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Virus-vector and Host Relationship of Rice Tungro Disease in Promising Rice Genotypes

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Thirty rice genotypes (30 Nos) collected from different places viz., India (9 Nos) , Indonesia (6Nos) , Srilanka (1No), IRRI, Philippines (12 Nos) and Bangladesh (2 Nos) were tested under glasshouse conditions. The results revealed that all rice genotypes expressed typical tungro symptoms except in five rice genotypes viz., Palisithari 601, Utri Merah, Utri Rajapan, ARC 11554 and IR 81366-124-1-2-2 which were found free of foliar symptoms except mild stunting. The initial symptoms in all rice genotypes expressed were stunting followed by interveinal chlorosis and initiation of leaf discolouration and twisting of discoloured leaves. Three rice genotypes showing moderately resistant reaction obtained from IRRI (IR 73546-20-2-2-2, IR 77298-5-6 and IR 81336-39-3-3-3) expressed resistant reaction with (score 3) with varied levels of resistance to the green leafhoppers. Resistant rice genotypes expressed high level of tungro resistance with a score of 1 or 3. The per cent tungro infection (6.6) was recorded in rice cultivars, Palisithari 601, Utri Merah and IR 81366-124-1-2-2. Rice cultivars, Palisithari 601 and ARC 11554 recorded (1 score) for both tungro and as well as green leafhoppers and rice genotypes Tjempo Kijik, Utri Merah an Utri Rajapan recorded (1 score) for tungro resistance and recorded (7 Score) total susceptibility to the leafhoppers.

Key words: Rice tungro, leaf hopper vector, rice, virus-vector relationship.

Rice (*Oryza sativa* L.) is the most important food crop of the world and is an ideal model crop plant due to its small genome size, extensive genetic resources and ease of transformation with other cereal crops. An average daily consumption of rice provides 20-80% of dietary energy and 12-17% of dietary protein for Asians. It is cultivated all around the world including more than 100 countries, except the region of Antarctica. Rice tungro disease (RTD) is a major constraint in production of rice (*Oryza sativa* L.) not only in India but in all South and Southeast Asia. In Andhra Pradesh, rice tungro disease (RTD) has been reported to occur in almost all the popular rice cultivars grown in Khammam, East Godavari, Ranga Reddy, West Godavari, Medak, Nalgonda, Nellore, Chittoor and Prakasam districts.Management of tungro virus disease can be achieved through vector control (Bae and Pathak, 1969; Shukla and Anjaneyulu, 1980; Satapathy and Anjaneyulu, 1984).

The interaction between the rice tungro virus and its vector *N. virescens* is characterized by an absence of a demonstrable incubation period or latent period, a gradual decrease of the vector's infectivity with time after acquisition feeding, trans-stadial blockage (loss of infectivity in the insect due to moulting) (Ling, 1966), and recovery of infectivity by re-acquisition feeding (Ling, 1972). RTSV and RTBV are transmitted in a semi-persistent manner by the leafhopper vectors so that they are

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retained by the insects for period up to 1 week. By contrast, RTBV may be transmitted only after leafhoppers feed on source plants infected with both RTSV and RTBV or when they acquire RTSV first and then RTBV (Cabauatan and Hibino, 1988).

The zigzag leafhopper, Recilia dorsalis has also been reported to be a vector of rice tungro viruses, but it is much less prevalent. The degree of occurrence of actively transmitting vector population varies due to agro-ecological conditions of the location (Mukhopadhyay 1984b). The disease causes deleterious effects in the normal physiological and biochemical processes of the infected plants. Studies on various host-virus combinations have indicated disruptions in various activities in diseased plants (Fraser, 1987). The present study was undertaken to understand the virus-vector and host relationship of rice tungro disease in promising rice genotypes.

MATERIALS AND METHODS

Adult leafhoppers (*Nephotettix virescens*) were collected from the experimental plots of Directorate of Rice Research, Hyderabad. The leafhopper species was isolated and pure colonies maintained in the green house on potted pots of the susceptible variety Taichung Native 1(TN1), in separate rearing cages. With the objective of studying virus-vector and host-plant resistance for both virus and vector, 30 varieties which were known to have different degrees of resistance to tungro virus complex have been selected. They included 10 resistant, 10 moderately resistant and 10 susceptible cultivars or donors from different origins (Table 1.1). These rice genotypes were first soaked in Petri plates (with water) for 24 hours and after germination these seedlings were transplanted into plastic pots which were filled with soil.

