

## Inheritance of Tolerance to Rice Tungro Disease (RTD) in F<sub>2</sub> Progeny of Cross Between Rice Varieties Radhunipagol and Pusa Basmati-1

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### Abstract

Disease tolerance/resistance breeding depends on available genetic variability in the vast collection of germplasm as a source of disease tolerance/resistance. Rice varieties behave differently in tungro epidemic according to their susceptible and tolerant nature. From earlier investigations it was found that traditional rice varieties of West Bengal viz. Dumursail, Radhunipagol, Raghusail and Tulaipanja were tolerant varieties with zero yield loss. The genetics of tolerance in the traditional rice variety, Radhunipagol was investigated in this present study by crossing tolerant variety Radhunipagol with a susceptible variety Pusa Basmati-1. The F<sub>2</sub> plants derived from this cross were evaluated in glasshouse and field experiments to determine the inheritance pattern of RTD resistance. A total of 14 F<sub>2</sub> lines comprising of 683 F<sub>2</sub> plants were evaluated for their reaction to rice tungro disease (RTD). A chi-square ( $\chi^2$ ) analysis for assessing segregation from F<sub>2</sub> led to the conclusion that the tolerance found in the F<sub>2</sub> progeny of this cross is determined by a recessive gene. It indicates a typical monogenic recessive gene is governing resistance and susceptibility reaction against RTD in rice. The information obtained in this study could be valuable for rice tungro disease tolerance breeding using traditional rice varieties of West Bengal. Furthermore the data could be used in planning a systematic breeding programme to incorporate the RTD tolerance into the susceptible cultivars.

**Keywords:** Rice tungro disease (RTD); F<sub>2</sub> segregating population; Chi-square ( $\chi^2$ ) analysis; Goodness of fit; Inheritance.

### Introduction

Breeding for resistance is the environmentally most sound and also most cost-effective approach to prevent losses caused by plant viral diseases. The green leafhopper (GLH) transmitted tungro virus results in one of the most economically important and wide spread viral disease of rice. Rice tungro disease (RTD) is one of the significant fears to sustainable annual rice productions in the world (Bunawan *et al.*, 2014). Management of RTD by the use of conventional tungro resistant rice cultivars has been the most important aspect of tungro research (Khush and Vinnani, 1985). It is a practical, cost-effective and environmentally sound way to stabilize rice yield and protect farmers' income.

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The first step in the study of genetics of viral resistance is to determine whether the resistant response is inherited, and if so, the number of genes involved and their mode of inheritance. (Shahjahan *et al.* 1990, Whitham and Wang, 2004.

Kang *et al.*, 2005). There have been several studies on the sources of host resistance to plant viruses and inheritance of resistance to plant viruses and viral disease (Diaz-Pendon *et al.*, 2004; Fraser, 1986, 1990). There have been several investigations on the underlying general trends or common mechanisms of virus resistance (Goldbach *et al.* 2003). Studies on the inheritance of tolerance/resistance have been carried out in some crop plants of economic importance viz. *Lycopersicon peruvianum* (Rosello *et al.* 1998), *Pisum sativum* (Provvidenti 1990; Provvidenti and Alconero 1988).

#### Host plant resistance

Host-plant resistance is the most effective and environment friendly approach to control the damage caused by insect pests and increase yield potential of cereal crops (Jena *et al.* 2006; De *et al.* 2012). Identification of genotypes resistant to tungro is part of the disease management programme (Latif *et al.* 2011). Localized outbreaks could then be managed by targeted deployment of relevant resistance genes to that particular environment (Azzam *et al.*, 2000).

Plant host resistance is achieved in two ways: one method involves dominant *Resistance (R)* genes and the other depends on recessive alleles of genes that are critical for plant viral infection (Masayosh *et al.*, 2016).

