Tospovirus Current situation on Brinjal, Chilli and Tomato – A way forward

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• Introduction
• Tospoviruses
• Distribution of Tospoviruses
• Tospoviruses in India
• Thrips transmission of Tospoviruses
• Development of Tospovirus resistance
• Strategies for Tospovirus Resistance
• The Way Forward
Nature of Tospoviruses

• Family: *Bunyaviridae*
• Genus: *Tospovirus* (*Tomato spotted wilt virus* as type species)
• Particle: spherical, enveloped, 80-120 nm diameter
• Genome: ssRNA, tripartite (8.9, 4.8, 2.9 kb)
• Vector Transmission: 16 thrips species in persistent, propagative manner
• Mechanically transmitted by sap
• Not seed transmitted
• Distribution: worldwide
Tospoviruses – A Challenge

• Tospoviruses are top ten important viruses:
  1. Exponential increase in losses caused
  2. Geographically Very wide distribution
  3. Wide - narrow host range
  4. Unique morphology and thrips transmitted but not seed (Except SVNV).
  5. Thrips are efficient vectors, polyphagous, difficult to control
  6. Thrips develop resistance to insecticide quickly
  7. Climate change and environmental influence on vector population build up and virus symptom variation
Tospovirus Emergence in Asia

Tospoviruses are emerging as significant limiting factors for Vegetable production

73% of the globally reported tospoviruses occur in Asia (21/29)

56% of the vector thrips species known globally occur in Asia (9/16)

Currently there is serious concern of Tospoviruses in Vegetable improvement in South and South East Asia
Tospoviruses Occurring worldwide

1. ANSV: Alstroemeria necrotic streak virus
2. BNeMV: Bean necrotic mosaic virus
3. CaCV: Capsicum chlorosis virus
4. CCSV: Calla lily chlorotic spot virus
5. CSNV: Chrysanthemum stem necrosis virus
6. GRSV: Groundnut ring spot virus
7. GCFSV: Groundnut Chlorotic fan spot virus
8. GBNV: Groundnut bud necrosis virus
9. HiCRV: Hippeastrum chlorotic ring spot virus
10. INSV: Impatiens necrotic spot virus
11. IYSV: Iris yellow spot virus
12. LiNRSV: Lisianthus necrotic ring spot virus
13. MSMV: Melon severe mosaic virus
14. MYSV: Melon yellow spot virus
15. MVBaV: Mulberry vein banding virus
   a. Tomato fruit yellow ring virus  Syn. Tomato yellow ring virus
   b. Groundnut bud necrosis virus syn. Peanut bud necrosis virus,
   c. Physalis silver mottle virus Syn. Melon Yellow Spot Virus
   d. Peanut yellow spot virus is syn. Groundnut yellow spot
16. PCSV: Pepper chlorotic spot virus
17. PNSV: Pepper necrotic spot virus
18. PoRSV: Polygonum ring spot virus
19. PYSV: Peanut yellow spot virus
20. TCSV: Tomato chlorotic spot virus
21. TNSV: Tomato necrotic spot virus
22. TSWV: Tomato spotted wilt virus
23. TNRSV: Tomato necrotic ring spot virus
24. TYRV, Tomato yellow fruit ring virus
25. TZSV: Tomato zonate spot virus
26. SVNαV: Soybean vein necrosis virus
27. WBNV: Watermelon bud necrosis virus
28. WSMV: Watermelon silver mottle virus
29. ZLCV: Zucchini lethal chlorosis virus.
## Global Distribution of Tospoviruses

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<th>Region</th>
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Genome Organization of Tospoviruses

Genome: Single stranded negative sense RNA

**L**

Large (L) RNA - RNA-dependent- RNA –Polymerase

8.9 kb

**M**

Medium (M) RNA - viral movement protein (NSm), common precursor to the glycoproteins (Gn)

4.8 kb

**S**

Small (S) RNA - codes in ambisense for a non-structural (NS) Protein and Nucleoprotein

2.9 kb
Thrips – The Challenge

• **Small size**: difficult to detect
• **Polyphagous**: feed on a board range of plant species, feed on different parts of the plant (pollen, flower structures, leaves, stems)
• **Show habitat infidelity**: extraordinary ability to adapt, can expand geographic range, can spread to new crops
• **Have superior reproductive out put**: produce many off springs
• **Have propensity to overwinter on a broad range of plant species**: survive through out the year
• **Vectors of viruses**: Spread virus diseases
Thrips Species implicated in Tospovirus transmission

