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# TOMATO BUSHY STUNT VIRUS AND TOMATO ADVANCED LINES/CULTIVARS

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**A B S T R A C T**

Thirty-three advanced lines/cultivars were screened against Tomato Bushy Stunt Virus (TBSV). Infected tomato plants found with leaf chlorosis and necrosis having bushy habit with an incidence of 9.09%. Thirty advanced lines/cultivars showed negative response against TBSV infection. One advanced line/cultivar LITTH-557 showed moderate response whereas two lines/cultivars, NSx6658 and Eurekaka showed the presence of TBSV with strong reaction. Most of the advanced lines/cultivars were found to be free from TBSV infection with high yielding potential.

**Keywords:** *Solanum lycopersicum,* Virus symptoms, Host plant, ELISA.

## INTRODUCTION

Tomato (*Solanum lycopersicum* Linnaeus) is one of the most widely produced plants in Solanaceae family. It is considered as a dominant crop over all other vegetables due to high nutritional value and its use in culinary preparations, ketchups and salads (Chisti *et al*., 2008). Brunt *et al*. (1996); Mughal and Khan (2002); Steven *et al.* (2007) reported more than 20 viruses attacking tomato crop around the globe and the most common are Tobacco Mosaic Virus / Tomato Mosaic Virus (ToMV/TMV), Cucumber Mosaic Virus (CMV), Potato Virus X (PVX), Potato Virus Y (PVY), Tomato Spotted Wilt Virus (TSWV), Tobacco Ring Spot Virus (TRSV), Tomato Yellow Leaf Curl Virus (TYLCV), Potato Leaf Roll Virus (PLRV), Alfalfa Mosaic Virus (AMV), Tomato Leaf Crumple Virus (TLCV) and Tomato Bushy Stunt Virus (TBSV).

TBSV is belonging to the genus Tombusvirus in Tombusviridae family. Tombusviruses were considered as economically important pathogens of tomato crop grown under controlled and open field conditions in many countries (Ohki *et al*., 2005). 1st time TBSV was reported on tomatoes by Smith in 1935 in England. It has a wide experimental and cultivated host range that include tomato, tobacco, pepper, eggplant, apple, pear, spinach, tulip and lettuce (Artelli *et al*., 2001). TBVS can cause stunting and bushy growth pattern, chlorotic spots, leaf crinkling, necrosis, deformation of tomato fruits and leaves (Gerik *et al*., 1990; Luis-Arteaga *et al.,* 1996). Fruit setting and fruit size is reduced than normal with symptoms of blotching, rings, line patterns and necrosis that minimize the economic value of the crop or make it unacceptable for the consumer. Yield losses in tomatoes can be up to 80% due to TBSV infection (Gerik *et al*., 1990). Photoperiod and temperature strongly influenced symptom expression especially in vegetable crops grown under controlled conditions conducive to symptoms expression (Cherif and Spire,1983, Hillman *et al*., 1985). No insect vector has been reported for transmission of TBSV from infected to healthy plants except spread via sap inoculation, contact with infected tissue or contaminated implements as well as through contaminated soil and irrigation water (Koenig, 1988). The virus can be transmitted with variable efficiency (4-65 %) through seeds of pepper, tomato and apple (Tomlinson and Faithfull, 1984). The virus can resist quite high temperature and remains established in certain soils, especially clayey soil where it can persist

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for up to five months. It is readily acquired by bait plants from soil without involving vector transmission (Martelli *et al*.,1988). Transplanting of healthy seedlings in contaminated soils previously cultivated with infected crops or their residues and infected plant sap resulted in 10 to 100% infection (Gerik *et al*., 1990). The study was undertaken to find out the incidence of TBSV and its status in tomato advanced lines/cultivars.

**MATERIAL AND METHODS**

The present studies were carried out during 2012-2013 at Vegetable Research Institute and Plant Virology Section, AARI, Faisalabad.

**Incidence of TBSV in tomato advanced lines/cultivars:** Thirty-three tomato advanced lines/cultivars were examined for disease symptoms (leaf chlorosis, necrosis, shriveling and yellowing of leaves along with bushy growth habit) produced by TBSV as described by Martelli *et al*. (1984) and Mughal and Khan (2002). Three leaf samples from each tomato advanced line/cultivar were collected at random in polythene bags and numbered as described by Mughal and Khan (2002, 2006) & Burhan *et al*. (2007) and stored at 4°C until processed. Total 99 samples from 33 tomato advanced lines/cultivars (Table 1) were collected and tested through ELISA and percentage of incidence was calculated as follows.

$$\% \textbf{ incidence} = \frac{\textbf{No. of plants infected}}{\textbf{Total No. of plants}} \textbf{X 100}$$

**Source of ELISA kit:** Antiserum, antibody conjugated with alkaline phosphatase (IgG conjugate) and positive control for TBSV purchased from Bioreba A.G. Christoph Merian Ring-7, CH-4153; Reinach BL1 Switzerland.

**Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA):** ELISA was performed following the protocols described by Clark and Adams (1977) to confirm the infection of TBSV. Microtiter plate wells (8 ∇ 12 flat-bottom wells of c. 400 µL/well) were coated with antiserum diluted in carbonate buffer (pH 9.6). Plates were incubated overnight at 4°C. IgG used was diluted with carbonate buffer in the proportion of 1: 1000. Following incubation plant extracts were added in the wells. The plant extracts were prepared in pestle and mortar by grinding the tomato leaves in extraction buffer at 1:10 ratio (1 g sample: 10 mL buffer). After adding the crude plant extract, plates were incubated overnight at 4 °C. The virus was detected by the corresponding antibody conjugated with alkaline phosphate diluted in conjugate buffer (PBS-TPO, pH 7.4). Plates were incubated overnight at 4°C and washed with phosphate buffer saline (PBS, pH 7.4) at each stage. Phosphate substrate buffer p-nitrophenyl was coated on the plates. Buffer and healthy samples were also charged as a control. The samples were left at room temperature (25 ºC) for 30 minutes. Reaction was recorded based on yellow color development, No yellow stain development = (-), Mild yellow stain development = (+), Moderate yellow stain development = (++), Strong yellow stain development = (+++). The sample was considered positive if yellow stain was observed.

**RESULTS AND DISCUSSION**

Infected tomato leaves produced signs of leaf chlorosis and necrosis along with bushy growth habit as described by Luis-Arteaga *et al*. (1996) and Mughal and Khan (2002). Nine plant samples out of 99 were found infected with TBSV with an incidence of 9.09%. Hafez *et al.* (2010) identified the TBSV serologically by ELISA and found its incidence up to 25.5% in Egypt. The disease incidence may be attributed to plants susceptibility to TBSV. According to Tomlinson and Faithfull (1984) and Koenig (1988) TBSV may also be spread through contact with infected tissue / contaminated implements. Kim *et al*., (2007) stated that the virus may spread through infected seed or contaminated soil and irrigation water. Many scientists have reported TBSV presence in tomato (Gigante, 1955; Pontis *et al*., 1968; Martinez *et al*., 1974; Fischer and Lockhart, 1977, Borges *et al*., 1979; Cherif and Spire, 1983; Gerik *et al*., 1990; Luis-Arteaga *et al*., 1996. Tunisia and Spain has already faced TBSV epidemic in eggplant and pepper (Cherif and Spire, 1983; Luis-Arteaga *et al*., 1996). Novák *et al*. (1981) and Yilmaz, (1981) identified TBSV in diseased lettuce plants. Ninety samples from 30 tomato advanced lines/cultivars (LITTH-539, LITTH-545, Jury F1, LITTH-551, LITTH-550, LITTH-514, Sohail F1, LITTH-561, LITTH-558, Money Maker, LITTH-555, LITTH-556, LITTH-560; LITTH-544, LITTH-559, LITTH-541, LITTH-508, LITTH-513, Sahil F1, 08532; 08516, 08504, Austra; 08502, 08517, 08518, 08542, 08543, Nagina and R Grande) showed no reaction against TBSV. The variety /line LITTH-557 showed moderate yellow stain development (++) whereas the varieties /lines “Eurekaka and NS x 6658” showed TBSV infection with strong yellow stain development (+++). Results are summarized in Table 1.

Table 1: ELISA based detection of TBSV in Tomato advanced lines/cultivars.

| Sr. No. | Variety/line | Presence of TBSV |
|---|---|---|
| 1 | LITTH-539 | - |
| 2 | LITTH-545 | - |
| 3 | Jury F1 | - |
| 4 | LITTH-551 | - |
| 5 | LITTH-550 | - |
| 6 | LITTH-514 | - |
| 7 | Sohail F1 | - |
| 8 | LITTH-561 | - |
| 9 | LITTH-558 | - |
| 10 | Money Maker | - |
| 11 | LITTH-555 | - |
| 12 | LITTH-557 | ++ |
| 13 | LITTH-556 | - |
| 14 | LITTH-560 | - |
| 15 | LITTH-544 | - |
| 16 | LITTH-559 | - |
| 17 | LITTH-541 | - |
| 18 | LITTH-508 | - |
| 19 | LITTH-513 | - |
| 20 | Sahil F1 | - |
| 21 | 08532 | - |
| 22 | 08516 | - |
| 23 | 08504 | - |
| 24 | NSx6658 | +++ |
| 25 | Austra | - |
| 26 | Eurekaka | +++ |
| 27 | 08502 | - |
| 28 | 08517 | - |
| 29 | 08518 | - |
| 30 | 08542 | - |
| 31 | 08543 | - |
| 32 | Nagina | - |
| 33 | R Grande | - |

**CONCLUSION**

It was concluded that most of the advanced lines/cultivars, used in tomato breeding program at Vegetable Research Institute, AARI, Faisalabad, are found free from TBSV with high yielding potential except LITTH-557, Eurekaka and NS x 6658.

**REFERENCES**

Artelli, G. P., M. Russo and M. Rubbino. 2001. Tomato bushy stunt virus. A.A.B. Descriptions of Plant Viruses, pp. 382.

