

# A Research Review on Tomato Bushy Stunt Virus Disease Complex

Hafiz Husnain Nawaz, Muhammad Umer, Sadia Bano, Anam Usmani, Memoona Naseer  
Institute of Agricultural Sciences, University of the Punjab, Lahore

\*corresponding e-mail: [umer.impp@gmail.com](mailto:umer.impp@gmail.com)

## Abstract:

Tomato Bushy Stunt Virus (TBSV) was firstly reported on tomatoes by Smith in 1935 in England. The virus belongs to genus *Tombusvirus* and family *Tombusviridae*, is a soil-borne virus with isometric particle about 30 nm in diameter. Tomato Bushy Stunt Virus can cause chlorosis, necrosis, stunting, leaf yellowing, leaf mottling, leaf crinkling and fruit setting may be reduced or become zero. These symptoms were depending upon the host morphology. Transmission of this virus is naturally through infected seeds, propagative material and manually by the use of infective cutting tools. A numbers of varieties were affected. But it's also observed that *Lycopersicon pimpinellifolium* not susceptible host plant. Gel Electrophoretic analysis shows that virus distantly related serologically with several other viral species in the genus *Tombusvirus*. In phosphotungstic acid, the particles show an angular outline and unresolved surface structure but when mounted in uranyl acetate, they exhibit a rounded outline and somewhat knobby surface and edges. The viral genome is monopartite and TBSV-Ch has been completely sequenced and shown to contain 4,776 nucleotides. The protein shell is constructed by 180 subunits, each subunit contain Mr 41,000 and made icosahedral surface lattice. These subunits show a dimeric clustering on the surface of particles, which give rise to 90 morphological units. These were located on the two-fold axes of the lattice. Each coat protein subunit contains 387 amino acid residues and has four regions.

**Keywords:** Tomato Bushy Stunt Virus, Genus *Tombusvirus*, Family *Tombusviridae*, Monopartite, Genome analysis of TBSV.

## Introduction

It is firstly describe by Smith (1935), then Ainsworth (1936) and Bawden & Pirie (1938). The virus belongs to family *Tombusviridae* and genus *Tombusvirus* (Russo *et al.*, 1994).

A soil-borne virus with isometric particles about 30 nm in diameter and rounded outline, occurring in economically important crops host such as *Lycopersicon esculentum*, *Capsicum annuum*, *Solanum melongena*, *Tulipa* spp., *Tolmiea menziesii*, *Malus* spp. and *Pyrus* spp etc. The virus shows that it have a wide host rage, But it is also observed that *Lycopersicon pimpinellifolium* is not susceptible host of TBSV. The virus restricted on their natural host range, comprising primarily vegetables and ornamentals. Infection of woody plants is less common.

However, the soil-borne nature of the virus and its tendency to remain localized in tissues, make it likely that a wider number of cultivated and wild plants are infected locally in the roots (Kegler & Kegler, 1980., and Cherif, 1981). The type strain of the virus was reported to infect 52 of 157 species (Schmelzer, 1958); and 45 of 62 species (Hollings & Stone, 1965); and the BS3 strain, 33 of 48 species (Cherif & Spire, 1983). Virus particles contain one major linear positive sense, ssRNA species of 4.7 kb and a single coat protein of Mr 41,000. These viruses were transmitted by mechanical inoculation to a number of experimental hosts. Natural transmission is through seed and soil, apparently without a vector. The biological, physicochemical, ultrastructural, and molecular properties of the virus have been reviewed frequently (Martelli *et al.*, 1989., and Russo *et al.*, 1994).

## Symptoms

Tomato Bushy Stunt Virus can cause stunting and bushy growth, chlorotic spots, leaf crinkling, necrosis, deformation of tomato fruit and leaves (Gerik *et al.*, 1990., and Luis-Arteaga *et al.*, 1996) (Fig.1); and pepper (Cherif & Spire, 1983).Fruits become smaller in size than normal and show blotching, rings, line patterns, necrosis and sometimes fruit setting reduced drastically, (Fig.2); that can also minimize the economic value of the crop, or make it unacceptable for consumer. In the case of tomatoes yield losses can be as high as 80% (Gerik *et al.*, 1990).

