Strains of Rice Tungro Virus

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ABSTRACT

Symptomatology, transmission, varietal reaction, and cross-protection tests of four distinct isolates of rice tungro virus collected from various parts of India were studied. Four strains of rice tungro virus designated RTV_1, RTV_{2A}, RTV_{2B}, and RTV_3 were identified. RTV_1 produced mild symptoms on Taichung (Native) 1, and did not infect any one of the six differential cultivars tested. RTV_{2A} and RTV_{2B} were indistinguishable on Taichung (Native) 1, on which they produced severe symptoms. RTV_{2A} infected Pankhari 203, Kamod 253, Ambemohar 159, and Ambemohar 102, and produced severe symptoms. RTV_{2B} infected Latisail, Pankhari 203, and Ambemohar 159, and produced mild symptoms. Kamod 253 and Ambemohar 102 were susceptible to RTV_{2A} but resistant to RTV_{2B}. RTV_3 produced severe symptoms on Taichung (Native) 1 in the initial stages, but the infected plant soon recovered and produced green leaves. This strain also infected five out of six differential cultivars. Kataribhog was resistant to all the four strains.

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Rice tungro virus (RTV) is a good example of non-propagative leafhopper-transmitted virus (3). The symptoms of tungro include stunting, incomplete emergence, and twisting of new leaves, which also display interveinal chlorosis or chlorotic spots. The older leaves exhibit yellow-orange discoloration from the tip downwards.

Transmission of the virus by *Nephotettix impicticeps* Ish. was first demonstrated by Rivera & Ou (4). Prior to 1967, existence of strains was not known. Rivera & Ou (5) reported the occurrence of two strains in The Philippines. They designated them as "S" and "M" based on differential symptomatology induced on a rice cultivar, FK 135. The cultivar Pankhari 203, reported resistant to both Philippine strains, was susceptible to the strain existing in East Pakistan, indicating that the latter may be different from the Philippine strains (2). Shastry et al. (7) reported the occurrence of two strains of tungro in India, and designated them as RTV_1 and RTV_2.

In order to determine strains occurring in India, comprehensive studies on various isolates collected from different parts of the country were made at the All-India Coordinated Rice Improvement Project (AICRIP), Hyderabad-30. These results are reported here.

MATERIALS AND METHODS.—Nonviruliferous adults of *Nephotettix impicticeps* Ish. were used for transmission studies. The isolates of RTV were collected from different parts of the country in the form of infected stubbles. Isolate 1 was the local collection maintained at the greenhouse of AICRIP; Isolate 2, from Tirupathi (Andhra Pradesh); Isolate 3, from Sasaram (Bihar), and Isolate 4, from Chinsurah (West Bengal). Nonviruliferous adults of *N. impicticeps* were fed on the stubbles for 24 hr; these were then inoculated on Taichung (Native) 1 seedlings in the 3- to 4-leaf stage, maintained under insect-proof conditions.

RESULTS.—Ten isolates were collected from different regions of Andhra Pradesh, Bihar, and West Bengal. All isolates were inoculated to Taichung (Native) 1 and Pankhari 203. Based on the severity of symptoms, such as stunting and leaf coloration, identification was made of four distinct isolates. They were maintained on Taichung (Native) 1.

TABLE 1. Symptoms of rice tungro virus isolates on the cultivar Taichung (Native) 1

Isolate no.	No. plants infected/ inoculated	Incubation period in days	Height of the plants 60 days after inoculation, cm	% Stunting	Color of leaves 60 days after inoculation[a]	
					Young	Old
1	30/40	6.4	14.0	76.7	Yellow	BO
2	34/40	8.3	44.3	26.2	DG	Yellow
3	23/40	6.8	14.2	76.3	Yellow	BO
4	34/40	6.2	31.4	47.7	Green	Orange
Healthy			60.0		DG	DG

[a]BO = bright orange. DG = dark green.

Symptoms on Taichung (Native) 1.—Most severe symptoms of stunting and yellowing of leaves were observed on plants inoculated by either Isolate 1 or Isolate 3 (Table 1). Plant growth was considerably restricted after infection. Forty to 50 days after inoculation, the plants ceased growing. Most plants, in the case of Isolate 1, and all, in the case of Isolate 3, were killed within 60 days. Plants infected by Isolate 2 exhibited mild symptoms. They recovered completely and produced dark-green foliage, although the presence of virus could be detected at any stage. Plants infected with Isolate 4 exhibited severe symptoms in the initial stages of infection, but within 1 month after inoculation, all plants recovered from the disease and the newly emerging leaves were green, with barely distinguishable mild chlorotic spots. Virus could be recovered both before and after recovery of the plants (Fig. 1, above).

