

# Breeding elite rice lines resistant against rice tungro virus disease

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Rice is the most important food crop in India and plays a key role in delivering global food security.

Due to changed cultivation practices and apparent changes in the climate, there has been increased incidence of many pests and diseases.



❖ Rice is a natural host for more than 25 viruses.










❖ **About 15 viruses may seriously affect rice yield.**

rice bunchy stunt,  
rice black-streaked dwarf,  
rice gall dwarf,  
**rice dwarf,**  
rice transitory yellowing,  
**rice grassy stunt,**  
rice giallume,  
rice necrosis mosaic,  
**rice ragged stunt,**  
rice stripe necrosis,  
rice stripe,  
rice hoja blanca,  
**rice tungro bacilliform,**  
**rice tungro spherical,**  
and rice yellow mottle viruses.

**The distribution of each virus is generally restricted to only one or two of the continents in which rice is grown**

**“yellow dwarf” a disease of mycoplasma origin.**

## Rice viruses- symptoms and vectors

S.No	Virus/Phytoplasma	Typical symptoms	Reports in India	Transmission	
1	Rice tungro spherical virus and rice tungro bacilliform virus		Severe stunting Reduced tillering Yellow to orange discolouration Interveinal chlorosis Twisting of leaf tip	1966 –till today Raychaudhuri et al 1967	Green leafhopper <i>Nephotettix virescens</i> 
2	Rice ragged stunt virus 		stunting, abnormal leaves with serrated edges twisted leaf tips, vein swelling or galls on the underside of the leaf blades	1979 Ghosh et al 1979	Brown planthopper <i>Nilaparvata lugens</i> 
3	Rice grassy stunt virus		severe stunting profuse tillering. leaves are stiff and narrow interveinal chlorosis & bronzing.	1972-84 Kulshreshtha et al 1974 Mariyappan et al 1984	Brown planthopper <i>Nilaparvata lugens</i> 
4	Rice necrosis mosaic virus		Stunting with reduced tillering Mosaic mottling on the upper leaves Necrotic lesions on the basal parts of the stems and sheaths	1979 (Ghosh 1979)	Mechanical and Soil transmission
5	Rice yellow dwarf (Candidatus Phytoplasma oryzae )		Yellowing of the newly formed leaves Stunted plant Increased tillering Conspicuous on the ratoon crop	1976 (Muniyappa and Ramakrishna 1976)	Green leafhopper <i>Nephotettix virescens</i> <i>N. nigropictus</i> 

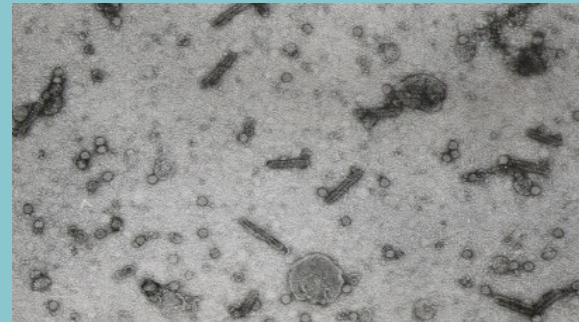
## Rice tungro virus disease

Rice tungro virus disease (RTD) – a destructive disease of rice.



Tungro is caused by two unrelated viruses

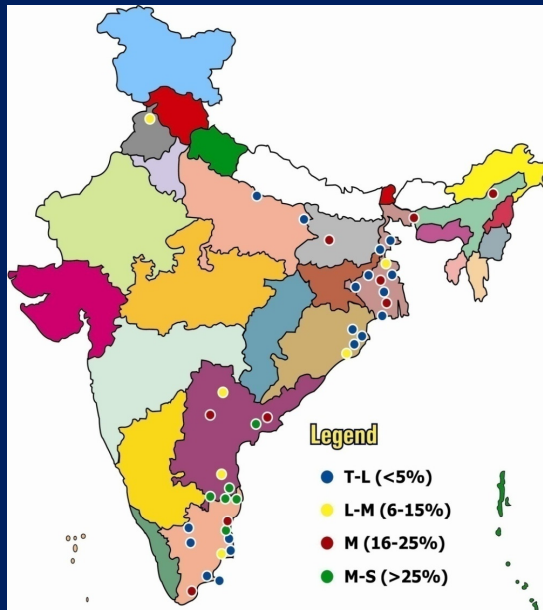
- *Rice tungro bacilliform virus* (RTBV), – a DNA virus,
- *Rice tungro spherical virus* (RTSV), an RNA virus.