In the case of eggplant, the virus induces stunting, leaf yellowing and mottling, poor fruit setting, and deformation and necrosis of the fruits (Koenig & Avgelis, 1983., and Luis-Arteaga *et al.*, 1996) (Fig.3 ); mosaic, leaf malformation, necrosis and sometimes death of statice (Krczal & Beutel, 1994); extensive necrosis of the leaves and petals of tulip (Mowat, 1972); stunting and mild mottling of the leaves in piggyback (*Tolmiea menziesii*) (Henriques & Schelegel, 1978); and fruit pitting, veinal necrosis and stunting in cherry (Allen & Davidson, 1967). The virus is also reported from apple (Allen, 1969). Photoperiod and temperature strongly influenced symptom expression, especially in protected vegetable crops (Hillman *et al.*, 1985., and Gerik *et al.*, 1990).

### Geographical Distribution

The TBSV reported by UK, USA, Morocco, Argentina and Tunisia. Also show presence in Portugal, France, Italy, Germany and Canada without evidence of their spread. Information regarding TBSV shows that it is not found from South East Asia.

### Transmission of TBSV

Transmission of Virus is necessary for cause a severe epidemics in an area to whole the growing region of susceptible host. The virus can be transmitted with variable efficiency (4-65%) through seed of pepper, tomato and apple (Tomlinson & Faithfull, 1984). It was also found in seed and pollen of sweet cherry (Allen & Davidson, 1967., and Kegler & Kegler, 1980); but no transmission was obtained to cherry trees hand-pollinated with infected pollen (Allen & Davidson, 1967). The virus is transmitted by grafting in vegetatively propagated crops (e.g. cherry) (Kegler & Schimanski, 1982). This virus is not transmitted by vector, either aerial or soil-inhabiting. The virus is not transmitted by aphids, either non-persistently or semi-persistently (Koenig & Avgelis, 1983).

### Relationship to other Species of Genus *Tombusvirus*

Agar Gel Electrophoresis of this virus and other viral genomes. The virus is distantly related serologically with several other virus species in the genus *Tombusvirus* (Jaegle & Van Regenmortel, 1985., and Koenig & Gibbs, 1986). ELISA was also used for measuring the serological cross-reactivity between this virus species and other species of the genus (Jaegle & Van Regenmortel, 1985). Nucleic acid hybridization with random primed cDNA probes was used for assessing the relationship between the virus and other members in the genus (Gallitelli *et al.*, 1985). And Koenig & Burgermeister, 1988); and for detecting genomic RNA, DI-RNA, and satellite RNAs in total RNA samples from leaves of naturally infected plants (Celix *et al.*, 1997). The genome organization is identical to that of members in the genus *Aureusvirus*, but differences exist in the overall genome size (4.7 versus 4.4 kb) and in the size and sequence homology of the polymerase cistron (Rubino & Russo, 1997). The strategy of expression is similar to that of members of other genera in the family (Russo *et al.*, 1994). The polymerase shows significant protein sequence similarity with comparable proteins of members of the other genera in the family, as well as with that of the genera *Enamovirus* and *Luteovirus* (Russo *et al.*, 1994).

### Particle Properties

Molecular weight:  $9.3 \times 10^6$  (Weber *et al.*, 1970). Sedimentation coefficient ( $s_{20, w}$ ): 132S at infinite dilution (Hollings & Stone, 1965). Buoyant density in CsCl:  $c. 1.35 \text{ g.cm}^{-3}$  (Mayo & Jones, 1973). Diffusion coefficient ( $D_{20} \times 10^{-7} \text{ cm}^2.\text{sec}^{-1}$ ): 1.26 (Schachman & Williams, 1959). Isoelectric point: pH 4.1 (McFarlane & Kekwick, 1938). Partial specific volume:  $0.71 \text{ cm}^3.\text{g}^{-1}$  (Schachman & Williams, 1959). Electrophoretic mobility:  $-4.65 \times 10^5 \text{ cm}^2/\text{sec.V}$  at pH 8.2 in veronal-HCl buffer ionic strength 0.02 (Ambrosino *et al.*, 1967). Zone electrophoretic R $\theta$  value: 0.34 (Quacquarelli *et al.*, 1966). Absorbance is 1 mg/ml, 1 cm light path: 4.5 at 260 nm (Ambrosino *et al.*, 1967).  $A_{260}/A_{280}$ : 1.66 (Ambrosino *et al.*, 1967). P content: 1.54% (Ambrosino *et al.*, 1967).

