5. INVESTIGATIONS ON RICE TUNGRO VIRUS IN INDIA

M. D. MISHRA*

The investigatins on paddy viruses were initiated at the Indian Agricultural Research Institute, New Delhi, in 1966, when occurrence of rice tungro virus (RTV) and some other virus like maladies were recorded from some districts of West Bengal and Orissa as the first record in India (Raychaudhuri *et al.* 1966, 1967 and 1969). The existence of RTV in the country remained obscure till 1969, when severe outbreak of RTV epidemic took a heavy toll of the rice crop in Uttar Pradesh and Bihar (John, 1970). Consequently, a systematic survey was conducted as a collaborative effort of the Department of Plant Protection and Quarantine, Indian Agricultural Research Institute, other constituent institutes of ICAR and the Ford Foundation during the year 1970, 1971 and 1972. These concerted efforts not only confirmed the presence of RTV in India, but also the existence of endemic pockets in West Bengal, Bihar and Uttar Pradesh. Subsequently, the RTV infection was also reported from Assam, Manipur, Tripura, Kerala, Andhra Pradesh, Mysore and Madras besides a few localities in Punjab and Western Utta Pradesh. It is evident, thereafter, that RTV is not only wide spread but also fairly well established in some states in the north-eastern region of India. As the disease is a potential menace and is apt to cause devastating damage within a short time, a thorough investigation into its various aspects was considered necessary to be forearmed against the malady. Accordingly, a research project was implemented at the Indian Agricultural Research Institute (IARI) and many interesting findings have since emerged which are briefly presented here.

The vectors

The virus is known to be transmitted by leaf-hopper vectors namely, *Nephotettix virescens* (Distant.) *N. nigropictus* (Motsch.) and *Recilia dorsalis* Motsch. in a non-persistant manner. Transmission tests with *R. dorsalis* at the IARI yielded only negative results. *N. virescens* has been found to be highly efficient vector irrespective of the strains of RTV involved. The minimum period of acquisition and inoculation access were found to be 15 min. and 5 min., respectively . Infectivity of the vector was usually lost within 2 days and was never retained more than 3 days. The minimum acquisition feeding period had earlier been reported to vary from 5 to 30 min. (Singh, 1969; Wathanukul and Weerapat, 1969), and minimum inoculation feeding period from 7 to 15 min. (Ling, 1968; Lim, 1969). Similarly, the maximum retention period was reported by others to vary from 2 to 5 days (John, 1968; Ling, 1966).

A very interesting phenomenon of differential transmission of RTV strains was encountered in the case of *N. nigropictus*. In the recent studies at IARI (unpublished), *N. nigropictus* selectively transmitted the RTV 4 strain (Aligarh isolate) but not RTV 3 strain (Dhaniakhali isolate). The percentage of transmission was to the extent of 36.12% with only 24 hours of aquisition feeding. The very low percentage of transmission of the Faizabad isolate of RTV by the same vector, reported by us earlier (Raychaudhuri *et al.*, 1971) appears to be due to the same reason.

* Virologist, Division of Mycology & Plant Pa hology, Indian Agricultural Research Institute, New Delhi-110012, India

The virus

The rice tungro virus was purified by Galvez (1968) and Saito *et al.* (1970), each following different method. The virus was purified at IARI by adopting a modification of the method, followed by Saito *et al.* The leaves of RTV infected paddy plants of cultivar Taichung Native 1 (TN-1) were cut into 0.5 cm small pieces. These freshly cut leaf pieces were impregnated in a solution of 0.2 M ascorbic acid in 0.1 M phosphate buffer of pH 7.0 and frozen overnight in the deepfreeze. Next morning after thawing, the leaf pieces were homogenized in a Waring blender at full speed. The homogenate was squeezed through the two layers of muslin cloth. After adding carbon tetrachloride (20 %v/v), the mixture was stirred vigrously on a magnetic stirrer for 15 minutes and then centrifuged at 10,000 rpm for 15 minutes. The water layer after separation was centrifuged at 10,000 rpm for 20 minutes. The supernatant was filtered and given a high speed centrifugation at 29,000 rpm for 90 minutes. The pellet was resuspended in 0.01 M EDTA or 0.1 M phosphate buffer pH 7.0 and clarified by centrifugation at 6,000 rpm for 15 minutes. A second cycle of high and low speed centrifugation was given for further purification of the sample, if required. The electronmicroscopic examination revealed presence of polyhedral particles of 30–35 nm diameter.