Transmitted by green leafhoppers (GLH)– *Nephotettix virescens* and *N. nigropictus* in a semi-persistent manner.



# Rice tungro virus distribution in India



**1967:** First reported from West Bengal.

**1969:** Eastern U.P., West Bengal and northern parts of Bihar.

Subsequently, the disease reported from A.P., Assam, Kerala, Puducherry, Tamil Nadu and other rice growing areas.

**1973 & 1974:** Many parts of Orissa

**1977:** Krishna district of A.P.

**1981:** Many parts of West Bengal

**1998:** Gurdaspur and Amritsar districts of Punjab, in an area of 0.45 million ha

**2003:** Nalgonda district of A.P.

**2003-2006:** Kanyakumari and Tirunelveli districts of Tamil Nadu.

**2007:** Karimnagar and Medak districts of AP in an area of >19000 acres.

**2010:** Kanyakumari, Tamil Nadu

**Besides obvious endemism, reasons for periodical epidemics are not known**

As chemicals are not very effective for the control of tungro.....,

.....host plant resistance has become the most important alternative for the management of this disease

Ever since the identification and confirmation of tungro virus incidence in India, attempts were made to identify donors and develop breeding lines resistant to tungro disease

## SES Scale (IRRI 1996) for RTV

Score	Description
1	No symptoms
3	1-10% plant height reduction with no distinct leaf discoloration.
5	11-30% plant height reduction with no leaf discoloration.
7	31-50% plant height reduction and yellow to orange leaf discoloration
9	More than 50% plant height reduction and yellow to orange leaf discoloration.



RTV screening



## Vikramarya



- ❖ **IET 7302**
- ❖ **Cross: RPW 6-13/ PTB 2;**
- ❖ **with long bold grains;**
- ❖ **Released in AP during 1986**
- ❖ **Duration: 130 days;**
- ❖ **Resistance to tungro, GLH and blast**

**Though varieties are bred/released for RTV resistance, these varieties are not popular and popular varieties are prone to RTD**

## Nature of resistance in rice genotypes.

Studies on genetics of inheritance of the donors suggest the involvement of single gene to multiple loci governing the resistance

<b>Name of the cultivar</b>	<b>Score (0-9 scale)</b>	<b>Source</b>
Pankhari 203	3	India
Ptb 8	1	India
TKM 6	3	India
Utri Merah	3	Indonesia
Utri Rajapan	1	Indonesia
Tjempo Kijik	1	Indonesia
ARC 11554	1	India
TN 1	9	Taiwan
Aguina Anarelo	1	Indonesia

Prasad GSV, Krishnaveni, D. Subramanian N, Neeraja CN and Muralidharan K. 2004. Genetics of resistance to rice tungro virus disease. J.Mycol.Pl.Pathol 34(3):846-849

