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Dispersal of Rice Tungro Associated Viruses by Leafhoppers in the Presence of Singly or Jointly Infected Source Plants under Caged Conditions

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Dispersal of rice tungro bascilliform virus (RTBV) and rice tungro spherical virus (RTSV) by leafhopper (*Nephotettix virescens* Distant) vector in leafhopper resistant IR56, moderately resistant IR36 and susceptible TN1 in the presence of singly or jointly infected sources in different proportions and combinations were studied under caged conditions. Percentage of leafhopper transmission increased as the number of source plants increased. Healthy plants kept with infected plants, were infected by both RTBV and/or RTSV but the percent infection of RTSV alone was maximum in presence of higher number of RTSV source. Generally challenge infection of RTBV infected plants with RTSV was higher than that of RTSV infected plants by RTBV. Percentage of challenged infection was always higher when source plants infected singly either by RTBV or RTSV of same cultivar was used in the combinations. Cultivar TN1 and IR36 served as a better source than IR56. The tungro viruses spread faster when density of infected plants was increased in the cages.

Key Words: Rice tungro virus, *Nephotettix virescens*, Vector pressure, Source density, Dispersal, Challenge infection

Introduction

Tungro is a disease complex associated with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (Hibino et al. 1991). Rice plants infected by both RTBV and RTSV develop the typical 'tungro' symptoms, RTBV causes milder tungro symptoms and RTSV causes no clear tungro symptoms except very mild stunting (Hibino et al. 1990). Both viruses are transmitted in a semipersistent manner by the green leafhopper (GLH) *Nephotettix virescens* (Distant) and some other leafhopper species (Ling 1972, Hibino et al. 1991). Transmission and retention of RTV by its vector is related with the rice cultivars and type of tungro particles (Chowdhury et al. 1990). RTSV can be transmitted independently by GLH from RTSV infected plants, while RTBV transmission from RTBV alone infected plants occurs only when GLH is allowed to feed on RTSV infected plants first and then on the RTBV source (Cabautan & Hibino 1985). Generally rice cultivars resistant to GLH are predominatly infected with RTBV alone when inoculated by GLH that has fed on

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plants infected with both viruses (Dahal et al. 1990). Joint infection by RTBV and RTSV is predominant when rice plants has no such resistance to GLH (Hibino et al. 1987, 1990).

The density of virus inoculum sources plays an important role in development and spread of tungro disease, it is also influenced by types of rice cultivar and virus isolate (Dahal et al. 1992). Relative proportion of source plants, variety and type of viruses associated with the source, would play an important role for disease outbreak and role of those components on the outbreak of disease have not been well documented. This report demonstrates the development of RTBV and RTSV in three rice cultivars in a cage in the presence of singly or doubly infected virus sources in different combinations and proportions.

Materials and Methods

Plants, Insects and Viruses

Leafhopper resistant IR56, moderately resistant IR36 and both tungro and GLH susceptibles TN1 (Taichung Native 1) were used to study the spread and probability of cross infection of RTBV by RTSV and vice-versa by vector GLH. The *N. virescens* colony used in this study was maintained on susceptible TN1 in the glass house of International Rice Research Institute, Philippines. GLH adults used for inoculation were made virus free by successive transfers on healthy TN1 seedlings. Tungro source was an isolate maintained on TN1 for several years by successive transfer with GLH, used as virus source during the period of study if not mentioned otherwise. Seedlings of TN1, IR36 and IR56 were inoculated with RTBV and/or RTSV by viruliferous GLH and inoculated plants were indexed by ELISA (Bajet et al. 1985) for confirmation. Plants infected jointly with RTBV and RTSV or individually by any of the component virus were used as source plants.

