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Indexing Rice Tungro Virus Disease—A Modified Approach

S K GHOSH, S GANGOPADHYAY and N K CHAKRABARTI
Division of Plant Pathology, Central Rice Research Institute
Cuttack 753006

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A method for indexing rice tungro virus disease is proposed. The method was found suitable both *in vivo* and *in vitro* in determining the intensity of the disease in a particular plant. The method, typically based on symptomatology, provides a detailed picture of the infection and its gradual development in a particular cultivar, in a particular plant and even in an individual leaf quantitatively. It is also useful in screening rice cultivars against the disease and evaluating assessment of loss due to rice tungro virus alone.

Key Words: Rice tungro virus, Disease intensity, Indexing

Introduction

A method for accurate measurement of the extent of disease in a single plant has long been needed. International Rice Research Institute (1976) proposed a system called "Standard Evaluation System" for rice (SES) for indexing rice diseases including rice tungro disease. For the rice tungro disease, the colour of the infected leaves, nature of flowering, tillering ability and height of the infected plant were used as different criteria for evaluating the severity of the disease. Such characters as: (a) area of discolouration and its subsequent development, (b) point or area of the origin of discolouration, and (c) extent of veins cleared, were not considered. Moreover, using SES the individual infected leaf on a single plant and the gradual disease development were not considered. Consequently, the severity of tungro disease in a single plant could not be scored. We attempted to develop a suitable method by which the disease intensity in a single plant would be ascertained.

Materials and Methods

Rice cultivar *T(N)* 1, highly susceptible to rice tungro virus (RTV), was raised in earthen pots, 4 seedlings/pot, and filled with 4 kg of well-sieved field soil mixed with 1.5 g of ammonium sulphate.

A virus-free stock of the green leaf hopper, *Nephotettix virescens*, was reared on healthy rice plants, cultivar *T(N)* 1, in large wooden cages covered with insect proof, galvanised wire mesh. Acquisition feeding of the virus-free green

Table 1 *Distribution of numerical values based on the importance of the factors in the proposed scale*

Grade	Symptom syndromes	Numerical Scale for the severity	Maximum numerical value
(A)	TYPE (DEVELOPMENT) OF FOLIAR SYMPTOM (DISCOLOURATION)		20
	No symptom	0	
	Started from apex	5	
	Started from single margin	10	
	Started from both margins	15	
	Irregular	20	
(B)	NATURE (COLOUR) OF DISCOLOURATION		20
	No discolouration	0	
	Light yellow	5	
	Yellow	10	
	Yellowish orange	15	
	Orange	20	
(C)	EXTENT OF VEINS CLEARED		10
	No clearing	0	
	Few	5	
	More	10	
(D)	AREA OF DISCOLOURATION		20
	Nil	0	
	0 — 2 cm	5	
	2.1 — 5 cm	10	
	5.1 — 10 cm	15	
	Above 10 cm	20	
(E)	REDUCTION IN TILLERING		15
	Nil	0	
	Mild	5	
	Moderate	10	
	Severe	15	
(F)	NATURE OF STUNTING		15
	Nil	0	
	Mild	5	
	Moderate	10	
	Severe	15	

leafhoppers was for 24 hr on tungro-diseased rice plants.

Thirty-day-old plants at the two-leaf stage, caged individually with mylar polyester tubes 25 × 5 cm with two nylon screen side windows, were inoculated by confining four viruliferous insects in each cage for 24 hr. Control plants were treated similarly with non-viruliferous insects. All plants were kept in an insect proof screened house.

Healthy seedlings of four cultivars namely *Ratna*, *Pankaj* (as resistant to RTV) and *T(N)* 1, and *Karuna* (as susceptible to RTV) were grown up to 30 days in an insect-proof condition. Then they were transplanted at distance of 15×15 cm (plant to plant/row to row) in the field. Each plant was then inoculated artificially by confining 4 viruliferous leaf hoppers/plant as described earlier. Development of the disease was noted in each plant of each cultivar.

Observations were recorded at weekly intervals from 10 to 38 days after inoculation. Disease development was noted chronologically according to the proposed scale (table 1). In the proposed scale, each symptom syndrome was given a numerical value based on their importance and noted with respect to each infected leaf (table 2). When the chlorosis developed irregularly, the size of the individual area of discolourations was noted and their average was used to grade the area of discolouration (table 1). In this way numerical values for each leaf of the individual plant were recorded separately and indexing was done according to the following formula:

$$RTV\ index = \frac{\left[\sum_{s=A}^{D} L_{1s} + \sum_{s=A}^{D} L_{2s} + \sum_{s=A}^{D} L_{3s} + \ldots\ldots + \sum_{s=A}^{D} L_{ns}\right] + G}{T_N}$$

L_1, L_2, L_3, L_n = number of affected leaves on a plant varying from 1 to n
s = score of a diseased leaf varying from A to D
L_{is} = total score on diseased leaves of a plant
G = growth habit of infected plant (i.e. E + F of table 1)
T_N = total number of leaves in a single infected plant

Results and Discussion

In a single plant not all the leaves had discolourations and even if discolouration were present, they were not necessarily of the same type because cells vary in their susceptibility (Furumoto & Mickey 1967a, b). The 0–9 scale of SES is confined only to considering infected leaf colour, tillering, height and flowering. These can be used for estimating disease intensity, but are insufficient to indicate the exact damage to the plant.