In ultra-thin section the pholyhedral particles were found arranged in rows and aggregated in groups within the nuclear membrane, probably representing intranuclear crystalline inclusions.

The strains

Several strains of RTV have been reported and designated as S,M and T strains in the Philipines and RTV 1, RTV 2A, RTV 2B and RTV 3 in India (Rivera and Ou, 1967; IRRI, 1970; Anjaneyulu and John, 1972). Recently, a new strain of the virus, designated as RTV 4, has been described from this laboratory (Mishra *et al.*, 1976). This strain was obtained as an isolate collected from Western U.P. (Aligarh) and is milder than the others.

The milder RTV 4 strain induced cross-protection against the infection with the severe RTV 3 strain (Mishra *et al.*, 1976). In such tests, the TN-1 test plants were first inoculated with the milder strain. Seven days later, when initial symptoms were noticeable the same test plants were again inoculated with the RTV 3 strain — " the challenging strain'. However, when the inoculation with "challenging strain" was done 24 hours after the first inocculation, the extent of stunting was found to be considerably more than induced by either of the strains individually. Such an additive effect was observed irrespective of the sequence of inoculations, but the extent of stunting was always more if the severe strain was inoculated first (Niazi *et al.*, 1975).

During these studies at the IARI, three distinct strains were detected from the four isolates collected from different parts of northern and north-eastern India. The isloates collected from Dhanikhali and Tarkeshware (in West Bengal) was identified as RTV 3 strain, from Mastipur (in Uttar Pradesh) as RTV 1 and from Aligarh (Uttar Pradesh) as the new RTV 4 strains. The severe strain seems to be prevalent in the main endemic regions of West Bengal, where more than one rice crop is grown.

The designation of these strains is presently based on the differentials which are different for the Philippine and Indian strains, leading to some confusion in the nomenclature and identification. All the five strains of RTV, recorded from India, behave like 'S' strain on the Philippine set of differentials but are not further separable on them. The following key has been prepared to give an idea about the latest position in this regard. The "T" strain and all the Indian strains have been grouped here

under "S" strain on the basis of the reaction on FK 135.

Key to the strains of RTV

A_1. Fk 135 shows mottling and no distinct interveinal stripes .....M. strain
A_2. Fk 135 shows distinct interveinal stripes .....M. strain
B_1. Produces distinct narrowing of leaves in TN 1 .....T. strain
B_2. No narrow of leaves in TN 1
C_1. Pacita susceptible. Latisail, if susceptible, shows foliar symptoms.
D_1. Does not infect Ambemohar 159 and Pankhari 203 .....RTV 1
D_2. Infects both Ambemohar 159 and Pankhari 203
E_1. Latisail resistant .....RTV 2A
E_2. Latisail susceptible
F_1. Ambemohar 102 and Kamod 253 susceptible. TN-1 severely affected initially but later produces green leaves. If not, at least Pankhari 203 and Latisail shows masking of symptoms .....RTV 3
F_2. Ambemohar 102 and Kamod 253 resistant. No such recovery or masking .....RTV 2B
C_2. Pacita resistant. Latisail symptomless carrier. .....RTV 4

Leaving the "M' strain as such, the various constituents of 'S' strain complex should be designated as 'S' in numerical sequence. Accordingly 'T', RTV 1, RTV 2A, RTV 3 and RTV 4 should be designated as S 1, S 2, S 3, S 4, S 5 and S 6, respectively. It will systematise the available information. A more comprehensive treatment of the subject is not possible unless RTV strains occurring in various countries are identified. The situation calls for immediate attention to the study of strainal differences on global scale with due care to maintain uniformity in experimental material and procedures. The standard set of differentials that we recommend for this purpose consists of Ambemohar 102, Ambemohar 159, FK 135, Kamod 253, Latisail, Pacita, Pankhari 203 and TN-1. The differentials should be inoculated individually at 2–3 leaf stage. Three adults of *N. virescens* should be allowed to feed on each test seedling for 6 hours after prior acquisition feeding of 24 hours. Data on the percentage of infection, the extent of stunting, foliar coloration and reduction of tilering should be recorded 30 and 60 days after inoculation (Basu, *et al.* in press).

