number, and plant height, and the lowest delay in days to flowering (Figs. 13-16). Reduction in filled grain number, effective tiller number, and plant height was also lower in Sigadis. In Balimau Putih, IR54 and Sigadis, the yield reduction, was generally higher in the plants infected with the viruses at 3 weeks after soaking. In TN1, the yield reduction in the plants infected at 3 weeks was as high as that in the plants infected at 1 to 5 weeks. In all the cultivars,the effect of virus infection was the lowest in plants infected at 7 weeks after soaking.

3 Virus concentration in plants

One week old seedlings inoculated with a mixture of RTBV and RTSV were transplanted in 15 cm pots. Seedlings infected with both viruses were selected. Uninoculated seedlings served as controls. At 1, 3, 5, and 7 weeks after inoculation, at least 5 RTBV + RTSV infected plants and 4 uninoculated plants of each cultivar were harvested. Plants were separately homogenized in PBS-T at 1/10 dilution (w/v). Homogenate was directly tested in ELISA.

RTBV concentration in doubly infected plants was generally high in BW 272-6B, IR36 and TN1, but low in Balimau Putih, Utri Rajapan and Jhingasail (Fig. 17). In Balimau Putih, FX-135, Jhingasail, Palasithari and Utri Rajapan, RTBV concentration was relatively high at 1 to 3 weeks after infection and later decreased to a low level. RTSV concentration was generally high in BW

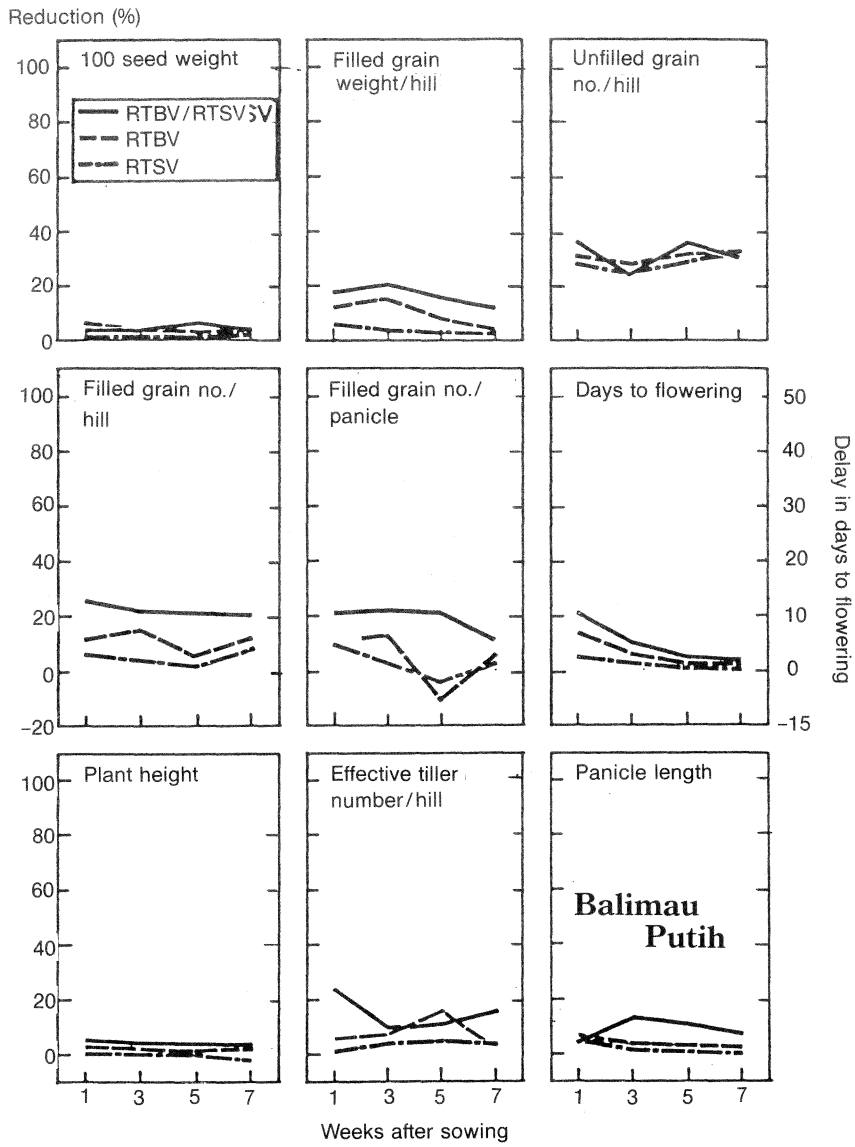

Fig. 13 Percent reduction in growth parameters and yield components and delay in days to flowering in Balimau Putih plants infected with RTBV and/or RTSV at 1, 3, 5 or 7 weeks after sowing.