Resistance to RTD

The plants were screened for their reaction to RTD in glasshouse (28±2°C, >95% RH) using a locally virulent population of *N. virescens*. The tungro isolate used was originally collected at experimental farms of DRR, Rajendranagar and maintained in TN1 by successive transfers with viruliferous leafhoppers. Initially newly emerged adult leafhoppers were allowed a acquisition access period on 45 to 60 day-old virus source plants for 12 hours. Immediately after acquisition feeding, these viruliferous adults were used for inoculation. Fifteen-day old seedlings of test entries were individually capped with a Mylar cage into which 3 viruliferous GLH were released for 24 hours. The observations on the time taken for symptom expression (incubation time) and the final resistant/ susceptible reaction was scored from 15 days up to 30 days. The evaluation of the reactions of these test plants was made on the basis of percent seedling infection and graded by adopting the standard evaluation method (IRRI 1996) in the scale: 1 = no symptom, 3 = 1-10% plant height reduction with no distinct leaf discoloration, 5 = 11-30% height reduction with no distinct leaf discoloration, 7 = 31-50% height reduction and/or yellow to orange leaf discoloration, and 9 = more than 50% height reduction and yellow to orange leaf discoloration. Further, the scores are grouped: 1and 3 as resistant or tolerant, 5 as moderate, and 7and 9 as susceptible to tungro virus disease. Then detailed observations were recorded on the sequence of events in symptom expression. Based on these data, incubation period, per cent seedling infection, stunting and reduction in tillering were calculated.

Back inoculation test

In order to confirm the presence of viruses in the inoculated plants, back inoculation test was conducted on 5 inoculated test plants of each cultivar by single plant inoculation method (Mishra et al., 1976). These back-inoculated TNI plants were observed for symptom expression. Plants showing tungro symptoms were recorded as positive and those not showing symptoms were considered as negative.

Resistance to GLH

At DRR, the seedling bulk damage rating test was used to ascertain scores for the varieties to GLH. The test seedlings were sown in trays and uniformly infested with second and third-instar nymphs of the insect at the rate of 5-7 insects per seedling. Scoring for reaction was made as soon as seedlings were killed in susceptible control (TN1). The standard evaluation method (IRRI 1996) in the scale: 0 = no damage, 1 = very slight damage, 3 = first and second leaves show yellowing, 5 = all leaves show yellowing, 7 = more than 50% of plants dead and remaining plants show severe wilting or stunting, and 9 = all plants are dead. Further, the

scores are grouped: 0-3 as resistant or tolerant, 5 as moderate, and 7-9 as susceptible, to green leafhoppers.

RESULTS AND DISCUSSION

Thirty rice genotypes (30 Nos) collected from different places viz., India (9 Nos) , Indonesia (6Nos) , Srilanka (1No), IRRI, Philippines (12 Nos) and Bangladesh (2 Nos) were tested under glasshouse conditions. The results revealed that all rice genotypes expressed typical tungro symptoms except in five rice genotypes viz., Palisithari 601, Utri Merah, Utri Rajapan, ARC 11554 and IR 81366-124-1-2-2 which were found free of foliar symptoms except mild stunting. The initial symptoms in all rice genotypes expressed were stunting followed by interveinal chlorosis and initiation of leaf discolouration and twisting of discoloured leaves. (Table 1.2)

Resistant rice genotypes expressed high level of tungro resistance with a score of 1 or 3. The per cent tungro infection (6.6) was recorded in rice cultivars, Palisithari 601, Utri Merah and IR 81366-124-1-2-2. Rice cultivars, Palisithari 601 and ARC 11554 recorded (1 score) for both tungro and as well as green leafhoppers and rice genotypes Tjempo Kijik, Utri Merah an Utri Rajapan recorded (1 score) for tungro resistance and recorded (7 Score) total susceptibility to the leafhoppers. The incubation period in the tungro resistant cultivars varied from 12 to 15 days. Most of the genotypes after inoculation developed typical tungro symptoms including orange foliar discolouration but with the advancement of the new growth were found completely symptomless. This was neither evident in other genotypes showing moderate resistance or susceptibility to tungro nor in the case of check variety TN 1.

In case of moderately resistant (MR) rice genotypes for tungro and leaf hopper vector, all the genotypes expressed tungro resistance with a score of (3 or 5). Three rice genotypes showing moderately resistant reaction obtained from IRRI (IR 73546-20-2-2-2, IR 77298-5-6 and IR 81336-39-3-3-3) expressed resistant reaction with (score 3) with varied levels of resistance to the green leafhoppers. Noticeable observations were made on the accession IR 77298-5-6, where in it has expressed good level of resistance to tungro but completely destroyed by the GLH. The incubation period in the tungro resistant cultures varied from 11 to 13 days. Among the MR rice varieties, maximum infection (60 per cent) was noticed in Shuli 2 (Acc 26527) followed by ARC 7140 and Seratus Hari T36 (Acc 26527) with 50 per cent RTD infection while the later two had succumb to the GLH damage. Accessions like ARC 7140, IR 73012-15-2-2-1, IR 81852-120-2-1-3 and IR 81178-29-2-3-2 expressed moderate level of resistance to both RTD and GLH.