Preservation of cultures

It is felt that cultures of all the above strains should be properly preserved and made available to the researchers for comparison and studies. We are able to preserve cultures of RTV strains, which remained viable and recoverable through the feeding *N. virescens* even after a storage of 20 months. For this purpose, the virus affected leaves were preserved as such in tubes containing $CaCl_2$, and stored at the frigidair temperature (5–7°C). After 20 months of storage, the preserved leaves were soaked overnight in 0.5% solution of Na_2SO_3 at cool temperature and placed upright on sterilized sand saturated with phosphate buffer (pH 7.0) and caged with adults of *N. virescens* for 5–18 hours. The test leafhopers could then infect healthy TN-1 seedlings providing ample evidence of efficacy of this technique to preserve RTV—affected leaves for at least 20 months (in press). The effect of longer periods of storage is under investigation. This technique is the modification of the method described by Bos (1969) for the preservation of saptransmissible viruses.

Symptoms of paddy plants

The rice tungro virus infection on paddy cultivars is generally expressed by such disease syndromes as yellowing/interveinal chlorosis of leaves, reduction in plant height (stunting), reduction in tiller numbers, delay in booting/flowering and mortality of

infected plants. Besides this, the percentage of infection is always taken into consideration for the expression of the virulence of infection. All these disease symptoms are expressed differentially with the genotypes of the cultivars, age of the plants at the time of infection and the viral strains involved. In two of the highly susceptible cultivars namely TN-1 and N 136 stunting and the reduction in the fresh weight induced as a result of RTV infection were found to be highly significant and indirectly related to the age of the plants at the time of infection. The tiller numbers, root length and booting also appeared to be affected, but not as significantly as the height and weight of the infected plants. The mortality was very high when infection was within 27 days of sowing, but none beyond 34 days as in the case of N 136 cultivar. We consider stunting being the most significant factor in the disease syndrome, is also the most important factor for determining the virulence of different strains of RTV. Besides it is also expressed differentially in different cultivars, maximum being in TN-1 plants i.e. to the extent of 75% when inoculated 10 days after sowing. Earlier, Ling and Palomar (1966) reported significantly different percentage of stunting in the plants infected at different ages.

The yield loss suffered by TN-1 was estimated to the extent of 75%, if the infection was within 45 days after sowing. Later infections were expressed only in the sprouts of the stubbles after harvesting (Ghosh *et al.*, 1975).

The host range

A number of graminaceous weeds were reported to be artificially inoculable with the rice tungro virus (Wathanakul, 1964; Rivera *et al.*, 1969). Attempts were made to search for the alternative hosts amongst the graminaceous weeds generally observed in the rice fields (Mishra *et al.*, 1975). Nine plant species, which were found to be artificially inoculable through the feeding of viruliferous vectors are, *Bothriochola odorata, Brachiaria reptans, Echinochloa colonum, Paspalam distichum, Pennisetum typhoides, Setaria verticillata, Dactyloctenium aegyptium, Digitaria adscendens* and an unidentified grass.

These grasses showed differences in expression of symptoms and also in the commencement and duration of recoverable phase of viral inoculum. The virus was recoverable one year after inoculation from *B. reptans, D. aegyptium, P. distichum, P. typhoides* and the unidentified grass, but not from others which were found to lose the infectivity within 45 days after inoculation. In the case of *D. aegyptium* the virus was recoverable only one year after inoculation, indicating thereby a very slow multiplication of the virus in the host.

Many of these graminaceous weeds were collected from the endemic regions of West Bengal and at the IARI experimental fields and tested for the presence of viral infection. *Eleusine indica, Hemarthria compressa* (*Rotebellia compressa*), *Polypogon monspeliensis, Sorghum helpanse* and *Sporolus tremulus* were found to harbour natural infection of RTV.

