Defensive Interaction of Different *Lycopersicum esculentum cvs* Infected by *Tomato Bushy Stunt Virus*

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Abstract: The *Tomato bushy stunt virus* (TBSV) P19 is one of a class of plant and animal virus proteins that are known to interfere with host defense mechanism, referred to as RNA silencing. Such proteins target foreign (virus and retrotransposon) RNAs in a sequence specific manner for degradation. Tomato, *Lycopersicum esculentum L*; Elisa, Super Strain-B (Super) and GS *cvs* were inoculated with the *Tomato Bushy Stunt Virus (*TBSVEgh). The P19 gene sequence was determined from both TBSVgh pure virus preparation and from virus inoculated tomato cultivars tissue. The sequence of the viral P19 gene showed similarity with P19 gene of Western United States TBSV with 98% identity. Differential display polymerase chain reaction (DD-PCR) was used for studying the up- and down-regulated genes in the three examined cultivars. High expression of Actin genes were observed in inoculated plants. The expression of plant defense genes was higher in *L. esculentum* Mill Elisa cultivar than cultivar GS. Results indicated that some suspected defense genes, against TBSV Egh, are *de novo* genes.
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1. Introduction

Plant viruses can cause huge economic losses in crop production. Viruses induce a series of physiological changes after invading the susceptible host plants which can lead to disease symptoms including systemic and local symptoms (Culver and Padmanabhan, 2007). Several innate defense pathways of the host and the counteracting virus encoded suppressor(s) for gene silencing also play important roles in virus infection (Ahlquist et al., 2003; Sanfacon, 2005; Nagy, 2008). *Tomato bushy stunt virus* (TBSV) is the type member of the genus Tombusvirus within the family Tombusviridae. In nature, TBSV isolates occur as different pathogenic strains that collectively infect many vegetable crops and also other plant species; including *Lycopersicon esculentum* (tomato), *Capsicum annuum* (pepper), *Cynara cardunculus* (artichoke), *Prunus avium* (cherry), *Vitis vinifera* (grapevine) and some *Rosa sericea* (ornamentals). The natural occurrence of TBSV is thus far known to be geographically distributed across central and Western Europe, North Africa and the Americas (Martelli et al., 2001).

For TBSV, the P19 gene is a cytoplasmic protein of 19 KDa, (Scholthof et al., 1995; Turina et al., 2003). TBSV and its P19 gene provide a suitable genetic model system to investigate the differences and similarities between virus-host interactions in susceptible versus resistant plants. P19-protein mediates three phenotypically distinct activities: it is required for systemic spread of TBSV in some hosts e.g., spinach (Lakatos et al., 2004) and the P19-protein induces a systemic lethal collapse (Scholthof et al., 1995). P19 is also a suppressor of post-transcriptional and virus-induced gene silencing (Voinnet et al., 2003). The seemingly separate biological activities require P19 to be expressed at high levels, and also crucial for its function are centrally located charged amino acids (Qiu et al., 2002). It is generally accepted that the possession of a gene-silencing suppressor by a plant virus is an important counter defense strategy for the virus to establish successfully a systemic infection (Voinnet et al., 2003).

Actin cytoskeleton, plays an important role in cell morphogenesis in plants as demonstrated by pharmacological, biochemical, and genetic studies (Kost and Chua, 2002; Mathur and Hülskamp, 2002). The actin cytoskeleton may be involved in the transportation of organelles and vesicles carrying membranes and cell wall components to the site of cell growth as in root hairs, trichome cells, and pollen tubes. Therefore, the actin cytoskeleton is essential for cell elongation and tip growth. Similarly, inhibition of F-actin elongation blocked the initiation of polar growth and elongation of root hairs (Miller et al., 1999). Furthermore, reduction in actin arrays resulted in dramatic reduction of root hair length and caused severe bulges in the *actin2* (*act2*) mutant and serious retardation of root growth in the *act7* mutant in *Arabidopsis thaliana* (Gilliland et al., 2003). Mis-expression of the reproductive *ACT11* gene in vegetative tissues of *Arabidopsis* altered morphology

of most organs in plants because of its effects on the proportion of different actin isovariants (Kandasamy et al., 2002).

The aim of this study is to test P19-mediated protein–protein interactions and its relation to RNA silencing. Comparison between the sequences of P19 gene isolated from purified virus particles and that of the mutated sequence in the infected plant tissues. Identification of plant defensin genes that resist viral infection and comparison of the reaction of TBSV tomato cultivar (GS) with that of the others (Elisa and Supper) used in the study.