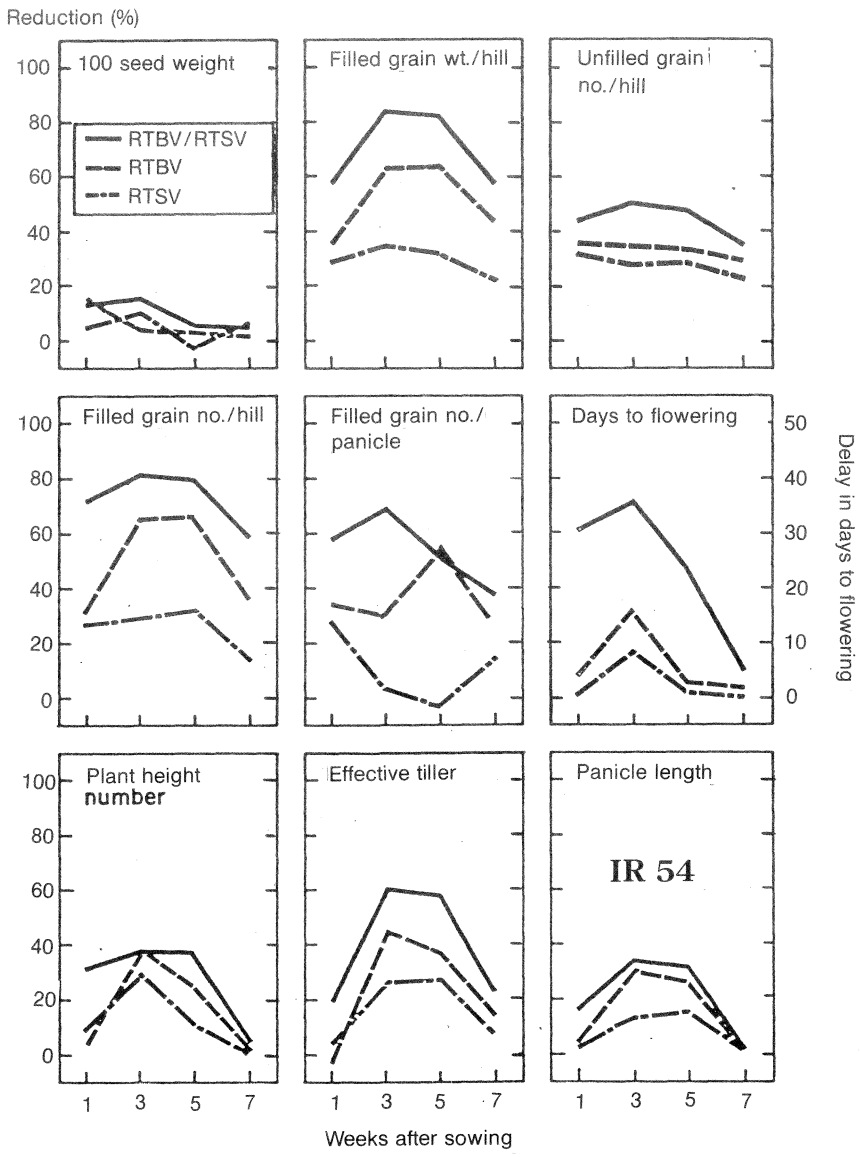

Fig. 14 Percent reduction in growth parameters and yield components and delay in days to flowering in IR54 plants infected with RTBV and/or RTSV at 1, 3, 5 and 7 weeks after sowing.