Dispersal of Tungro Associated Viruses

A total of 20 plants including healthy, RTBV and/or RTSV were placed in a metal cage of 52 × 52 × 73 cm in size, at randomly in split plot design in 6 combination and 3 relative proportions. The combinations were S: B: H; B: S: H; S: B + S: H; B: B + S: H; B + S: S: H; B + S: B: H (where B = RTBV, S = RTSV and H = healthy) and the proportion of healthy and tungro infected plants included 12: 2: 6; 8: 2: 10 and 4: 2: 14. For each of the combination and proportion separate cages were used. In each cage 40 virus free GLH adults were released and two days after the release of GLH healthy plants placed earlier in the cages were removed and grown in a screen house. After 25 days plants were serologically tested to access the extent of transmission of two virus components.

In a separate trial singly infected plants by RTBV or RTSV of TN1, IR36 and IR56 were placed in different combinations amongst the varieties and released RTV free GLH adult for two days acquisition as well as inoculation access feeding. After 2 days of confinement, infectivity of GLH were tested for each combination using 1-wk old TN1 seedlings as test plants. Formerely plants infected either by RTBV or RTSV were also removed from the cages and tested after 3-wk to measure the probability of challenge or cross inoculation among themselves.

Dispersal of Virus and Density of Source

Tungro infected (RTBV + RTSV) 3-wk old source plants of IR36, IR56 and TN1 were combined with healthy plants of same age to have a 10, 30 and 50% density of RTV infected source plants. Plants were placed in a cage in randomized block design with three replications and 40 non-viruliferous GLH adults were released in the cage. Two days after the release, healthy plants were

removed from the cage and grown in the screen house and after one month scored for tungro incidence based on symptoms and by latex serology.

Serology

Both the latex flocculation test and ELISA were used for confirmation of RTV infection in inoculated plants. The latex test followed the method described by Omura et al. (1984) and ELISA the method of Bajet et al. (1985).

For ELISA, leaf samples were homogenized separately with 0.02M phosphate buffer saline (*p*H 7.4) containing 0.05% Tween 20. The volume of the buffer solution was adjusted to obtain extracts of 20 times dilution. Extracts of healthy, and doubly infected TN1 served as the controls. Samples with absorbance at 405 nm more than three times the absorbance (means of four wells) of healthy control were considered positive.

Results and Discussion

Dispersal of Tungro and Type of Source Viruses

When RTBV, RTSV and RTBV + RTSV sources of TN1 were combined with healthy TN1 plants and exposed to virus free GLH for 2 days in a cage, formerly healthy seedlings had infection with the viruses and developed tungro symptoms (figure I). Irrespective of combination and proportion of sources, subsequent percentage of infection increased with increase in number of source plants. Percentage of subsequent infection on healthy TN1 plants appeared to be higher when singly infected RTBV source was combined with singly infected RTSV source than when it was combined with RTBV + RTSV infected source. Percentage of plants infected with RTSV alone was higher under the presence of more RTSV source, when RTBV + RTSV source was