## Molecular mapping of resistance genes

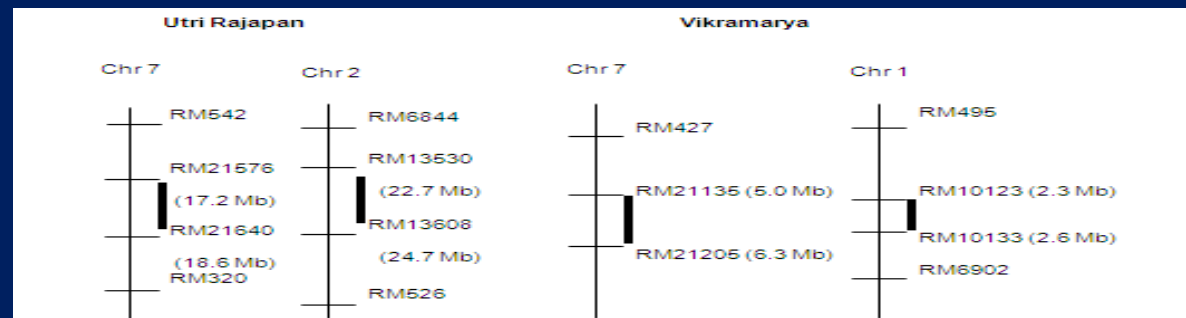
In variety ARC11554, RTSV and green leafhopper (GLH) resistance was reported on chromosome 4 and being fine mapped.

In Utri Merah, RTV resistance was mapped on chromosome 7.

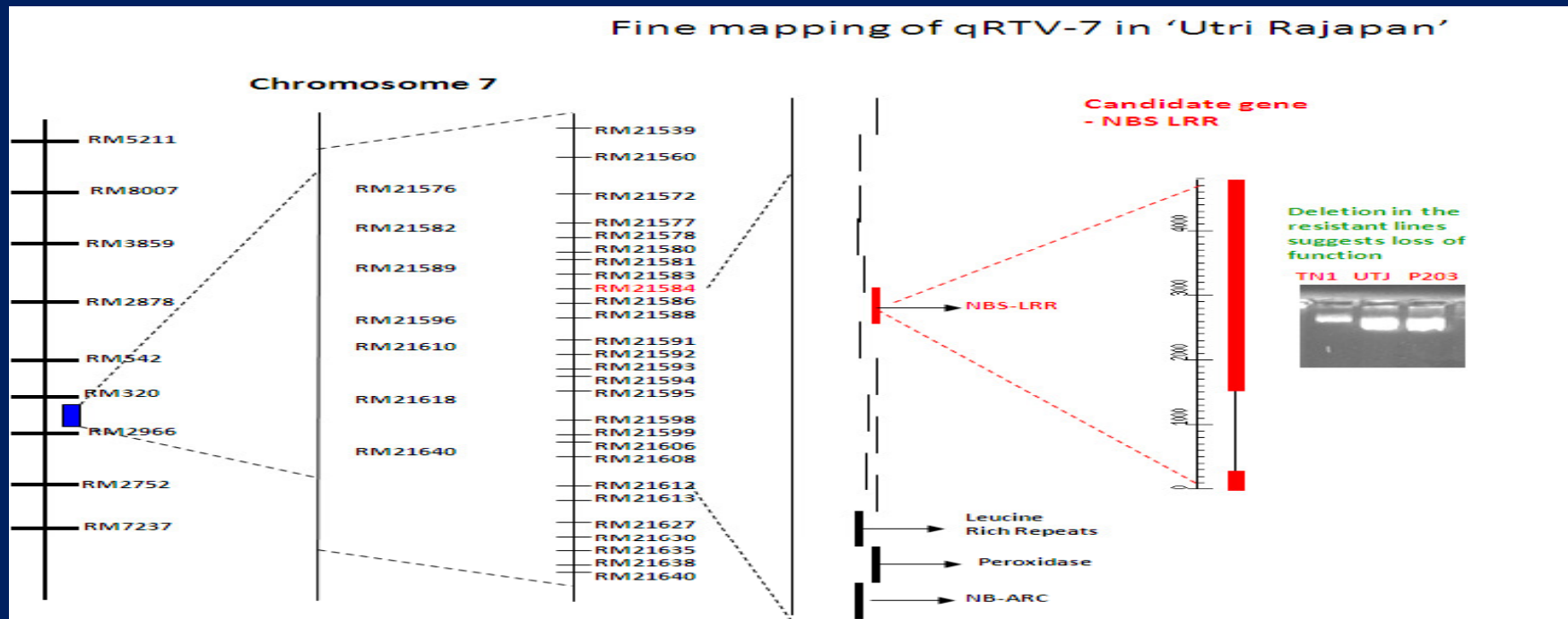
Two well known donors for Rice Tungro Disease resistance 'Utri Rajapan and 'Vikramarya' were targeted for mapping.