2. Materials and Methods

Propagation of TBSV Egh in Three Different *L. esculentum* Cultivars

TBSV was propagated in three different cultivars of *L. esculentum* by mechanical inoculation from virus infected leaves. Plants used for inoculation were 10-15 days old. *L. esculentum cvs* used were Elisa, Super Strain-B (Super) and GS. Inoculated plants were sustained for 35 dpi under greenhouse conditions. Confirmation of infection was by symptoms and DAS-ELISA kit (Agdia, USA).

Extraction of Viral RNA From Pure Virus Preparation and From Inoculated Host Plant Tissue

Extraction of genomic viral RNA was performed by QIAamp Viral RNA Mini column for purification of viral RNA (Qiagen Int; USA). Total RNA was extracted from plant tissue using Plant RNA Extraction kit (Omega bio-tek, USA).

Amplification of P19 From TBSV Egh Isolate and From Inoculated Host Plant Tissue

cDNA was synthesized from both viral RNA and total RNA extracted from the three inoculated tomato cultivars. The reaction consists of 2.5 µl RNA combined with 5 µl of a 2x reverse transcription mixture, 2.5 µl dNTPs (4 mM), 1 µl of the P19F**:** 5`-AGC TCG AGC CAT GGA ACG AGC TAT-3', 1 µl (50unit/µl) of Murine Leukemia Virus (MLV) reverse transcriptase and volume was completed up to 20µl of RNAs free water. The reaction was incubated at 37°C for 1 hr, followed by one cycle for inactivating the enzyme at 75°C for 10 min. The 2nd PCR reaction was performed in a total volume of 25 µl containing 2.5 µl (10x) buffer, 1.5 µl 25 mM $MgCl_2$, 1µl dNTP mixture, 0.25 µl Taq polymerase (Red Hot Taq DNA Polymerase, Thermo Fisher Scientific (Abgene), USA) and 1 µl of P19 F primer: 5'-AGC TCG AGC CAT GGA ACG AGC TAT-3', 1 µl of P19PstR primer: 5'-AGC TGC AGT TAC TCG CTT TCT TTT TCG-3', 2 µl cDNA and dH_2O up to 25 µl. The PCR reaction conditions; 94°C for 3min, followed by 35 cycles consists of 94° C for 20 sec, annealing at 67°C for 30 sec, and extension at 72°C for 1 min and ending with extension at 72°C for 5 min then hold at 4°C.

Cloning and Sequencing of TBSV P19 Genes and Accession Numbers

The amplified P19 genes from the purified viral RNA genome and from the total RNA of the three inoculated tomato cultivars were cloned into the vector (PCR 2.1-TOPO, Invitrogen, USA). The PCR amplicones were subjected to DNA sequence by using the forward primer P19F (Macrogen Inc, Korea) and the sequences were submitted to the Genebank.

Sequence Analysis and Phylogentic Construction

Computer-based sequence comparison of the obtained DNA nucleotide sequence of P19 genes with other P19 genes in different known TBSV strains was carried out by sequence alignment ClustalW (1.82) software**.** Phylogenetic tree was generated using Mega 4 program.

Up-Down Regulated Genes in the Three Examined *L. esculentum* Cultivars Inoculated with TBSV Egh

Leaf samples were collected in duplicates from the inoculated host plant cultivars at different time intervals: 1, 2, 7, 14, 21, 28, and 35 days post inoculation (dpi). PCR reaction was performed for the extracted RNA in a total volume of 25 µl (2.5 µl 10x Taq buffer, 1 µl $MgCl_2$ (100 mM), 2 µl (20 mM) dNTP mixture, 1 unit Taq polymerase (DFS-Taq DNA polymerase, bioron, GmbH, 1µl from each primer (Actin-f -5' GGC GAT GAA GCT CAA TCC AAA CG 3' and Acti r- 5' CCT CAC GAC CAA GAT CAA GAC G 3' consecutively, 2 µl cDNA and the volume was completed by sterile distilled H_2O). The reaction mixture was subjected to amplification as follows: 94°C for 5 min, 40°C for 5 min, and 72°C for 5 min in 9700 thermal cycler (Perkin-Elmer), followed by 40 cycles through 94°C for 1 min, 65°C for 1 min and 72°C for 2 min, final extension cycle at 72°C for 10 min.

Up-down Regulation Genes Isolation, Sequencing and Sequence Analysis

The PCR products were purified, cloned and sequenced as previously mentioned and the obtained DNA sequences were submitted to the Genebank. Sequence alignment was performed using ClustalW 1.4 program and the phylogentic construction was performed using the MEGA4 program.