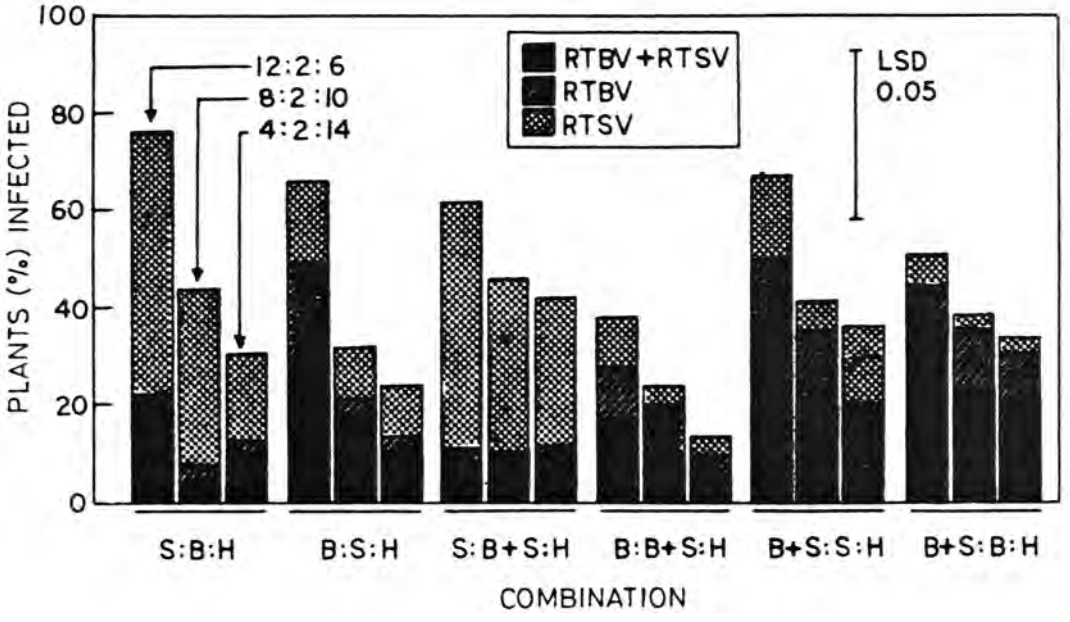

Figure 1 Percentage of subsequent infection with RTBV and RTSV of healthy plants when healthy (H) RTBV + RTSV - infected (B + S), RTBV - infected (B) and RTSV - infected (S) plants in six combinations and three proportions were confined in a cage with virus free *N. virescens* for 2 days

combined with RTBV source, the infection was mostly with both RTBV and RTSV, although percentage of subsequent infection was lower. Presence of tungro source in the field is most important for spread of the disease. Shukla and Anjaneyulu (1982) observed that presence of one infected plant in a population of 81 plants resulted a rapid spread of disease with presence of 2 GLH per plant. RTSV alone or RTBV + RTSV infected plants served as a better source for the spread of tungro disease and present results indcate that the presence of higher number of RTSV infected plants in the field will increase the chances of RTBV spread.

Singly infected RTBV and RTSV plants of three cultivars were confined in a cage with different combination for 2 days with virus free GLH, they had challenge infection with other viruses (table 1). Generally percentage challenge infection of RTBV infected plants by RTSV was higher than that of RTSV infected plants by RTBV when individually infected plants were combined in the same cage. The rate of challenge infection appeared higher when RTBV or RTSV infected plants of the same cultivar were combined rather than when each cultivar was combined with susceptible TN1.

A higher percentage of challenge infectior within the same cultivar may be due to higher movement of GLH. Generally movement of GLH is more in resistant cultivars than the susceptible one (Ling & Carbonell 1975) under field conditions. In GLH resistant cultivars percentage of RTBV infected plants are usually high, though some percentage of plants are also infected by RTSV. In presence of high vector population possibility of challenge infection of RTBV infected plants by RTSV may be high. High movement of GLH on resistant cultivars may intensify the chances of cross infection as it happend with IR56 in the present study.

Virus Acquisition by GLH Under Confinement with Singly Infected RTBV and RTSV Source

GLH adults that had confined in a cage for 2 days with RTBV infected plants of TN1, IR36 or IR56 and RTSV infected TN1 plants 24 each, were infective of RTSV alone in high percentage (table 2). GLH that had confined in a cage with RTBV infected IR56 and RTSV infected TN1 were infective of only RTSV in high rates. GLH that were infective of both viruses were obtained but at lower rates when confined with RTSV infected TN1 and RTBV infected TN1 or IR36. Higher percentage of RTSV infection in respect of all the combination is due to

Table 1 *Percentage of cross infection within RTBV or RTSV infected plants of three rice cultivars when placed in different combinations and exposed to virus free GLH adult in a cage*

Cultivars		% RTBV infected plants cross infected with RTSV	% RTSV infected plants cross infected with RTBV
RTBV-infected plants	RTSV-infected plants		
IR36	TN1	33	16
IR56	TN1	12	8
TN1	IR36	50	20
TN1	IR56	16	8
TN1	TN1	54	16
IR36	IR36	62	20
IR56	IR56	48	20