Two QTL for RTD resistance were identified in 'Utri Rajapan' on chromosomes 7 and 2 explaining 40.8% and 21.6% of the phenotypic variance.

In 'Vikramarya', another two QTL for RTD resistance were detected on chromosomes 7 (different location) and 1 explaining 18.7% and 16.4% of PV



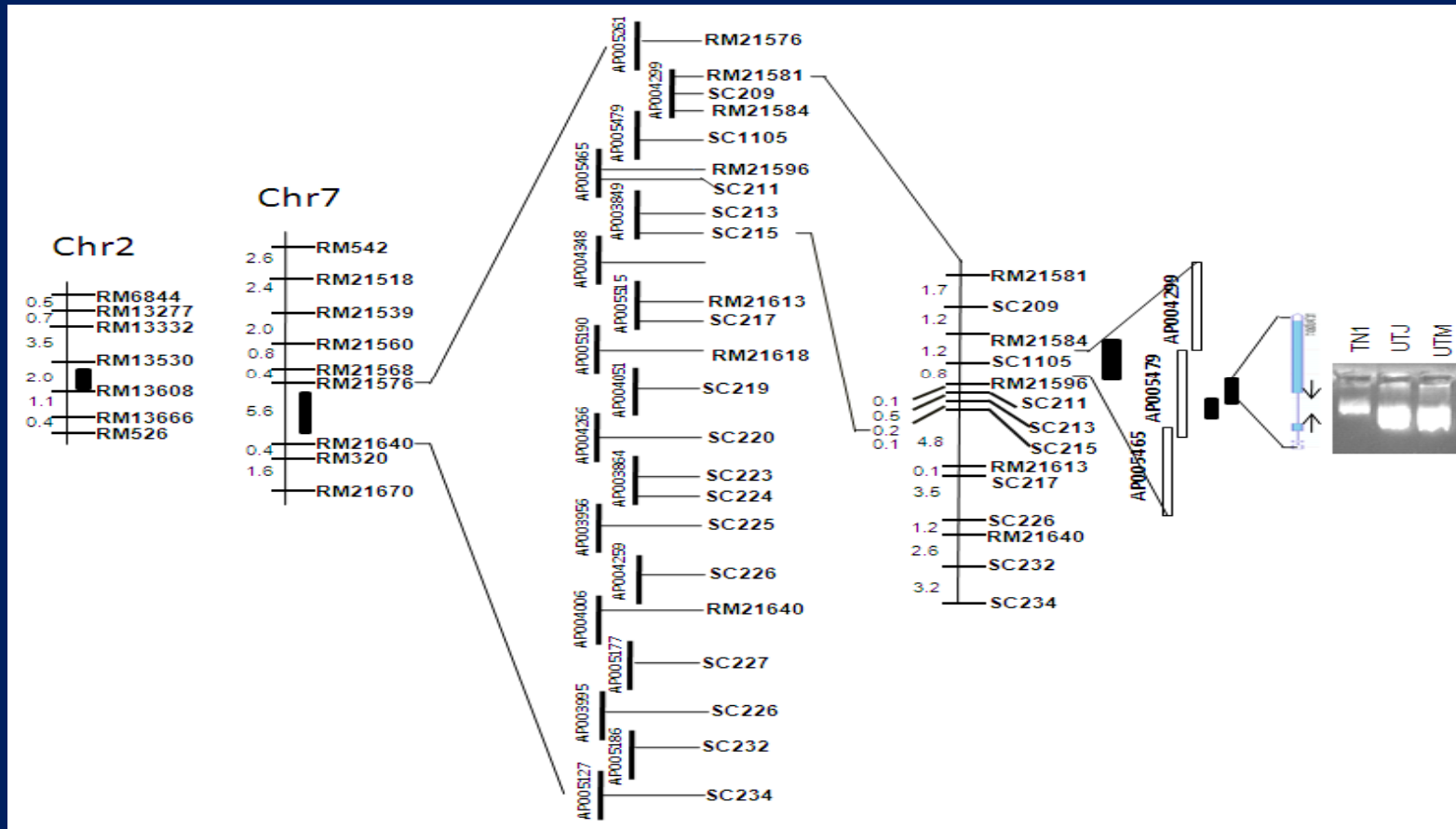
## The major QTL *qRTV-7* from *Utri Rajapan* was fine mapped.