The period of survival of the vector on these natural hosts was considerably longer being 18–25 days, as against other artificially inoculable host, where the vectors did not survive for more than 6 days (Mishra *et al.*, 1973).

The epidemic

The spread of rice tungro virus is essentially dependent on the influx of the principal leafhopper vector, *N. virescens,* besides the presence of viral inoculum and a suspectible cultivar at vulnerable stage. The "Survey reports" on the basis of visual observations, indicated appearance of the virus infection only after the vector population reaches the level of one or two adults per plant in a crop. Further increase in the

vector population results in the spread of the viral infection (Lowe and Nandi, 1972). In our experiments, both infection and spread of the disease could be caused by lesser population of vectors in a susceptible cultivar.

Generally, the leafhoper population reaches its peak in the middle of later Kharif crop (Monsoon-crop sown in July or August) season, i.e. September or October. A survey of *Nephotettix* population in Delhi revealed that the green leaf-hoppers (*Nephotettix* spp.) appear towards the end of July and are abundant by about September end, (Mishra *et al.*, 1971). In fact humidity, i.e. frequency of summer or pre-monsoon rains play an important role in the magnitude and time of the first influx of vector population in the Kharif crop. This might account for the early appearance of vectors in Eastern and North-eastern India, where summer is milder and much more humid than in Delhi and rice is available as a host of these vectors all the year round. In Purnea District of Bihar, the vector population was detected as early as in May. The time of appearance and magnitude of vector population are of utmost importance since study build up of leafhopper population during the nursery stage or shortly after transplantation may result in heavy RTV infection. Such infections were detected in parts of West Bengal, Bihar, Uttar Pradesh and Orissa. These endemic pockets may further serve as a focal point for spread of infection in vast areas during 'tungro year's as happened in 1969 and to some extent in 1971.

At IARI, the development of RTV epidemic in suspectible cultivars, namely, N 136 and TN-1 was studied. Both viral inoculum and vector population were built up early in the crop season by periodic sowing of TN-1 and releasing laboratory-bred viruliferous vectors. Care was taken to provide irrigation as and when necessary. The crop in the plot, which harboured both RTV inoculum and the leafhopper vector population, was harvested just after the test cultivars were sown in adjoining plots. Periodic counts of the disease as well as the vector were taken and the progress of the epidemic was analysed and charted.

Analysis of disease progress curve in different rice cultivars revealed differential progress of epidemic. While cultivars like 6636 (Assam collection) behaved as "fast tungro" type, IET 1991, Pusa 2–21 and 10596 (Assam collection), etc. proved to belong to "slow tungro" category (unpublished).

We have also been studying the dynamics of the spread and the progress of RTV infection in the healthy crop. These interesting investigations are in progress at the moment.

Perpetuation of RTV and the vectors

Surveys between croping season in endemic areas of West Bengal revealed that the infected stubbles of the harvested crops play a very significant role in the perpetuation of viral inoculum.

This does not minimise the importance of several grassy weeds recorded by us earlier as naturally infected. Our intensive observations on the common winter grass, *Polypogon monspeliensis* have proviled convincing evidence of the importance of the grass as reservoir for both the virus and the vector to tide over the winter months.

Periodic counts of the vectors in our experimental plots in Delhi during winter revealed the population to be quite low, the nymphs being more predominant than the adults. This surviving population, however, could carry over the RTV inoculum to the next rice crop sown in the neighboring plots in March. The nymphal stage of *N. virescens* was found to be greatly lengthened during winter. The insect could withstand long periods of fasting on moist soil, indicating markedly reduced metabolism (Basu *et al.*, 1976).

Varietal screening

For field screening of rice varieties in Delhi, we developed a technique to put them under adequate disease pressure by creating artificial epidemic to overcome the uncertainties of natural outbreaks. The principle is to initiate RTV epidemic in isolated fields through release of viruliferous vectors and subsequent maintenance of the 'tungro garden' by round the year cultivation of rice (Raychaudhuri *et al.*, 1971). This method was found to be very satisfactory and is being tried every year with modifications as and when necessary.