Table 2 *Percentge of N. virescens that transmitted RTBV and/or RTSV on TN1 test seedlings after 24 hr acquisition access feeding from a mixed population of RTSV-infected TN1 and RTBV-infected TN1, IR36 and IR56 under caged conditions*

Combination of cultivars		*N. virescens* (no.) tested	% *N. virescens* transmitted		
RTBV-infected plants	RTSV-infected plants		RTBV + RTSV	RTBV	RTSV
TN1	TN1	34	6	9	38
IR36	TN1	52	4	8	46
IR56	TN1	50	0	0	70

[1] Plants were indexed by ELISA

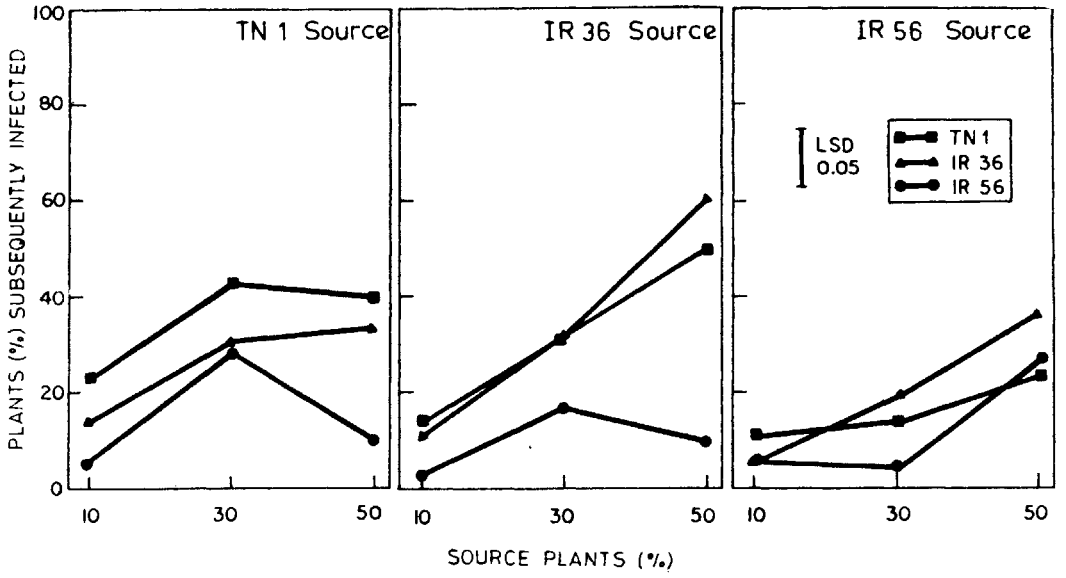

Figure 2 Percentage of tungro infection when 25-day-old healthy seedlings of TN1, IR36 or IR56 and tungro infected seedlings of TN1, IR36 or IR56 at the same age were combined in 9:1, 3:7 or 5:5 proportion and then confined with virus free *N. virescens*

susceptibility of TN1 variety to both virus and vector.

Dispersal of RTBV and RTSV in GLH Resistant Cultivars

Plants of each cultivar were infected with tungro when confined with GLH vector and virus source but the percentage of infection in each cultivar varied depending on the cultivars used as virus source and its density (figure 2). Cultivars TN1 and IR36 appeared to be a better source to disperse the viruses. Whereas IR56 plants combined 1:1 with tungro source of IR36 or IR56 had low levels of subsequent infection than TN1. During the confinement TN1 and IR36 showed higher percentage of joint infection with RTBV and RTSV, while IR56 generally had higher percentage infection with RTBV alone. The results of this experiment

confirmed the previous observations of Ling(1975)and Rao and Anjaneyulu (1979) that all the rice cultivars do not serve as equally good source for the spread of tungro disease. Susceptible cultivars to both GLH and RTV serve as a better source for virus transmission. Our observation also indicated that susceptible TN1 and intermediate IR36 served as better tungro source than IR56.

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