3. Results

TBSV Egh Inoculation Into the Three Different *L.esculentum cvs*

Inoculation of the three different *L.esculentum* cultivars Elisa, Super, and G.S with TBSV Egh under greenhouse conditions, resulted in the appearance of different intensity of symptoms as shown in Figure 1. Symptoms included chlorotic lesions with different patterns, bushy growth and stunting with malformations of newly formed leaves.

Figure 1. (A) Symptoms of TBSV Egh infection on *L. esculentum* under greenhouse conditions. Cultivars are; G.S, Elisa and Super, **(**B) RT-PCR products for the P19 gene at different intervals post inoculation. Lane M: 1 Kb plus DNA ladder, lane1:1dpi, lane 2: 2dpi, lane 3:7dpi, lane 4: 14dpi, lane 5: 21dpi, lane 6: 28dpi and lane 7: 35dpi.

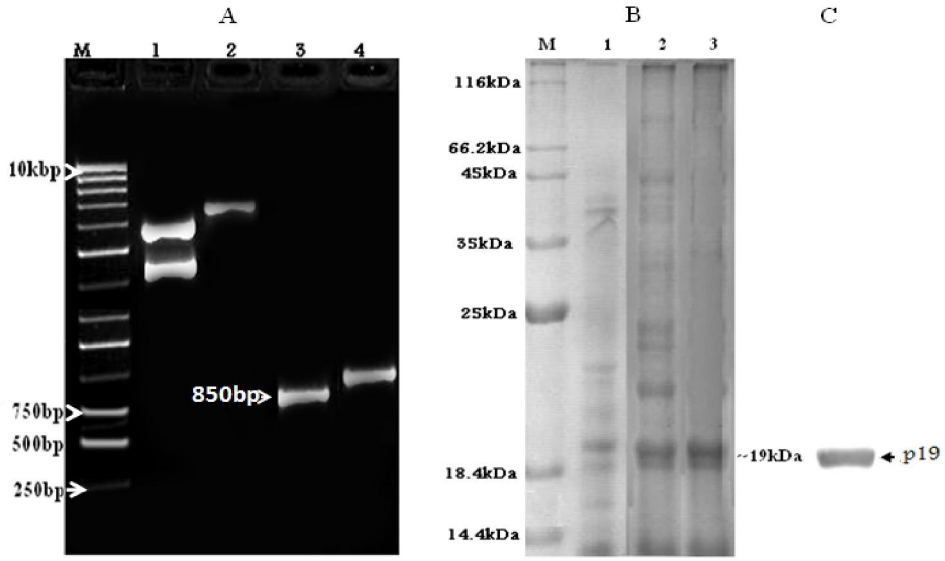


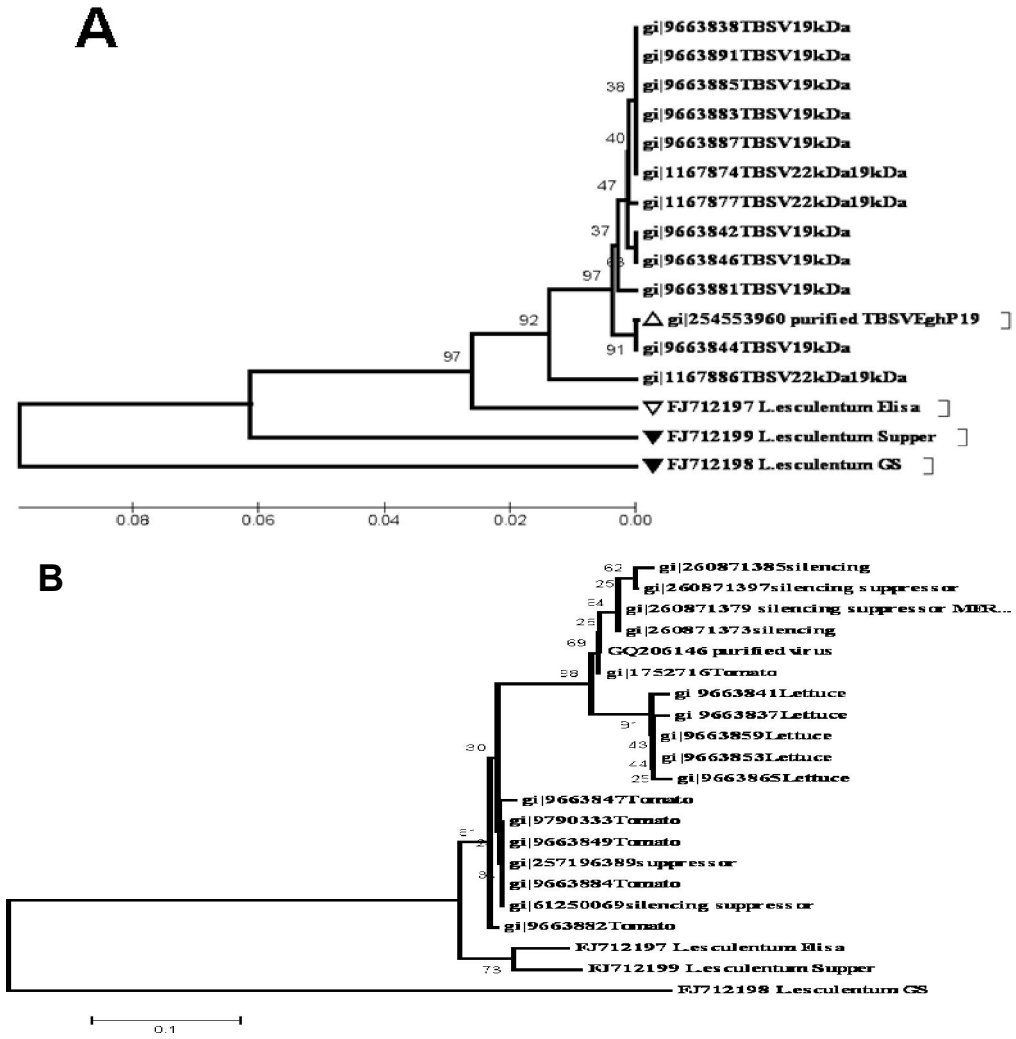
Figure 2. (A) PCR amplification for cloning of the P19 Egh gene. Lane M: Molecular weight standard 1 Kb plus DNA ladder (Sib Enzyme); Lane1: circular pCR 2.1-TOPO plasmid vector; Lane2: P19 Egh recombinant plasmid; Lane3: amplified P19 Egh gene by specific P19 primer from recombinant plasmid and Lane4: amplified P19 Egh gene from recombinant plasmid by M13 primer, (B) SDS-PAGE for the recombinant p19 fractions and purifications, (C) Western blot for the purified P19 using the TBSV antiserum.

Resistance of *L.esculentum* Cultivars to the Egyptian Isolate of TBSV Based on P19 Gene Expression