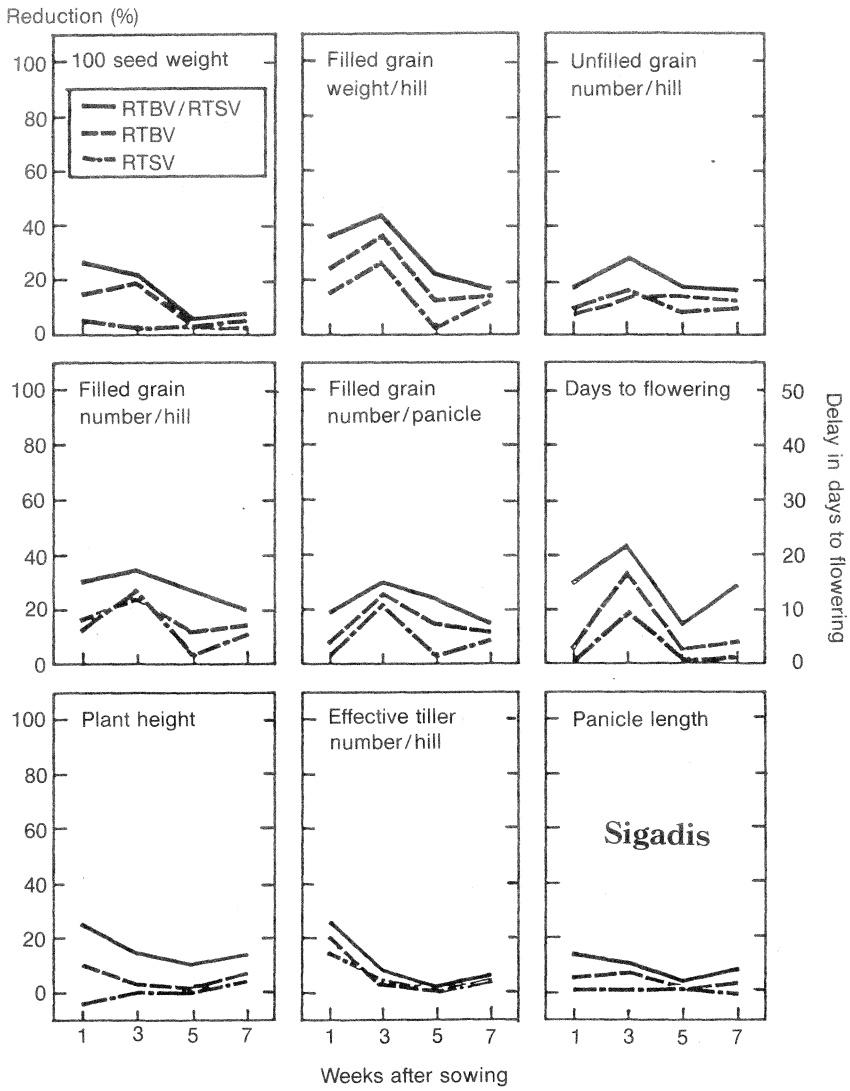

Fig. 15 Percent reduction in growth parameters and yield components and delay in days to flowering in Sigadis plants infected with RTBV and/or RTSV at 1, 3, 5, and 7 weeks after sowing.