#### Varietal tolerance

Varieties behave differently in tungro epidemic according to their susceptible and resistance nature (Dahal *et al.*, 1992). Extensive breeding programmes, conducted at International Rice Research Institute (IRRI), based on the screening of rice germplasm collections led to the identification of a number of rice cultivars resistant to RTD (Hibino *et al.*, 1990; Khush *et al.*, 2004). Among the land races of rice, Latisal, Dudshar, Ashanlaya and Nagra show mild symptoms. Indrasail, Rajmalati, Kalamkathi, Madhumalati and Dhushri remain symptomless (Mukhopadhyay, 1980). From earlier investigations it was found that traditional rice varieties of West Bengal viz. Latasail, Sonajhuli and Tulsibhog were found to be moderately tolerant with only 6%, 7% and 9% yield reduction respectively. Dumursail, Radhunipagol, Raghusail and Tulaipanja were tolerant varieties with zero yield loss (Dey and De, 2016). Tolerance was also observed in advanced rice breeding lines following mass screening and forced inoculation methods with *Nephotettix virescens* (Distant), the insect vector (Dey *et al.*,

2016). The tolerant varieties could be used as future tolerance donors in rice breeding programs.

More than 80% of reported viral resistance is monogenically controlled; the remainder shows oligogenic or polygenic control (Kang *et al.*, 2005). Many major resistance genes have been identified that condition race-specific resistance rice tungro (Azzam and Chancellor, 2002).

#### Rice Tungro Disease (RTD)

Tungro virus disease occurs if a susceptible variety, virus inoculum and the vector, green leafhopper that carries the virus are available in a rice field (Muralidharan *et al.*, 2003). The virus is transmitted mainly by leafhoppers *Nephotettix virescens* (Distant) and *Nephotettix nigropictus* (Stal) (Rivera and Ou, 1965, Azzam and Chancellor, 2002). The leafhopper transmitted tungro virus results in one of the most economically important and wide spread viral disease of rice. Rice tungro disease (RTD) caused by the co-infection of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) is a devastating viral disease of rice prevalent in Southeast Asia with outbreaks affecting thousands of hectares (Dai and Beachy, 2009).

The disease cause distinct stunting of plants, discolouration of leaves, reduction in tiller number and ultimately loss of yields. The discolouration starts from the tip and extend to the lower part of the leaf blade. Young leaves may have a molted appearance and old leaves show rusty specks of various size. The colour of leaves may be yellow or orange, slightly rolled outward and somewhat spirally twisted. The symptoms become more prominent in low nitrogen content of the soil (Mukhopadhyay, 1980). The disease adversely affects the yield components viz. height, tiller number, number of panicles/hill, number of grains, grain weight etc (Chowdhury and Mukhopadhyay, 1970). In case of severe infection by green leaf hopper (GLH) the plant look unhealthy, growth retarded, leaves turn yellow and the crop dried up. The plant may show reduction in height and tiller number when infested at tillering stage.

After high-yielding rice varieties were introduced in the early 1960s throughout South and Southeast Asian countries and double-rice cropping and staggered planting became more common in irrigated areas, tungro became an increasingly important disease (Hibino, 1996). At most of sites within each country, the genetic composition of the virus population was not significantly

different over the two or three cropping seasons. The result suggested that the geographically isolated populations are genetically stable over the sampling time (Azzam *et al.*, 2000).

The disease is caused by a complex of two viruses, RTBV (Rice Tungro Bacilliform Virus) and RTSV (Rice Tungro Spherical Virus). The RTBV is DNA virus, responsible for the development of the symptoms in leaves whereas RTSV is RNA virus, responsible for the transmission of the disease. Both the virus is non-enveloped. RTBV is a virus of 30-35nm in diameter and 160-220 nm long, whereas RTSV is of 30 nm in diameter. The virus infection results a drastic reduction in chlorophyll, amount of sugar increase whereas starch decrease in the grain. Enhanced understanding of transmission, inheritance pattern and biological control of these viruses makes tungro disease very significant in terms of plant virology, molecular biology and entomology, with the focus on achieving the ultimate goal of improved management strategies for control of RTD in order to reduce the economic damage to global rice production (Bunawan *et al.*, 2014).