### Particle Structure

In phosphotungstic acid, the particles show an angular outline and unresolved surface structure (Lovisolo *et al.*, 1967); but when mounted in uranyl acetate, they exhibit a rounded outline and somewhat knobby surface and edges (Francki *et al.*, 1985) (Fig.4). The protein shell is constructed by 180 subunits, each subunit contain Mr 41,000 and made icosahedral surface lattice. These subunits show a dimeric clustering on the surface of particles, which give rise to 90 morphological units. These were located on the two-fold axes of the lattice (Harrison *et al.*, 1978) (Fig.8). Each coat protein subunit contains 387 amino acid residues (Hopper *et al.*, 1984., and Hillman *et al.*, 1989); have four regions, internal region have positive charged domain (R) which contain 66 residues, together with an 36 residues long arm, lay on the N-terminal region of the polypeptide. This region is flexibly linked to two distinct globular parts, 168 residues of domain S, which forms the shell of viral particle and the other 127 residues of domain P protrudes on the surface of the particle. The P domains giving rise to the 90 projections in clustered pairing that lay on the units that are contrasted by negative staining in the electron microscopy. Coat polypeptides can assume three distinct packing orientations, designated A, B and C because of their flexibility (Harrison, 1984., and Hopper *et al.*, 1984). The connecting arms of the 60 subunits in orientation C are folded in an orderly manner at the bottom of the respective S domains, interlocking to form an internal framework which determines particle size (Olson *et al.*, 1983). The N-termini of the remaining 120 subunits in orientations A and B, and the positively charged residues of the inward-facing S domain surfaces, are potential sites for RNA binding (Hopper *et al.*, 1984) (Fig.5). Stability of particle consists on the P domain stable dimer contacts and on trimer interactions between the A, B and C subunits of S domains. This provide strength due  $\text{Ca}^{++}$  ions (two for each pair of interacting subunits), but not  $\text{Mg}^{++}$  ions (Robinson & Harrison, 1982., and Hogle *et al.*, 1983). Induction of reversible swelling of particle is due to removal of  $\text{Ca}^{++}$  occurring at about pH 7.

These are partially sensitive to protease but are not sensitive to RNAase (Kruse *et al.*, 1982). Within the protein shell genomic RNA is tightly packed, probably being located mostly in the space between the S domains and the internal concentric shell of N-terminal regions (Chauvin *et al.*, 1978).

### Particle Composition

**Nucleic acid:** Virus particles contain a positive sense and linear single-stranded RNA, accounting for about 17% of the total particle weight. The RNA has a mol. wt of *c.*  $1.67 \times 10^6$ . Molar percentages of nucleotides are reported as: G28.6, A26.3, C21.2, U26.3 (De Fremery & Knight, 1955) or G27.7, A27.0, C20.7, U24.5 (Ambrosino *et al.*, 1967).

**Protein:** The viral capsid is about 83% of the particle weight and composed of a single polypeptide of Mr 42,000 calculated by polyacrylamide gel electrophoresis (Gallitelli & Russo, 1987), or Mr 41,000, as deduced from the amino acid sequence of the coat protein gene (Hearne *et al.*, 1990).

### Genome Properties

The viral genome is monopartite and that of TBSV-Ch has been completely sequenced and shown to contain 4,776 nucleotides (accession No. M21958) (Hearne *et al.*, 1990). The genome contains five functional ORFs. ORF 5 is completely nested in ORF 4, in a different reading frame (Fig.6).