Without going in details, some of the interesting findings are briefly mentioned here. During the field screening trial of 1975, Pusa 2–21 and Pusa 33, two of the varieties developed at the IARI, indicated 4.8 and 7.8% infection, respectively as against 94.6% infection noticed in TN-1. The performance of BJ-1, Ratna, IET 1991, M Sung Song, NC 1626, Kataribhog, Intan, Bhadas 1303, Latisail, IR 20, IR 22, etc. proved to be promissing from the standpoint of resistance against RTV. Greenhouse tests invariably magnified susceptibility of the varieties, obviously due to forced feeding by the vector.

Control

Our approach to the control of tungro disease has been twofold namely, the use of resistant varieties like Ratna, Sona, Pusa 2-21 and Pusa 33 and secondly, the evaluation of insecticidal measures to keep the vector population in check particularly during the vulnerable stage of the crop. The efficacy of Furadan as seed dressing and seedling soak has long been reported from our laboratory (Mitra *et al.*, 1970), which is now being widely used against the leafhoppers. In our recent studies, Lannate was also found to be useful as seed dressing in reducing vector population and thereby the incidence of RTV infection.

Conclusion

This account of rice tungro virus is just an attempt to let you know what we have been doing about the tungro menace, which is a grave problem of the South-eastern Asiatic countries. Although a lot of work has been done during the last decade, there still remains much to be desired. For instance, our knowledge is still meagre in respect of many important aspects such as the extent of strainal variations in the region, their epidemiological potentialities, the mode of perpetuation in different ecoclimates and the factors governing devastating epidemics etc. A meeting like this which the Japanese Government has taken the lead, is often necessary for filling up the communication gap, pooling our information and revitalizing our energies. Further the exchange of active workers in this field would enable us to have insight into the problems and modify our approaches accordingly.

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Discussion

D. A. Benigno, Philipines: You mention strains by letters like S,M,T, what do these letters stand for?

Answer: I have suggested S & M strain which produce distinct interveinal stripes on cultivar FK135, and M, as mild strain which do not produce stripes on FK135. The other strains according to us are considered as substrain of S. This includes T strain also and these substrains are referred as S_1, S_2, S_3, S_4, S_5 and S_6 for the convenience in its nomenclature instead of RTV_1, RTV_{2A}, RTV_{2B} and so on.

K. Sogawa, Japan: How are you forecasting the occurrence of tungro virus disease in India?

Answer: Forecasting as principally based on forecasting the occurrence of its vectors. These may be 'tungro years' or "non-tungro years" depending on the build up of vector population during the vulnerable stage of the rice plant i.e. when they are at nursery stage the rice plants are most susceptible.

There is no other way by which we can forecast the RTV epidemic. Low and Dandi have worked out some sort of formulae of number of hoppers per plant to be responsible for initiation and spread of RTV infection. We are of opinion that even a lower population than what has suggested may bring about RTV infection and spread to a considerable extent.

Y. Saito, Japan: Are tungro virus particles exist within the nuclear membrane?

Answer: It appears to me like this, I would however like to have your opinion — whether the structure which I am getting as somewhat a nuclear inclusion and having a lettuce like structures is of viral nature?

I. N. Oka, Indonesia: 1. What commercial varieties are you recommending to combat the tungro virus disease in India? 2. What is the gene source(s) for resistance to tungro virus disease in your recommended varieties?

Answer: 1. The varieties recommended for use in the RTV affected areas are Ratna, Sona, and also two varieties Pusa 2-21 and Pusa-33 (bred at IRRI). They are though not absolutely resistant, but can considered as tolerant. 2. Pankhari 203, and Kataribhog, especially the latter one are reported to be resistant cultivars as used as gene source for resistance besides a few others.

Y. Nagai, Japan: How long does the effect of chemical "Lannate" dressing lasts for the control of the vector of tungro?

Answer: It lasts for about a month. In one of our field trials, application of 800 mg/100 gms of TNI seeds, the incidence of RTV was reduced by approximately 90% as from the control.