## Materials and Methods

To determine the mode of inheritance of the rice tungro disease (RTD) tolerance, a tolerant landrace of West Bengal, Radhunipagol was taken as the male parent and crossed with the RTD susceptible Pusa Basmati-1 rice variety as the female parent. To identify gene(s) involved in RTD tolerance the association of genotypic and phenotypic variation for RTD resistance was examined in a F2 population derived from cross between a susceptible variety Pusa Basmati-1 and one tolerant variety Radhunipagol. The F2 plants derived from these crosses were evaluated to determine the inheritance pattern of RTD tolerance.

### Rice population

Sowing of parent material and crossing program were performed during aman season. F1 seeds are collected and raised to build up segregating F2 population in rabi season. A total of 683 F2 plants planted in 14 lines were examined to analyze the pattern RTD tolerance inheritance. The screening was done according to IRRI Standard Evaluation System method (Gomez and Gomez, 1984) to assess their reaction against Rice Tungro Disease in Bose Institute Experimental Farm at Madhyamgram, West Bengal.

### Evaluation of RTD reaction

The screening of the germplasm was done in three ways as follows:

#### A. Mass Screening in Seed Bed

1. The seedlings were sown directly on raised seedbeds in single lines flanked by one line of TN 1 (susceptible check). A spacing of 10 cm is left in between the lines. The seeds were sown in the 1<sup>st</sup> week of October so as to coincide with the peak level of natural occurrence of *Nephotettix virescens* (Distant) population. One line of TN 1 is sown lengthwise in both sides of the varieties. Three lines of tungro infected tillers of Jaya1 (provided by Bidhan Chandra Krishi Visvavidalaya, West Bengal) were transplanted in the longitudinal channels between the beds. The disease score were taken from 30 – 60 days.

#### B. Mass screening in Field Condition

The same TN 1 encircled screening procedure was maintained for the transplanted plants in field condition.

#### C. Forced Inoculation method

The seedlings of different F2 lines were planted in 10 inch pots. The pots were placed inside mosquito nets to form insect-proof enclosures in the greenhouse of 6 x 7 ft area. Infected TN 1 plants were placed inside the mosquito net. 500 *Nephotettix virescens* (Distant) were released inside the mosquito net periodically. The pots were taken out after 7 days and observed for further development of the symptoms.

### Statistical analysis

The F2 plants derived from this cross were evaluated in glasshouse and field experiments to determine the inheritance pattern of RTD resistance. The RTD occurrence was recorded among the plants of F2 population of the cross based on the visual scores. The numbers of resistant and susceptible plants among the F2 generation were counted per line. A total of 14 F2 lines comprising of 683 F2 plants were evaluated for their reaction to rice tungro disease (RTD). Observations recorded in segregating generations were subjected to the chi-square ( $\chi^2$ ) analysis of goodness of fit, using standard formula at 5% level of significance. The Null hypothesis ( $H_0$ ) was taken as 3:1 ratio (susceptible: resistance).



## Results

To deduce the inheritance pattern of RTD tolerance rice (*Oryza sativa* L.) cross between tolerant traditional variety Radhunipagol × susceptible variety Pusa Basmati-1 were evaluated using Chi-square analysis ( $\chi^2$ ) at 5% level of significance. The test was performed to analyze the expected deviation from the Mendelian segregation ratio in the segregating F2 generation. The results are presented in Table 1. All information pertaining to RTD tolerance confirmed that the F1s of the cross showed no symptoms to RTD and this marked clearly that the resistance was susceptible over dominance. With respect to observed: expected F2 segregation ratio for resistance: susceptible chi-square test showed non-significance chi-square value between the probability of 0.9 - 0.8. The Chi square ( $\chi^2$ ) analysis confirmed that the expected ratio (Null hypothesis), is a good fit with 3:1 ratio (susceptible: resistance) in F2 progeny at 5% level of significance. It indicates a typical monogenic recessive gene is governing resistance and susceptibility reaction against RTD in rice for this cross combination.

The findings were as follows

1. The tungro tolerance seems to be governed by single recessive gene in the tolerant variety Radhunipagol.
2. The F2 segregated into susceptible: tolerant as 3:1 ratio and is supported by Chi square ( $\chi^2$ ) test (Table 1).