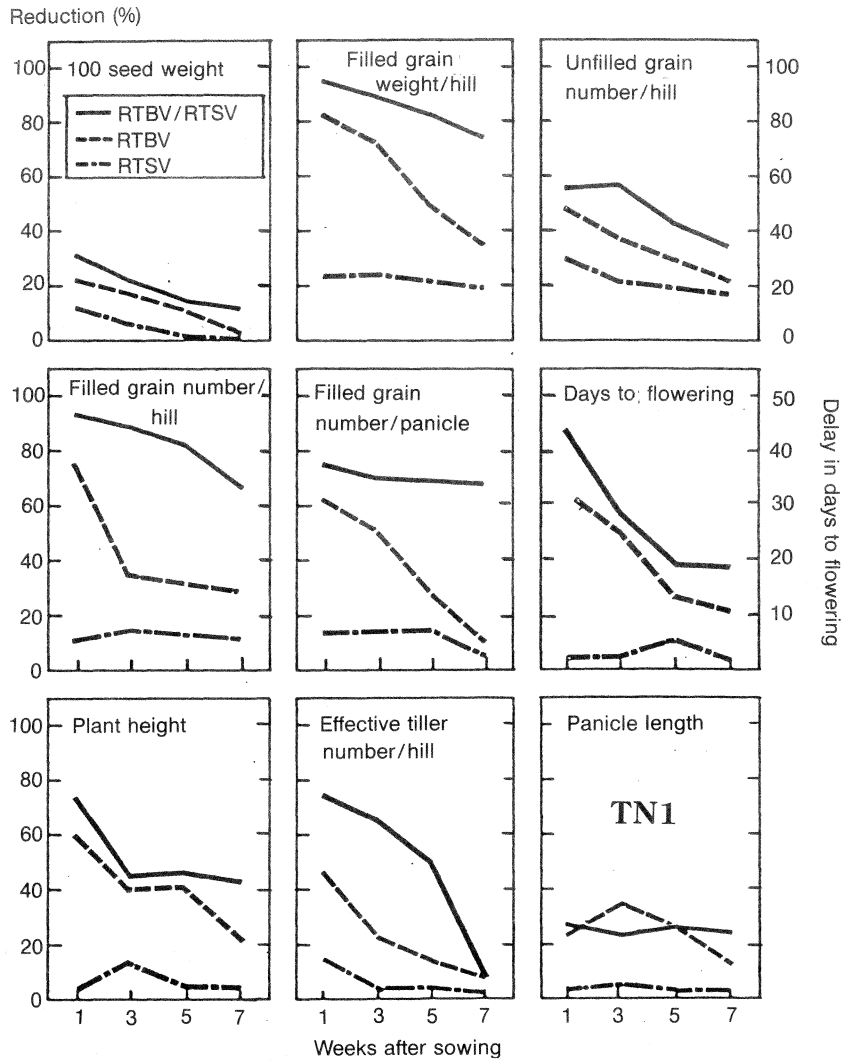

Fig. 16 Percent reduction in growth parameters and yield components and delay in days to flowering in TN1 plants infected with RTBV and RTSV at 1, 3, 5, and 7 weeks after soaking.

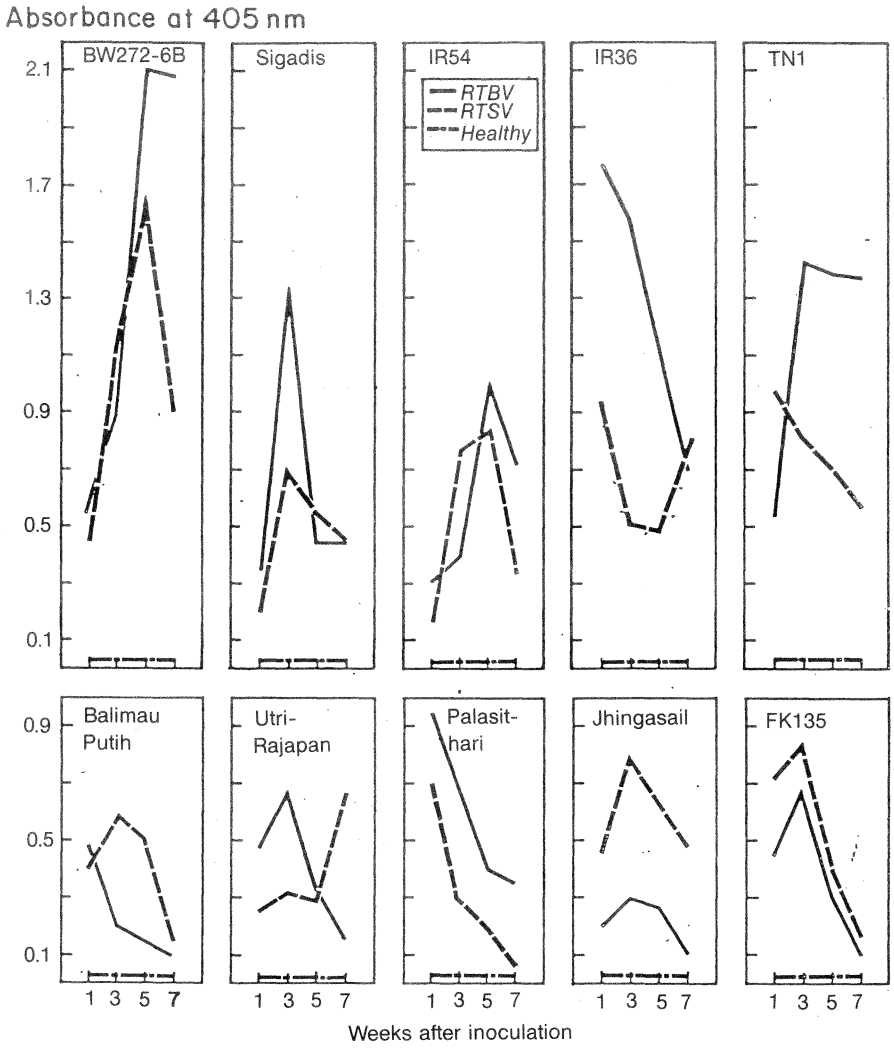

Fig. 17 Relative amounts of RTBV and RTSV in doubly infected plants of ten cultivars at 1, 3, 5 and 7 weeks after inoculation.