Out of two QTLs identified for tungro disease resistance, the major QTL, *qRTV-7* was fine mapped using rice genome sequence information. This QTL was located within ~17.69 and 19.39 Mb of chromosome 7 genome sequence spanning 18 BAC clones corresponding to ~1.74 Mb of the sequence (AP008213).

# Cloning of the candidate gene

The complete gene (LOC\_Os07g29820) covering 5.2 kb is being successfully amplified after standardization of several combinations of primers.



Bioinformatics of three BACs viz., AP004299; AP005479 and AP005465 showed 52 annotated genes in the region in addition several hypothetical and unknown proteins

Two candidate genes viz., LOC\_Os07g29810 and LOC\_Os07g29820 with predicted product of putative nucleotide-binding leucine-rich-repeat proteins were more focused because of their reported involvement in disease resistance.

Several candidate gene based primers were designed for detection of structural polymorphism and screened for their utility as markers

<b>Primers/Markers studied</b>	<b>Number of primer pairs</b>
RM microsatellite markers	32
Designed microsatellite markers	39 + 29
LOC_Os07g29810	22
LOC_Os07g29820 (primers for sequencing)	48
Designed candidate gene based markers (other than two disease resistance genes)	140 targeting 48 genes
RT primers for expression analyses (including candidate genes)	120

## Expression analysis of candidate gene

The expression analysis of candidate gene (LOC\_Os07g29820) in the infected and healthy tissues has been standardized

Differential gene expression is detected in resistant (Utrirajapan) and susceptible (TN1) and it was found that there is differential expression after six hours of infection in susceptible and resistant genotypes

## Breeding elite rice lines with identified RTV resistance genes

From the Production Oriented Survey (DRR), a few regions across country were identified as endemic for RTV.

The popular varieties grown in these regions which are found to be susceptible to RTV were selected for introgression of *qRTV-7*.

This objective has been fulfilled with five popular varieties introgressed with *qRTV-7* with foreground and background selection.

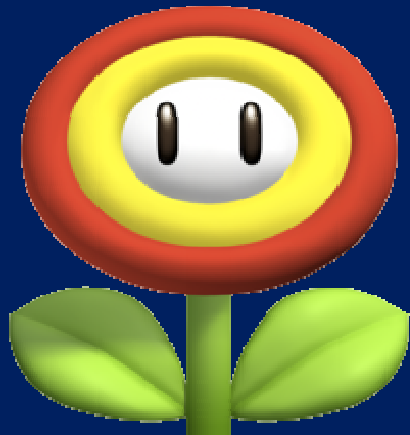


## Details and stage of backcross populations

The major QTL *qRTV-7* was being introgressed into IR64 (BC2F2), BPT5204 (BC2F2), MTU1010 (BC2F2), ADT39 (BC2F2) and CR1009 (BC1F2).

Female Parent	Male Parent	Stage of BC
IR64	Utri Rajapan (Resistant parent)	BC2F4
BPT5204	Utri Rajapan	BC1F4
MTU1010	Utri Rajapan	BC2F4
ADT39	Utri Rajapan	BC1F4
CR1009	Utri Rajapan	BC1F1





Thank you !!