Table 1. Different cultivars/accessions /donors tested for RTD and vector resistance

S.No	Designation	Origin
Resistant donors/cultivars for RTD		
1.	Pankhari 203 (Acc 5999)	India
2.	ASD 7 (Acc 6303)	India
3.	Palisithari 601 (Acc12069)	Srilanka
4.	Tjempo Kijik (Acc16602)	Indonesia
5.	Utri Merah (Acc16680)	Indonesia
6.	Utri Rajapan (Acc 16684)	Indonesia
7.	ARC 11554 (ACC 21473)	Indonesia
8.	Aguiha Anarelo	Indonesia
9.	IR 81366-124-1-2-2	IRRI
10.	Nidhi	DRR, India
Moderately resistant donors/cultivars for RTD		
11.	ARC 7140	India
12.	Seratus Hari T36 (ACC 5346	Indonesia
13.	Shuli 2 (ACC 26527)	Bangladesh
14.	IR 73012-15-2-2-1	IRRI
15.	IR 73546-20-2-2-2	IRRI
16.	IR 77298-5-6	IRRI
17.	IR 81852-120-2-1-3	IRRI
18.	IR 81178-29-2-3-2	IRRI
19.	IR 81336-39-3-3-3	IRRI
20.	Improved Samba Mahsuri	DRR,India
Susceptible donors/cultivars for RTD		
21.	ASD 8 (ACC6393)	India
22.	Habiganj DW8	Banglaesh
23.	IR 22	IRRI
25.	IR 36	IRRI
25.	IR 42	IRRI
26.	IR 56	IRRI
27.	IR 52	IRRI
28.	ARC10343	India
29.	TKM9	India
30.	ADT 36	India
Check varieties		
Vikramarya		Resistant
check	Local check	
TN 1	Susceptible check	Local check

Susceptible rice genotypes expressed high level of susceptibility to tungro with maximum RTD infection in rice cultivars, ADT 36 (96.6 %) followed by ASD 8 (Acc 6393), IR 42 (93.3 %), TKM 9 (92.8 %) and Habiganj DW 8 (90%). It was observed that all the susceptible genotypes to tungro infection was recorded the incubation period of less than 10 days except in IR 42 which took 11 days time to express the symptoms. Unlike in the case of resistant genotypes, there was no recovery of symptoms observed on the tungro susceptible genotypes.

Table 2. Reaction of rice genotypes against rice tungro disease and green leafhoppers (*N. virescens*).

S. No	Rice genotypes	No of RTD infected plants / inoculated	RTD infection (%)	Incubation period (days)	Reaction/ Score (1-9 scale)	GLH score (1-9 scale)
Resistant donors/cultivars for Rice tungro disease (RTD)						
1	Pankhari 203 (Acc 5999)	5/30	16.7	15	3	5
2	ASD 7 (Acc 6303)	4/29	13.8	13	3	1
3	Palisithari 601 (Acc12069)	3/30	6.6	15	1	1
4	Tjempo Kijik (Acc16602)	5/29	17.2	12	1	7
5	Utri Merah (Acc16680)	2/30	6.6	14	1	7
6	Utri Rajapan (Acc 16684)	2/29	10.3	14	1	7
7	ARC 11554 (ACC 21473)	4/28	14,3	14	3	3
8	Aguiha Anarelo	5/25	20.0	13	1	5
9	IR 81366-124-1-2-2	3/30	6.6	14	3	5
10	Nidhi	6/29	20.6	13	3	5
Moderately resistant donors/cultivars for RTD						
11	ARC 7140	12/24	50.0	12	5	5
12	Seratus Hari T36 (ACC 5346	15/30	50.0	12	5	9
13	Shuli 2 (ACC 26527)	15/25	60.0	13	5	9
14	IR 73012-15-2-2-1	12/22	54.5	12	5	5
15	IR 73546-20-2-2-2	13/24	54.2	12	3	3
16	IR 77298-5-6	8/28	28.6	12	3	9
17	IR 81852-120-2-1-3	7/29	24.1	13	5	5
18	IR 81178-29-2-3-2	8/28	28.6	12	5	5
19	IR 81336-39-3-3-3	8/26	30.8	11	3	5
20	Improved Samba Mahsuri	14/30	46.7	12	5	7
Susceptible donors/cultivars for RTD						
21	ASD 8 (ACC6393)	29/30	96.0	12	7	7
22	Habiganj DW8	27/30	90.0	13	5	7
23	IR 22	25/29	86.2	10	5	7
24	IR 36	21/25	84..0	10	7	5
25	IR 42	28/30	93.3	11	7	5
26	IR 56	26/29	89.7	10	7	5
27	IR 52	25/30	83.3	10	7	5
28	ARC10343	22/28	78.6	9	5	5
29	TKM9	26/28	92.8	9	5	5
30	ADT 36	29/30	96.6	10	7	7
Check varieties						
	Vikramarya (R)	13/30	43.3	12	3	3
	T N 1(S)	30/30	100.0	10	7	7