## Discussion

Recessive resistance, is also widely exploited in many crops (Truniger and Aranda, 2009; Wang and Krishnaswamy, 2012). In fact, about half of the alleles responsible for virus-resistance in crops are recessive (Kang *et al.*, 2005). Resistance with recessive inheritance, mostly acquired via the alteration of key host factors required for the viral infection cycle, is also recognized as an effective antiviral resistance mechanism (Robaglia and Caranta, 2006). Recessive resistance traits can be introduced into crop species by crossing, or random mutagenesis and selection (Piron *et al.*, 2010). Recessive resistance breeding has the practical advantages of not requiring the introduction of transgenes and not being restricted by the selection of naturally occurring traits only.

The result of genetic analysis for RTD tolerance in this study is consistent with other studies that resistance of tolerant Utri Merah rice variety to RTSV (strain A) is controlled by a single recessive gene, although additional genes in Utri Merah may also be involved in resistance against other strains of RTSV (Azzam *et al.* 2002).

For any monogenic trait, the segregation of plants in F2 generation should follow 3:1 ratio (resistant: susceptible). The goodness of fit was used to calculate Chi square ( $\chi^2$ ) for 3:1 ratio at 5% level of significance. The ratio of susceptible and resistant plants in F2 generation of susceptible X resistant cross combination were tested for goodness of fit

**Table 1:** Chi - Square ( $\chi^2$ ) Values of Segregation of Resistant/Susceptible F2 Plants to Rice Tungro Disease (RTD) from the Cross Pusa Basmati-1 ♀ X Radhunipagol ♂

Line No.	Total Plants	Sus.	Exp. Sus.	$\chi^2$	Res.	Exp. Res.	$\chi^2$	$\chi^2$ T	Probability
1	50	31	37.5	1.126	19	12.5	3.38	4.5	0.02- 0.05
2	50	36	37.5	0.06	14	12.5	0.18	0.24	0.05- 0.75
3	52	42	39	0.23	10	13	0.69	0.92	0.25-0.5
4	48	38	36	0.11	10	12	0.33	0.44	0.5
5	51	37	38.25	0.04	14	12.75	0.122	0.16	0.5-0.75
6	50	38	37.5	0.006	12	12.5	0.02	0.026	0.75-0.9
7	50	38	37.5	0.006	12	12.5	0.02	0.026	0.75-0.9
8	51	38	38.25	0.0016	13	12.75	0.004	0.005	0.9-0.95
9	54	44	40.5	0.302	10	13.5	0.907	1.209	0.25-0.5
10	54	43	40.5	0.154	11	13.5	0.46	0.61	0.25-0.5
11	51	37	38.25	0.04	14	12.75	0.122	0.16	0.75
12	46	31	34.5	0.35	15	11.5	1.06	1.41	0.1-0.25
13	49	35	36.75	0.083	14	12.25	0.25	0.33	0.5-0.75
14	29	24	21.75	0.23	5	7.25	0.69	0.92	0.75-0.9
Total	683	510	512.25	0.0099	173	170.75	0.0295	0.039	0.8-0.9

SUS=Susceptible, Exp Sus= Expected susceptible,  $\chi^2$  = Calculated  $\chi^2$  value,  
RES= Resistant, EXP RES= Expected Resistant,  $\chi^2$  T= Total  $\chi^2$  value

to the expected segregation and all the crosses were in agreement with the expected 3:1 ratio with high degree of confidence ( $p = 0.8-0.9$ ). This confirms the presence of one gene in the tolerant parent Radhunipagol.

### Conclusion

The data generated here may be used as base line data in determination of the tolerant races against tungro disease. With proper serological and PCR based detection of the presence of RTV in the plants may help in screening these land races in search of resistance. The resistance against rice tungro disease may be transferred through breeding and a disease resistant plant may be developed which is evident from the crosses made. As the resistance is governed by a single gene, it may be relatively easy to raise a resistant plant against tungro with proper screening method.

### Limitations of the study

The detection was based on the morphological symptoms, supported by forced inoculation technique only. The serological and/or PCR based detection was not possible due to lack of infrastructure.

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