As pointed out by Joshi (1973), severity is determined by visual observations

Table 2 *Distribution of different numerical values and calculations of RTV index values using equation 1/2 at different stages of pathogenesis [cv. T(N) 1]*

Leaf position (bottom to top)	Days after inoculation	Grade				Reduction in tillering (E)	Nature of stunting (F)	Growth habit of infected plant (G) (E+F)	Foliar score		RTV index
		A	B	C	D				Individual $\sum_{s=A}^{D} L_{is}$	Integrated $\sum_{i=1}^{n} \sum_{s=A}^{D} L_{is}$	$\left[\frac{\sum_{i=}^{n} \sum_{=A}^{D} L_{is}}{T_N}\right] + G$
1	2	3	4	5	6	7	8	9	10	11	12
L_1	10	0	0	0	0	0	0	0	0		
L_2		5	5	5	5				20		
										20	10.0
L_1	17	0	0	0	0	5	0	5	0		
L_2		5	15	5	10				35		
L_3		10	10	0	10				30		
L_4		10	10	0	5				25		
										90	23.7
L_1	24	0	0	0	0	10	10	20	0		
L_2		20	20	0	15				55		
L_3		20	20	0	15				55		
L_4		20	15	0	20				55		
L_5		20	15	0	15				50		
L_6		20	10	0	15				45		
L_7		20	15	0	15				50		
L_8		15	15	0	15				45		
L_9		10	20	0	15				45		
										400	46.6

1	2	3	4	5	6	7	8	9	10	11	12
L L_1	(Died) 31	—	—	—	—	10	10	20	—		
L_2		20	20	0	20				60		
L_3		20	20	0	20				60		
L_4		20	20	0	20				60		
L_5		20	20	0	15				55		
L_6		20	20	0	15				55		
L_7		20	15	0	20				55		
L_8		20	15	0	15				50		
L_9		20	15	0	15				50		
L_{10}		20	20	0	10				50		
L_{11}		20	20	0	10				50		
										545	56.50
L_1	(Died) 38	—	—	—	—	15	15	30	—		
L_2		20	20	0	20				60		
L_3		20	20	0	20				60		
L_4		20	20	0	20				60		
L_5		20	20	0	20				60		
L_6		20	20	0	20				60		
L_7		20	20	0	20				60		
L_8		20	20	0	20				60		
L_9		20	20	0	20				60		
L_{10}		20	10	0	15				45		
L_{11}		20	10	0	15				45		
										570	60.0

which are not likely to be absolutely accurate. In the case of rice tungro virus, the disease is a systemic type and always shows many types of symptoms, making scoring with Cobbs scale (Joshi 1973) difficult or impossible. However, Anjaneyulu (1975) proposed a scale for screening varieties which was related entirely on visual observation and with that scale also the quantification of RTV infection was not made possible. Moreover, susceptibility of one leaf differs from another along with the infected areas differ between leaves (Kado 1972).

The RTV index at different stages of pathogenesis (table 3) shows that the tungro infection value varied from 10.0 to 60.0 in case of *T(N)*1 under pot condition. Under field condition also quantification of the disease per plant was made possible by the proposed scale. In case of resistant cultivars like *Ratna* and *Pankaj* the infection value varied from 7.5 to 17.0 and 0.0 to 16.0 at different stages of pathogenesis respectively whereas, in case of susceptible cultivars like *T(N)*1 and *Karuna* the same varied from 12.5 to 56.9 and 20.0 to 58.2 respectively (table 4). Furthermore, if we take the lowest and highest possible values calculated by the proposed scale, we would find that the infection value (RTV index) varies from 10.0 to 63.0 in *T(N)*1. So based on the proposed scale

Table 3 *RTV index at different stages of pathogenesis in cultivar T(N) 1 under laboratory conditions*

Days after inoculation	Total leaf No.	Total infected leaf	Total tiller No.	Tungro infection value (RTV index)	Score according to SES
10	2	1	1	10.0	1
17	4	3	1	23.7	3
24	9	8	3	46.6	4
31	10	10	3	56.5	7
38	10	10	4	60.0	9

Table 4 *RTV index at different stages of pathogenesis in different rice cultivars under field conditions*

Days after inoculation	Tungro infection value (RTV index) in cultivars							
	Ratna		*Pankaj*		*Karuna*		*T(N) 1*	
	Reaction type (SES)	Index value (proposed scale)	Reaction type (SES)	Index value (proposed scale)	Reaction type (SES)	Index value (proposed scale)	Reaction type (SES)	Index value (proposed scale)
10	1	7.5	1	0.0	3	20.0	2	12.5
17	3	20.0	2	11.0	4	33.0	4	31.2
24	3	19.0	2	10.5	5	40.0	5	42.5
31	3	16.5	2	11.2	7	46.5	7	48.0
38	3	17.0	3	16.0	9	58.2	9	56.9

a new approach to classify different degrees of disease reaction in case of RTV like 0–15, 15.1–30 and 30.1–63.0 ranges as tolerant (since resistant word is confusing and bilingual), moderately susceptible and susceptible respectively is proposed.

According to SES scale when the infection reaches the value of 7 (i.e. about 60%) and up to 9 (i.e. about 100%), this has been considered as the extreme case of severity. But the present evaluation system suggests that infection value of 63.0 is a stage after which further gradation is of no use because at value of 63.0 the plant gains the maximum infection. So, the recording of flowering habit need not be considered, as in the case of severe infection there is no flowering. As cent per cent infection (scale 9 of SES) is very rarely noticed under naturally infected plants, so we have stopped gradation at the value of 63.0. With the present system we can safely recommend that the infection value of 63.0 is no less than cent per cent infection in nature. As, by cent per cent infection, quantification is not clearly emphasised, so our gradation of 63.0 infection value is more clear for the maximum infectivity.

Our proposed model takes into consideration of all the possible factors associated with the RTV disease incidence and its gradual development in a particular cultivar, in a particular plant, and even in the individual leaf. The system which is typically based on symptomatology can provide a detailed picture of the infection quantitatively. Gradual development of the disease and the cumulative effect of all factors are the most important criteria to designate a single plant about its severity of infection.

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