The resistances to *N. virescens* used so far were unstable and many of these resistant cultivars have succumbed to severe tungro after few or several years of intensive cultivation (Hibino *et al*., 1987). Many of the rice cultivars recorded less scores in the screenings have resistance merely to the leafhoppers. The present results are in agreement with the findings of Hibino *et al*, (1990) and Dahal *et al.* (1990). Rice cultivars, Tjempo Kijik, Utri Merah an Utri Rajapan showed no or limited resistance to leaf hoppers but gave low scores and indicating their resistance to the tungro viruses. It was also observed that three moderately resistant rice genotypes obtained from IRRI (IR 73546-20-2-2-2, IR 77298-5-6 and IR 81336-39-3-3-3) expressed resistant reaction with (score 3) with varied levels of resistance to the green leafhoppers. The accession IR 77298-5-6 expressed good level of resistance to tungro but completely destroyed by the GLH.

There are leafhopper resistant cultivars which appeared to have resistance to tungro complex. Since, leafhopper- resistance in cultivars affects their reactions to tungro infection, specification of virus resistances in those GLH resistant cultivars is generally difficult (Hibino et al 1987; Hibino et al 1988). In the present study, rice genotype (ASD 7) expressed the resistance score of 1 with corresponding tungro resistance score of 3. It may be due to the ability to acquire and transmit virus in green leafhopper populations may varied (Krishnaveni *et. al*. 2004). The differential pattern of segregation in these donors was attributed to variation in the population and viruliferous nature of *Nephotettixs* spp. used in different studies. The symptom-less carriers, confusion in the identification of symptoms, the variations in screening procedures adopted in glasshouse or field, the host tissue nutrient level affects virus infection (Seetharaman *et al*., 1976; Muralidharan *et. al.*2003).

Resistance to tungro disease has always been an important breeding objective for rice improvement in India and many other Asian countries (Anjaneyulu *et al*., 1982; Khush and Virmani, 1985; Ling 1974). Many cultivars bred as tungro resistant had resistance to GLH and they did not last long (Dahal *et al.*, 1990).

Lack of appropriate diagnosis also made the analysis of resistance of rice cultivars against tungro a difficult task. Differentiation of resistance to the virus and GLH has been of a great concern to develop screening methods for stable resistance. Because of complex virus-vector and host interactions, the methods used to differentiate virus resistance and GLH resistance was not conclusive, until serological indexing was used.

Tungro has been managed mainly by cultivar resistance and application of insecticide to reduce GLH populations. The insecticide application was not always efficient, and the instability of resistant cultivars has been the major obstruction in the use of cultivar resistance. Stable resistance for tungro has long been anticipated to solve the tungro problem. Some of the cultivars / genotypes that showed their resistances or tolerance to both tungro and green leafhoppers can be used as sources of resistance.

CONCLUSIONS

In case of virus - vector and host relationship of rice tungro disease, the incubation period in the tungro resistant cultivars ranged from 12 to 15 days and recorded a score of 1 or 3 whereas moderately resistant cultivars with incubation period of 11-13 days showed a score of 3 or 5 and susceptible cultivars showed symptoms < 10 days of inoculation with RTD with a disease score of 7 or 9. Resistant rice cultivars, Palisithari 601 and ARC 11554 recorded 1 score for both tungro and green leafhoppers. Rice genotypes showing moderately resistant reaction of IRRI cultures (IR 73546-20-2-2-2, IR 77298-5-6 and IR 81336-39-3-3-3) recorded 3 score with varied levels of resistance to the green leafhoppers. Rice cultivars, Tjempo Kijik, Utri Merah an Utri Rajapan exhibited limited resistance to leaf hoppers but recorded low score against RTD indicating their resistance to the tungro viruses. Whereas moderately resistant genotypes obtained from IRRI (IR 73546-20-2-2-2, IR 77298-5-6 and IR 81336-39-3-3-3) expressed resistant reaction with 3 score with varied levels of resistance to the green leafhoppers. Unlike resistant rice genotypes there was no recovery of symptoms observed on the tungro susceptible genotypes. Rice genotypes which showed resistant reaction against rice tungro disease were found promising and can be utilized as sources of resistance in the breeding programmes.

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