The 5' terminus does not have a VPg (Mayo *et al.*, 1982); nor, by analogy with the related *Carnation Italian ringspot virus*, a cap (Russo *et al.*, 1994), and the 3' terminus is not polyadenylated. The 5' region initiates with an untranslated sequence (UTR) of 165 nucleotide, and the 3' region terminates with an UTR of 351 nt. The 5'-proximal ORF1 (nt 166-1056) codes for a 33 kDa protein. Readthrough of the amber stop codon of ORF1 results in the translation on a 92 kDa fusion protein (ORF 2, nt 166-2622) (Fig.6). The 33 kDa protein does not contain motifs of any known viral function, but they read through domain in the 92 kDa protein contains the conserved motifs of the RNA-dependent RNA polymerase (RdRp) of positive strand RNA viruses. ORF 3 (nucleotide 2652-3818) encodes the coat protein (41 kDa). The protein encoded by ORF 4 (nucleotide 3856-4425) (22 kDa) is required for spread of the virus from cell to other cell (Scholthof *et al.*, 1993) (Fig.6). The 19 kDa protein encoded by ORF5 (nucleotide 3888-4406) may be involved in the induction of necrotic symptoms (Scholthof *et al.*, 1995a); and in the long-distance spread of the virus, depending on the host (Scholthof *et al.*, 1995b). The strategy of expression is based on direct translation from genomic RNA of the 5' proximal genes (33 and 92 kDa proteins) and translation of the downstream genes through two 3' co-terminal subgenomic RNAs of 2.1 kb (coat protein) and 0.9 kb in size (22 and 19 kDa proteins) (Hearne *et al.*, 1990).

**1. Features of the genome:** Sub-genomic *mRNA*, satellite RNA, and defective interfering RNA (DI RNA) is present in non-genomic nucleic acid of virions. In infected cells sub-genomic *mRNA* and 4 virus specified dsRNA species were found. The largest virus specified dsRNA is 4.733 kbp (corresponding to the genome); 2<sup>nd</sup> is 2.188 kbp (first subgenomic); 3<sup>rd</sup> is 0.936 kbp (2nd subgenomic); 4<sup>th</sup> is 0.62 kbp (satellite RNA); and 5<sup>th</sup> is 0.499 kbp (DI RNA) in size.

**2. Features of proteins:** Virion proteins were not glycosylated and not phosphorylated. Three proteins were found virus coded non virion. First is *M<sub>r</sub>* of 92000, 2<sup>nd</sup> is *M<sub>r</sub>* of 33000 and 3<sup>rd</sup> is *M<sub>r</sub>* of 22000.

### Satellites RNA

The virus supports the replication of a linear satellite RNA (satRNA) lacking coding capacity, and with a size of about 0.6 kb (Gallitelli & Hull, 1985). Virus particles of Spanish isolates from tomato and eggplant were found to contain two types of satRNA of 0.8 and 0.6 kb (Celix *et al.*, 1997); whereas a single species of satRNA of 0.6 kb was encapsidated by particles of the static isolate from Germany (D. Galetzka & M. Russo). Comparison of the sequences of the three satRNAs and of the satRNA associated with *Cymbidium ringspot virus* infections (Rubino *et al.*, 1990); showed the lack of substantial homology with the viral genome, except for the 5'- and 3'-termini and for a central block of about 50 nucleotides.

This latter sequence is conserved in the genome of all members of the genus *Tombusvirus* and in the defective interfering RNAs (DI-RNA) (Rubino *et al.*, 1995); and is thought to be a signal necessary for RNA replication (Russo *et al.*, 1994). SatRNAs are not thought to interfere greatly with virus symptom expression. DI-RNAs are smaller than genomic molecules and are generated *de novo* in the course of infection of experimentally inoculated hosts through recombination events whereby fragments of the viral genome are deleted stepwise (White & Morris, 1994). Their presence interferes with virus replication, thus modulating symptoms. Four regions of the viral genome are conserved in DI-RNAs: the 5' leader sequence of 168 nt; 200-250 nt from the polymerase gene; about 70 nt from the 22 kDa and 19 kDa cistrons; and about 130 nt from the 3' terminal noncoding region (Knorr *et al.*, 1991). DI-RNAs of about 0.4 kb and 0.7 kb were found associated with TBSV-Ch in infected *N. clevelandii* (Hillman *et al.*, 1987, and Celix *et al.*, 1997). A DI-RNA about 0.4 kb in size was

identified also in naturally infected static plants (Galetzka *et al.*, 2000), which may account for the variability of symptoms observed in the field (Krczal & Beutel, 1994).