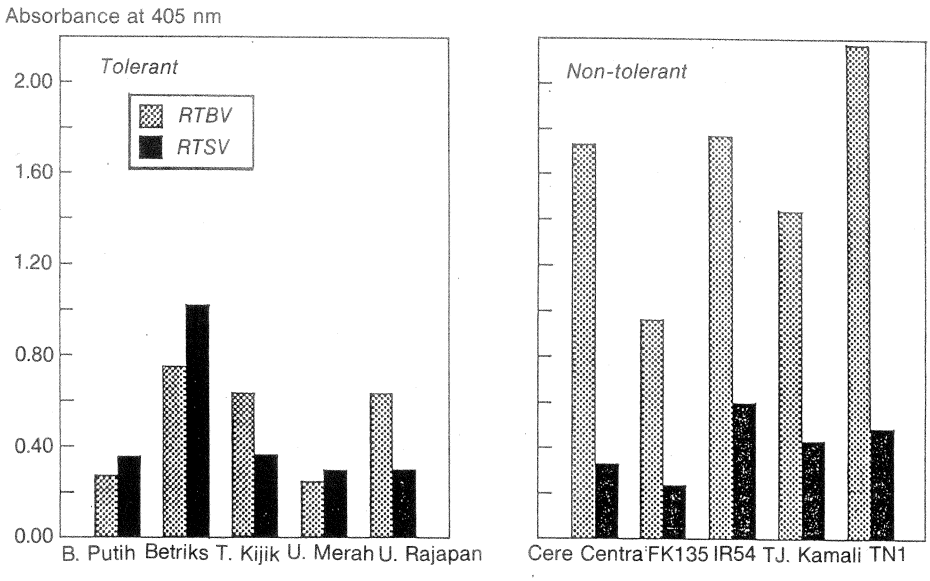

Fig. 18 Relative amounts of RTBV and RTSV in doubly infected plants of tolerant and non-tolerant cultivars infected at 5-7 days after soaking. Tested in ELISA at 3 weeks after infection.

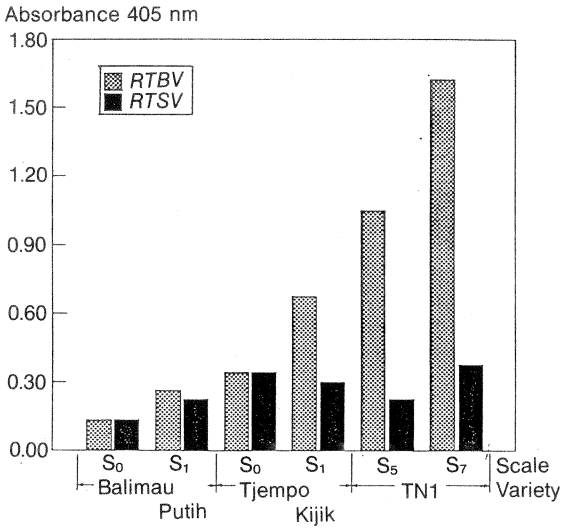

Fig. 19 Relative amounts of RTBV and RTSV in doubly infected plants with different symptoms severity (S0, S1, ... S5, S7) as detected in ELISA at 3 weeks after infection.

272-6B but low in Balimau Putih, Utri Rajapan, Sigadis and Palasithari.

Concentration of RTBV and RTSV in plants at 3 weeks after infection was compared among cultivars with mild and severe symptoms. RTBV concentration was low in plants of cultivars with mild symptoms, while it was high in plants of cultivars with severe symptoms (Fig. 18). RTSV concentration in plants was not significantly different between cultivars with severe and milder symptoms. Among the plants of each cultivar, the symptoms caused by double infection were more or less different. Plants of each cultivar with milder symptoms always showed higher RTBV concentration than plants of the same cultivar with milder symptoms (Fig.19). RTSV concentration was not significantly different between plants with more severe and milder symptoms.