TBSV symptoms in inoculated plants were most severe on the cultivar G.S, less severe on cultivar Elisa and moderate on the cultivar Super. Leaf samples harvested from inoculated plants at 1, 2, 7, 14, 21, 28, and 35 dpi, were used as template for the amplification of the P19 gene. Results presented in Figure 2 showed that the gene expression of the P19 increased with the increase of dpi. It has been observed that there was a noticeable differential increase in P19 gene expression in different cultivars. For the cultivar G.S a marked increase was seen at 7 dpi, whereas in cultivar Super the increase was noticed at 21 dpi, and in cultivar Elisa at 35 dpi. A phylogenetic tree was constructed based on the DNA sequence of the isolated P19 genes and their deduced amino acids (Figure 3) and the

results revealed that the P19 purified from the TBSV Egh virus was similar to Elisa P19 with identity 86% (based on the DNA nucleotide sequence and 100% based on the amino acids sequence). On the other hand P19 gene sequence of Super cultivars was less similar. In addition, the G.S P19 gene showed far less similarity with the other P19 genes as well as it formed a separate cluster.

Figure 3. Phylogenetic tree of the P19 genes Egh compared to that of P19 and P22 genes of reported TBSV isolates. (A) the phylogeny based on the DNA nucleotide sequence, (B) Deduced amino acids sequence alignment between the four obtained P19 genes and other P19 genes of tomato, lettuce, and silencing genes, (C) Sequence alignment between the four P19 genes amplified from pure TBSV and from the three inoculated tomato cultivars by the use of ClustalW 1.4.