### Replication

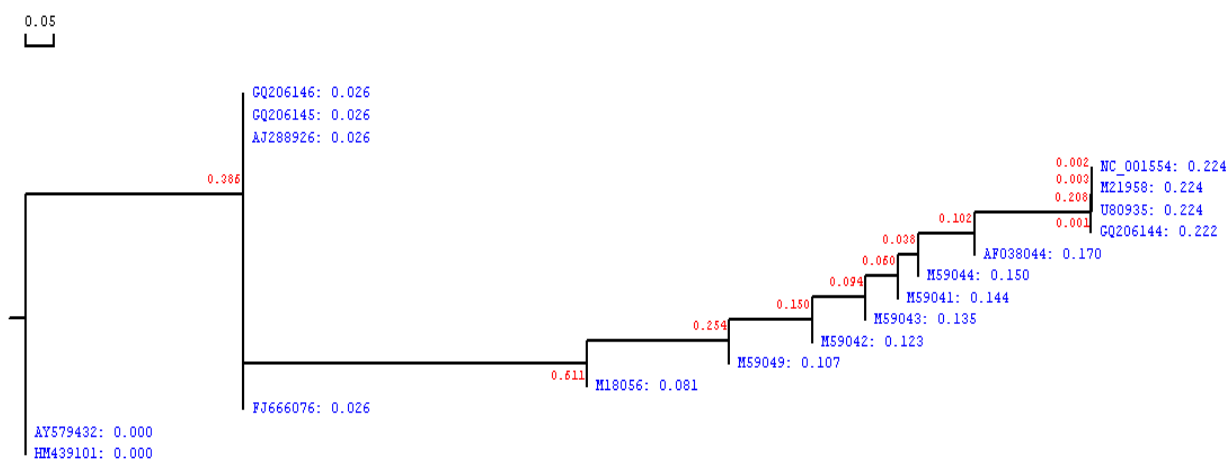
Genome replication occurs in cytoplasm with association of multivesicular bodies. *mRNA* of coat protein is translated in the cytoplasm. Satellite RNA can act as helper virus but replication of virus does not depend on a helper virus.

### Management of TBSV

The virus is firmly established in certain soils, especially clays, where it persists in an infective form for up to five months (Kegler & Kegler, 1980); resisting high temperatures (e.g. autoclaving at 121 °C for 2h) (Kegler & Kegler, 1980), and from which it is readily acquired by bait plants apparently without the mediation of a vector (Martelli *et al.*, 1988). Transplanting of healthy seedlings in contaminated soils which contain previous infected crop residues or watering with virus suspensions and infected plant sap resulted in 10 to 100% infection (Gerik *et al.*, 1990). So, simply we manage it by using IPDM techniques.