- *Frankliniella bispinosa*
- *F. cephalica*
- *F. gemina*
- *F. fusca*
- *F. intonsa*
- *F. occidentalis*
- *F. schultzei*
- *F. zucchini*
- *Ceratothriptoides claratris*
- *Dictyothrips betae*
- *Microcephalothrips abdominalis*
- *Neohydatothrips variabilis*
- *Scirtothrips dorsalis*
- *Thrips palmi*
- *Thrips setosus*
- *Thrips tabaci*

16 Species
Geographic Expansion of Thrips Vectors

Western flower thrips: *Frankliniella occidentalis* - Native to the Southwestern USA. Spread through global trade in ornamental greenhouses plant around the world from mid 1980s.

Melon thrips: *Thrips palmi* - Native to South East Asia. Expanded its geographic range in 1970s and 80s.

Chilli thrips or yellow tea thrips: *Scirtothrips dorsalis*, is an extremely successful invasive species of thrips which has expanded rapidly from Asia over the last twenty years, and is gradually achieving a global distribution.
Global Tospovirus Emergence

Frankliniella occidentalis
Alstromeria necrotic streak virus
Chrysanthemum stem necrosis
Groundnut ring spot virus
Impatiens necrotic spot virus
Tomato chlorotic spot virus
Tomato spotted wilt virus
Tomato Zonate spot virus

Thrips palmi
Capsicum Chlorosis virus
Calla lily chlorotic spot virus
Groundnut bud necrosis virus
Melon yellow spot virus
Tomato necrotic ring virus
Watermelon bud necrosis virus
Watermelon silver mottle virus

• Polyphagous and shows habitat infidelity, has superior reproductive propensity to ‘overwinter’ on a broad range of plant species
Thrips transmission of Tospoviruses

Overlapping stages in the life cycle can account for continuous virus spread in nature.
Tomato spotted wilt virus - Historical

* Spotted wilt of tomato was first described in 1915 in Australia.

* This disease was associated with a virus then named as *Tomato spotted wilt virus* (TSWV) in 1930.

* The properties of particle morphology, host range, and transmission by thrips, TSWV was classified as the type member of “tomato spotted wilt virus group” in 1970.

• This virus group designated as the genus *Tospovirus* belonging to the family *Bunyaviridae* by the “International Committee on Taxonomy of Virus” (ICTV) in 1991.
• Today it is one of the 10 most economically destructive plant viruses.
• Found in temperate, subtropical and tropical regions of the world.
* Mosaic disease of Groundnut was first described in 1963 by Nariani & Dhingra, 1963; Sharma, 1966 in Delhi.

* The name "Bud Necrosis" was given in 1968 which was found in Andhra Pradesh (Reddy et al. 1968).

• Subsequently this virus was found to infect several pulses like Cowpea, Mungbean, and Pea (Prasad Rao et al., 1980, )

• This was transmitted through thrips Franklinella scultzi which later confirmed as Thrips palmi (Vijayalakshmi, 1994)
Groundnut bud necrosis virus – Tomato, Potato
GBNV – Hot & Sweet Pepper

Hot Pepper

Sweet Pepper
GBNV – Brinjal (Egg Plant)
GBNV – Wide Host range

Cowpea  Pea  Groundnut  OKra

Onion  Bitter gourd
Diversity of GBNV population and host range

GBNV Vs Tospoviruses

TSWV Vs Tospoviruses

[Graphs showing nucleotide positions for L RNA, M RNA, and SRNA for both GBNV and TSWV]
GBNV Nucleocapsid structural diversity

GBNV.Tomato

GBNV.Brinjal

GBNV.Potato

GBNV.Chilli

GBNV.Cowpea
Tospoviruses in India

GBNV: Wide hosts

WBNV: Cucurbits

PYSV

IYSV

CaCV: Pepper, Tomato

MYSV
Diversity of CaCV population and host range

CaCV Vs Tospoviruses

TSWV Vs Tospoviruses
Diversity of WBNV population and host range

WBNV Vs Tospoviruses

TSWV Vs Tospoviruses
Detection of GBNV

A. Indicator hosts

B. Electron microscopy
Available Tospovirus Resistance

- Tolerance is short lived: Platense, Pearl harbor, Redpernose, Manzana and others resistant to TSWV are susceptible to GBNV.