All isolates were readily transmitted by the vector *N. impicticeps.* The four isolates used could be inoculated on to the test plants immediately after acquisition by the insect, a point clearly demonstrating that there was no incubation period for the four isolates. The percentage of transmission in the case of individual isolates varied from 58 in Isolate 3 to 75 in the case of Isolate 1 and 85 in the case of Isolates 2 and 4 (Table 1).

Varietal reaction.—Shastry et al. (6) reported that six cultivars, viz, Kataribhog, Latisail, Pankhari 203, Kamod 253, Ambemohar 159, and Ambemohar 102 were resistant to rice tungro virus. These cultivars were used as differentials in the current study. Inoculations were done on 20-day-old seedlings. Height measurements were taken 30 days after inoculation.

Kataribhog was resistant to all isolates, and Latisail was resistant to Isolates 1 and 2 (Table 2). Isolates 1 and 4 generally produced severe symptoms. The percentage of infection also was high. In contrast, Isolate 2 did not infect any of the cultivars tested. The reaction to Isolate 3 was intermediate. Latisail, Pankhari 203, and Ambemohar 159 were susceptible; the remaining cultivars were resistant. Percentage of infection was low, however. The symptoms induced by Isolate 3 on Pankhari 203 and Ambemohar 159 were less severe, as compared to those induced by Isolate No. 1.

Reaction of Philippine differential cultivars to the isolates.—Three cultivars of rice FK 135, Acheh, and Pacita used in The Philippines to differentiate the "S" and "M" strains of tungro virus were inoculated with the four isolates. Among these, only FK 135 showed distinct interveinal yellow striping symptoms. Pacita and Acheh showed mild striping symptoms. None of these cultivars revealed the characteristic yellow orange discoloration of leaves. The degree of striping on FK 135 depended on the virulence of the isolate. Isolate 1, for example, caused severe striping as compared to Isolate 2.

Cross-protection tests.—(i) *Within the plant.*—Severity of symptoms produced by each isolate on the susceptible Taichung (Native) 1 varied considerably. Two months after inoculation, Isolates 1 and 3 caused considerable (76%) stunting, whereas plants infected by Isolates 2 and 4 were stunted only

Fig. 1. (Above) Characterization of four isolates of rice tungro virus on rice cultivar Taichung (Native) 1. Isolates 1 and 3 cause extreme stunting and foliar discoloration. Isolates 2 and 4 are milder, 2 being the least virulent. (Below) Cross-protection studies with isolates of rice tungro virus on susceptible Taichung (Native) 1. IS 2 and IS 2 + IS 1 show mild reaction whereas IS 1 shows virulent reaction.

TABLE 2. Varietal reaction to four isolates of rice tungro virus

Cultivar	Isolate 1[a]			Isolate 2			Isolate 3			Isolate 4		
	A	B	C	A	B	C	A	B	C	A	B	C
Kataribhog	0/25	0	–	0/25	0	–	0/24	0	–	0/25	0	–
Latisail	0/15	0	–	0/15	0	–	8/15	11.8	+	12/14	28.8	+
Kamod 253	15/18	50.6	+	0/18	0	–	0/17	0	–	16/18	43.7	+
Pankhari 203	25/30	52.3	+	0/28	0	–	20/30	26.1	+	26/30	54.7	+
Ambemohar 102	10/12	45.7	+	0/12	0	–	0/12	0	–	10/12	40.0	+
Ambemohar 159	11/12	54.4	+	0/12	0	–	6/12	26.5	+	12/12	50.0	+

[a]A = plants infected per inoculated; B = percentage of stunting; C = indexing of the virus; + = present; – = absent.

to the extent of 26 and 47%, respectively. Infection by Isolates 1 and 3 caused yellowing of plants, whereas Isolates 2 and 4 caused green to light-green coloration of foliage. Isolate 3 produced milder symptoms on Pankhari 203 as compared to Isolate 1. These differences were used as criteria in assessing cross-protection tests.

Three rows of 15-day-old seedlings of Taichung (Native) 1 (9 seedlings/row) were transplanted in five boxes, and Pankhari 203 was similarly planted in one box. Five days after planting, left and middle rows were inoculated by milder isolates. When plants were beginning to exhibit symptoms, middle and right rows were inoculated with severe isolates. The extreme left row thus represented the control of the milder isolate, the extreme right row the control of the severe isolate, and the middle row a combination of both, the severe isolate having been superimposed on the milder one.