Borges, M. D. L., J. C. Sequeira and D. Louro. 1979. Aparecimento em Portugal do virus do emanjericado do tomateiro (tomato bushy stunt virus) Hospedeiros, morfologia e localizaçao nas celulas de Pimenteiro. Phytopathol. Mediterr. 18, 118 – 122.

Brunt, A. A., K. Crabtree, M. J. Dallwitz, A. J. Gibbs and L. Watson. 1996. Viruses of Plants, Description and lists from the VIDE Database. CAB International. 1284-1323.

Burhan, M., M. A. Khan, M. Irfanullah, M. Ishfaq, M. E. Javed. 2007. Comparison of seed potato produced from different rapid multiplication sources against PVX, PVY and PVS through ELISA. J. Agric. Res. 45 (1): 67-71.

Cherif, C. and D. Spire. 1983. Identification of tomato bushy stunt virus on tomato, pepper and eggplant in Tunisia: some characteristics of the Tunisian strain. Agronomie 3: 701–706.

Chisti, S. A. S., A. A. Khan, B. Sadia and I. A. Khan. 2008. Analysis of combining ability for yield, yield

componenets and quality characters in tomato (*Lycopersicon esculentum*). J. Agric. Res. 46 (4): 325-332.

Clark, M. F. and A. M. Adams. 1977. Characteristics of micro plate method of enzyme linked immunosorbent assay for the detection of plant diseases. J. Gen. Virol. 34: 475-483.

Fischer, H. U. and B. E. L. Lockhart. 1977. Identification and comparison of two isolates of tomato bushy stunt virus from pepper and tomato in Morocco. Phytopathology 67, 1352 – 1355.

Gerik, J. S., J. E. Duffus, R. Perry, D. C. Stenger and A. F. Van Maren. 1990. Etiology of tomato plant decline in California desert. Phytopath. 80: 1352-1356.

Gigante, R. 1955. Il 'rachitismo cespuglioso' del pomoro. Boll. Stn. Patol. Veg. Rome 12, 43 – 56.

Hafez, E. S. E., G. A. Saber and F. A. Fattouh. 2010. *Tomato Bushy Stunt Virus* (TBSV) infecting *Lycopersicon escluntum* (Zeitschrift) Fur Naturforsching Section. J. Biosci. 65c (9-10): 619-626.

Hillman, B. I., D. E. Schlegel and T. J. Morris. 1985. Effects of low molecular weight RNA and temperature on tomato bushy stunt virus symptom expression. Phytopath.75: 361-365.

Koenig, R. 1988. The Plant Viruses: Vol. 3. (New York, NY: Plenum Press). 13-72.

Luis-Arteaga, M., E. Rodriguez-Cerezo, A. Fraile, E. Saez and F. Garcia-Arenal. 1996. Different tomato bushy stunt virus strains that cause disease out breaks in solanaceous crops in Spain. Phytopath. 86: 535-542.

Martelli, G. P., A. Di Franco and M. Russo. 1984. The origin of multivesicular bodies in Tomato Bushy Stunt Virus-infected Gomphrenaglobosa plants. J. Ultra structural Res. 88, 275-281.

Martelli, G. P., D. Gallitelli and M. Russo. 1988. Tombus viruses. Plant Viruses R. Koenig. ed. Plenum Press, New York. 3: 13-72.

Martinez, J. A., J. A. Galindo and R. M. Rodriguez. 1974. Estudio sobre la enfermedad del 'Pinto' del jitomate (*Lycopersicon eculentum* Mill) en la region de Actopan, HGO. Agrociencia 18, 71 – 78.

Mughal, S. M. and M. A. Kan. 2006. Symptoms of Viral Diseases in Plants in: Laboratory Manual of Plant Virology, Department of Plant Pathology, University of Agriculture, Faisalabad. 9-15.

Mughal, S. M. and S. M. Khan. 2002. Viral diseases of tomato. Monthly Nida-e-Kisan.17-19.

Novák, J. B., J. Nováková and J. Lanzová. 1981. Dukaz viru zakrslosti rajcete (tomato bushy stunt virus) na hlavkovem salatu. Sb. UVTIZ Ochr. Rostl. 17, 241 – 245.

Pontis, R. E., O. Gracia, and J. M. Feldman. 1968. Tomato bushy stunt virus on tomato crops in Argentina. Plant Dis. Rep. 52, 676 – 677.

Smith, K. M. 1935. Strains of tomato bushy stunt virus. Annals of Applied Biology 22, 731-741.

Steven, T. K., G. Peter and A. O. Paulus. 2007. Viral disease, *Solanum lycopersicum* (tomato) in: Vegetable Diseases, A Colour Hand Book. 361-366.

Tomlinson, J. A. and E. M. Faithfull. 1984. Studies on the occurrence of tomato bushy stunt virus in English rivers. Ann. Appl. Biol. 104:485-495.

Yilmaz, M. A. 1981. Virus particles associated with diseases of tomato and lettuce in Turkey. Phytopathol. Mediterr. 20, 79 – 80.