- Sw-5 dominant gene introgressed from *S. Peruvianum* has broad spectrum resistance to TSWV and others TCSV and GRSV, is ineffective against GBNV.

- Sw-7 gene introgressed from *S. chilence* resistant to TSWV was found ineffective against GBNV.

- 14 *S. Peruvianum* accessions multiplied and distributed by AVRDC, tested in hot spots of GBNV showed little success

- Mechanical Inoculation protocols are inconsistent
Rapid screening Technique for Resistance
Resistance breaking is it a Problem in GBNV

NSm gene: 0.5-2.5% (0.7-10.2%)

NSs gene: 0.7-2.8% (0.7-11.7%)

N gene : 0.3 – 2.4% (0.4 – 7.3%)
Host Diversity of N Protein

GBNV.Tomato
WBNV.Tomato
WSMoV.Watermelon
CaCV.Tomato
TSWV.Tomato
TSWV.Pepper
NSs Protein Structural diversity

GBNV.Potato
GBNV.Tomato
GBNV.Chilli
GBNV.Cowpea
GBNV.Brinjal
Host Resistance Mechanisms

- Majority of virus resistant genes from plants – Monogenic dominant and 80% of the virus resistant genes are monogenic, the rest oligo or polygenic.

- Application of RGA based markers for identification of Tospovirus Resistance

- Plant resistance (R) genes containing domains of N-terminal binding sites (NBSs) and C-terminal leucine-rich repeats (LRRs)

- **Sw-5** gene conferring resistance to *Tomato spotted wilt virus* (TSWV) in Tomato (Aramburu & Martí, 2003; Ciuffo *et al.*, 2005) and in Pepper (Kang, 2014) have CC-NB-LRR type resistance.
Identification of Tospovirus resistance through NBS-LRR RGAs in Chilli

• 102 genotypes of chilli germplasm lines screened against GBNV, six lines are identified as resistant through mechanical sap inoculation and confirmation by ELISA and PCR.

• Five sets of degenerate RGA primers designed, amplified 0.5kb DNA from resistant genotypes but not from susceptible genotypes

Cloning, Sequencing and analysis indicated identification of 18 TIRNBS-LRR type RGAs and 12 Non-TIR-NBS-LRR type RGAs
Identification of TIR-NBS-LRR type RGAs

- 12 clones showed 96 to 100% aa identity with known RGAs of Capsicum and two clones showed 73 to 86% with *C. annuum*, *N. tabacum*, *N. tomentosiformis*, *Solanum aculeatissimum*, *S. bulbocastanum*, *S. esculentum*, *S. pennellii* and *S. tuberosum* TMV resistance protein N like and 82 to 85% with *Luffa aegyptiaca* and *L. siceraria* NBS-LRR resistant protein.
10 RGAs have high sequence homology with known Non-TIR-NBS-LRR type RGAs. The highest homology of 84 to 99% with known RGAs of *Capsicum annuum* and 73 to 87% with putative late blight resistance from *N. tabacum*, *N. tomentosiformis*, *S. lycopersicum*, *S. pennellii* and *S. tuberosum*. 

Conserved amino acid motifs identified within the chilli plants in NON TIR RGAs sequences of NBS domains through MEME software analysis. The black line represents the length of different chilli plants RGAs and coloured boxes represent the motifs along the length of each chilli plants RGAs.

Sequence signature of Seven major conserved motifs in Chilli RGAs NON-TIR-NBS region along with their e-values.
Breeding for Insect Vector Resistance

1. **Host Antixenosis**: Presence of morphological or chemical factors that alters insect behavior

   - Trichome density and cuticle thickness-thirps (Firdaus et al., 2011)
   - Acylsugars in leaves of Tomato reduces silvering due to thrips (Mirnezhad et al., 2010)

2. **Host Antibiosis**: Factors that increase insect mortality, developmental period and decrease in reproduction (Maris et al., 2004)

3. **Host Tolerance mechanisms**: Marketable yield in spite of insect attach
The Way Forward

• Tospovirus/vector resistant varieties will be an important component of Tospo IPM strategy

• A quick solution is unlikely, GBNV in tomato a difficult problem that requires long term commitment

• Mechanical inoculation alone may not result to resistance, thrips transmission coupled with natural field simulation based on weather condition is required

• Better understanding of viral biology and diversity, Epidemiology, Vector Ecology for effective management strategies

• Success depends on best collaborate team approach between Public and private is required
Thank you