Results indicated that the middle rows in all the boxes which were inoculated by a mild isolate followed by a virulent isolate developed symptoms of the left row only, i.e., those induced by inoculating with the mild isolate only (Fig. 1, below). Plants in the middle row exhibited less stunting, and the leaves were green as compared to the virulent control. Indexing also revealed that only the milder form of the virus could be recovered (Table 3). These results indicate that the milder form of virus protected the plants from subsequent infection by the severe isolate. Similar results were obtained when Pankhari 203 was first inoculated with the milder Isolate 3, and later by the more virulent Isolate 1.

(ii) *Within the vector.*—Cross-protection tests within the vector were attempted between Isolates 1 and 2. The vector *N. impicticeps* was confined on plants infected by both isolates individually for 24 hr and, after this feeding period, the leafhoppers from plants infected with Isolate 1 were transferred to those infected by Isolate 2 and vice versa for another 24-hr period. Then the leafhoppers were released individually on Taichung (Native) 1 seedlings for inoculation feeding for 24 hr. The plants were scored for symptoms 2 months after inoculation. Most of the inoculated plants produced symptoms typical of those induced by the latest acquired isolates, indicating lack of cross protection by the two isolates in the vector.

DISCUSSION.—Among the four isolates studied, the reaction pattern falls into two broad groups, two isolates inducing a milder reaction on Taichung (Native) 1 and the other two a severe reaction. In this respect, the original classification of RTV_1 and RTV_2 as mild and severe strains, respectively, is sustained. Based on the cross-protection studies and the reaction on different cultivars, it is clear that the four isolates varied considerably from each other, and hence they have been considered as different strains of rice tungro virus.

RTV_1 as reported by Shastry et al. (7) exhibits mild symptoms on Taichung (Native) 1 and did not infect Pankhari 203, Ambemohar 159, Ambemohar 102, and Kamod 253. In our study, Isolate 2 behaves similarly, and hence this isolate has been equated with RTV_1. Isolates 1 and 3 produce similar and severe symptoms on Taichung (Native) 1, like RTV_2. Even though these two isolates are indistinguishable on Taichung (Native) 1, they could be differentiated very easily on differential cultivars. Hence, these two strains have been designated as RTV_{2A} (Isolate 1) and RTV_{2B} (Isolate 3). Isolate 4 has a distinct reaction on Taichung (Native) 1. It first produces severe symptoms, and within 1 month after inoculation, the infected plants recover and produce green leaves. It is distinctly different from Isolate 2 based on its reaction on differential varieties. Hence, this isolate has been designated as RTV_3.

TABLE 3. Cross-protection tests between the different isolates of rice tungro virus

Cultivar	Isolate used[a]		Final symptoms characteristic of isolate
	First inoculation	Second inoculation	
Taichung (Native) 1	2	1	2
Taichung (Native) 1	2	3	2
Taichung (Native) 1	2	4	2
Taichung (Native) 1	4	1	4
Taichung (Native) 1	4	3	4
Pankhari 203	3	1	3

[a]First inoculation 5 days after planting; second inoculation made when plants began to show symptoms resulting from first inoculation.

The cultivars used in differentiating the S and M strains from The Philippines failed to differentiate any of the four Indian strains discussed here. The cultivar FK 135 shows distinct interveinal yellow stripes for all the strains studied here, although the virulent strains cause more intense reaction. From this comparison, it may be assumed that the four strains reported come under the S strain of The Philippines (5). Recently, Strain T has been reported from The Philippines (Annual Report of the International Rice Research Institute, Manila, 1970, p 263). This strain produces narrow leaves on Taichung (Native) 1. None of the strains reported here produces such distinct narrow leaves on Taichung (Native) 1.

The Indian strains, although distinct from each other in many respects, are similar to other viruses which cause transitory yellowing (Taiwan), Penyakit merah (Malaysia), Mentek (Indonesia), and yellow-orange leaf (Thailand). For instance, strain RTV_3 (Isolate 4) causes a severe reaction on Taichung (Native) 1 initially, followed by a rapid recovery, resembling the transitory yellowing disease of Taiwan (1). But the absence of incubation period for the virus in the vector, the nonpersistent nature of the virus, and transmission by *N. impicticeps* show that this strain is different from transitory yellowing. Whether the tungro-like diseases in countries like Thailand, Indonesia, and Malaysia are caused by viruses resembling any of the four strains discussed here is a subject for further research.

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