### Genome Characterization

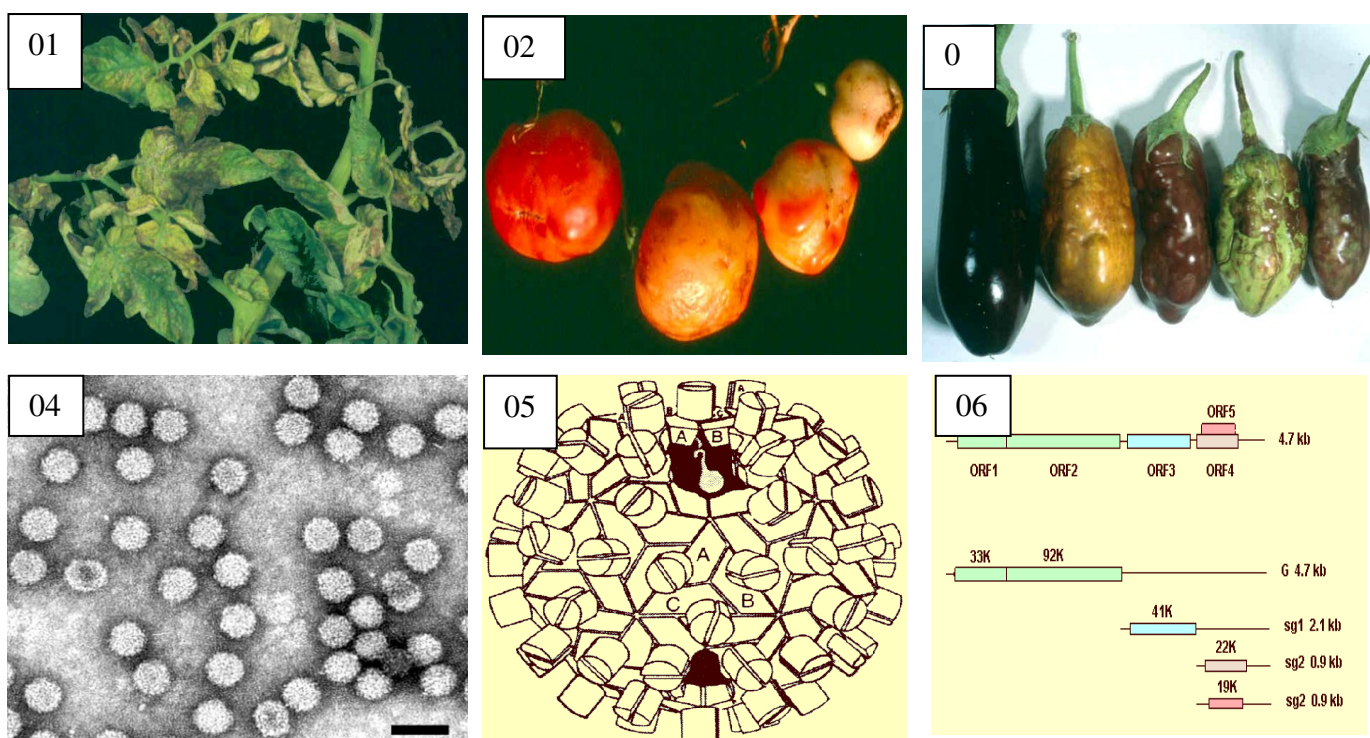
Genome characterizations are necessary for evaluation and identification of strains of TBSV and their mode of infection and also propagation. The NCBI-DATABASE is stands for National Center of Biotechnology Information and located in United States of America. It has information about all organism genome and structure. This information is useful to make and analyzed genome sequence. The 17 different sequences of Tomato Bushy Stunt Viral genome were taken by NCBI-DATABASE, these sequences were putted in DNAMAN software to observe their "Maximum Likelihood and Divergency" by making their Phylogenetic Tree. This tree shows us the differences and likelihood between different sequences.



### Conclusion

Tomato Bushy Stunt Virus is serious problem for wide range of plants. So, its Genome characterizations are necessary for evaluation and identification of strains of TBSV and their mode of infection and also propagation. This information will helpful for making strategies, which will show results in the form of maximum yield returns, betterment of farmer's livelihood and control the diseases of tomato bushy stunt virus.

## Figures



**Figure # 01:** Deformation and necrosis of the leaves of naturally infected tomato, **Figure # 02:** Chlorotic blotching and deformation of fruits from a naturally infected tomato plant, **Figure # 03:** Severe deformation and extensive necrosis shown by fruits from naturally infected eggplant plants, **Figure # 04:** Virus particles mounted in uranyl acetate, **Figure # 05:** Diagrammatic representation of a TBSV particle, **Figure # 06:** Virus genome organization showing the relative positions of the Open Reading Frames and their translational products. (Courtesy of F. Garcia-Arenal, M. Luis-Arteaga and Hopper *et al.*)

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