Discussion

In these experiments, grain yield reduction and plant growth retardation caused by the infection with RTBV and/or RTSV were generally less pronounced in the cultivars with milder symptoms, but greater in the cultivars with more severe symptoms. Generally in each cultivar, the reduction was the largest in the plants infected with both RTBV and RTSV, followed by the plants infected with RTBV alone, and then the plants infected with RTSV alone. RTBV concentration was low in infected plants with milder symptoms. RTSV concentration in plants was not significantly different between those with mild and severe symptoms. These observations agree with the fact that RTBV causes tungro symptoms (Hibino *et al.*, 1978; Hibino, 1983b; Cabauatan and Hibino, 1985). Beside the symptom severity, the RTBV concentration could be a good criterion to differentiate and assess cultivars for their tolerance to tungro.

Grain yield reduction due to RTSV infection was as high as 40 %. Recent studies have indicated that RTSV spread as an independent disease in the Philippines (Bajet *et al.*, 1986). RTSV incidence was often high in the fields when tungro symptoms were not visible (Cabunagan *et al.*, 1986; Tiongco *et al.*, 1987). RTSV, called rice waika virus, caused once an epidemic in Kyushu, Japan (Furuta, 1977) as well as grain discoloration and some grain yield reduction (Maejima *et al.*, 1977). More attention has to be directed to RTSV, although symptoms it induces are not clear in the fields.

Yield loss in the plants infected with both RTBV and RTSV at the seedling stage was less than 20 % in Balimau Putih and less than 40 % in Palasithari, Sigadis, and Utri Rajapan. When these cultivars were infected at 5 or 7 weeks after soaking, the loss was even lower. Tungro infection generally occurs after transplanting which is generally practiced at 4 to 6 weeks after soaking the seeds (Tiongco *et al.*, 1988). Crop loss caused by tungro in the field would be very low in these cultivars.

Plants of cultivars tolerant to tungro served as equally efficient virus sources as those of non-tolerant ones (Hasanuddin, 1987). These tolerant cultivars would provide virus sources in the field. This risk seems to have a limited importance, as RTBV and RTSV are present throughout the year in tungro endemic areas where rice double cropping is commonly practiced. Nevertheless, the tolerance can be combined with vector resistance or resistance to virus infection, and the possibility that tolerant cultivars serve as a tungro disease source can be minimized. As the vector resistance is unstable and the virus resistance may not be stable either (Hibino, 1986), the combination of these resistances with tolerance would protect crops when the resistances are overcome. Successful attempts to use tolerant cultivars to control plant viruses have been reported (Russel, 1964; Glendening *et al.*, 1966; Catheral *et al.*, 1970; Palham *et al.*, Walkins and Hider, 1976; Soto *et al.*, 1982; Makkouk and Laterrot, 1983).

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Discussion

Thresh J.M. (UK): Is it possible that the difference in virus content between tolerant and sensitive varieties is being underestimated? The relationship between OD values and virus concentration is not linear and "saturation" effects could be important at high virus contents. This problem could be overcome by appropriate dilutions of the extracts being assayed.

Answer: Yes, you are right. As we know that ELISA is only an indirect method to determine the relative amounts of the virus, the samples (antigen) were diluted 1:10 and the absorbance at 405 nm was analysed in the ELISA minireader II.

Whittle, A.M. (FAO, Indonesia): Having established a link between rainfall and vector population peaks and then proposing an advance of two months in transplanting, what is the effect of this advance on the date of vector population peak?

Answer: I presume that there would be little change as many factors affect the population of *Nephotettix virescens* (GLH). For instance mass-screening in the greenhouse indicated that at certain times it is difficult to maintain the population of GLH.

John, V.T. (IITA): Ever since the first tungro outbreak took place in India in 1969-1970, which affected mostly the variety Patna, the ICAR organized a regular monitoring team to study the population of *N. virescens*. These activities were effective since prophylactic measures could be adopted when the vector population was still small. Is such a system being implemented in Indonesia?

Answer: Such a system is not implemented in Indonesia. The strategy adopted is to try to prevent the build-up of a high population of the vector and promote the rotation of varieties to enable to maintain a certain level of resistance. We are also monitoring the incidence of tungro virus in the field.