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TBSVP19          ATGGAACGAGCTATACAAGGAAACGACG-CTAGGGAACAAGCTAACAGTGAACGTTGGGA 59
ElisaP19         --------------GCAGGGAGACGACGACAAGGGAACAAGATTACAGTGAAAGTTGGGA 46
SupperP19        --------------AAGGGAAGGGGGCAGTAGGGGACGAGTCAAACAGTGGACGTTGGGA 46
GSP19            ------------------GCCCAGCACACCTGGGA---AGCTTAAT----GACGTTGGGA 35
                    *       *     **    *     *      * *******

TBSVP19          TGGAGGATCAGGAAGTT-CCACTTCTCCCTTCCAACTTCCTGACGAAAGTCCGAGTTGGA 118
ElisaP19         TGGAGGATCAGGAAGGTGCCACTTCTCCCTTCAAACTTCCAGACGAAAATCCGAATTGGA 106
SupperP19        TGGAGAGTCAAGG-GGTGCCGCTTCTCCCGTCAAACTTCCTGACGAAAGTCGGAGTTGGA 105
GSP19             TGGAGGATTAAGGAGGT-CCACTTCTCCCTTCCAACTTCCTTACGAAAGTCCGAGTTGGA 90
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TBSVP19         CTGAGTGGCGGCTACATAACGATGAG-ACGAATTCGAATCAAGATAATCCCCTTGGTTTC 177
ElisaP19        CAGAGAGGCGGCAACATAACGAAGAG-GCGAATTCGAATCAAGATAATCCCCAAGGTTTC 165
SupperP19       CTGAGTGGCGCCTGCGTAACCATGAGAGCGGATTGGAATCAAGATAATCCCCTTGGTTTA 165
GSP19           CTTGGTGGCGCCTACATTACGATTTG-ACGAATTCCAATCCAGATTATCCCCTTGGTTTC 149
                *   * ******  * * ** *   *  ** ***  ****************  *****

TBSVP19         AAGGAAAGCTGGGGTTTCGGGAAAGTTGTATTTAAGAGATATCTCAGATACGACAGGACG 237
ElisaP19        AAGGAAAGCTGGGGTTTCGGGAAAGTTGTATTTAAGAGATATCTCAGATACGACAGGACG 225
SupperP19       AAGGGAAGCTGGGGTTTCGGGGAAGGTGTATTAAAGAGATATCTAAGATACGACAGGACG 225
GSP19           AAGGAAAGCTGGGGTTTCGGGAAAGTTGTATTTTGGAGATATCTCAAATACGACAGGACG 209
              **** ***************** *** ******   ********* * *************

TBSVP19         GAAGCTTCATTGCACAGAGTCCTTGGATCTTGGACGGGAGATTCGGTTAACTATGC-AGC 296
ElisaP19        GAAGCTTAACTGCACAGAGTCCTTGGATCTTGGACGGGAGATTCGGTTAACTATGC-AGC 284
SupperP19       GAAGCTTCACTGCACAGAGTCCTTGGATCTTGGACGGGAGATTCGGTAAACTATGC-AGC 284
GSP19           CAAGCTTCACTGCACAGAGTCCTTGGATCTTGGACGGGAGATTCGGTTTACTATGCCAGC 269
              ****** * ************************************  ******* ***

TBSVP19         ATCTCGATTTTTCGGTGTCAACCAGATCGGATGTACCTATAGTATTCGGTTTCGAGGAGT 356
ElisaP19        ATCTCGATTTTTCGGTTTCGACCAGATCGGATGTACCTATAGTATTCGGTTTCGAGGAGT 344
SupperP19       ATCTGGATTTTTCGGTTTGGACCAGATCGGATGTACCTATAGTATTCGGTTTCGAGGAGT 344
GSP19           CTCCCGATTTTTCGGTTTCCACCAGATCGGATGTACCTCTGGTATTCGGTTTCGAGGAGT 329
              **  *********** *  ****************** * ******************

TBSVP19         TAGTGTCACCATTTCTGGAGGGTCGAGAACTCTTCAGCATC-TCTGTGAGATGGCAATTC 415
ElisaP19        TAGTATCACCGTTTCTGGAGGGTCGCGAACTCTTCAGCATC-TCTGTGAGATGGCAATTC 403
SupperP19       AAGTATAACCGTTTCTGGAGGGTCGCGAACTCTTCAGCATC-TCTGTGAGATGGCAATTC 403
GSP19           TTGTATCCCCGTTTCTGGAGGGTCGCGCACTCCTCAGCACCCTCCGCGAGATGGCCACTC 389
              ** *  ** ************** * **** ****** * ** * ******** * **

TBSVP19         GGTCTAAGCAAGAACTGCTACAGCTTACCCCAGTCGAAGTGGAAAGTAATGTATCAAGAG 475
ElisaP19        GGTCTAAGCAAGAACTGCTTCAGCTTGCCCCAATCGAAG-GGAAAGTAATG-ATCAAGAG 461
SupperP19       GGTCAAAGCAAGAACTGCTACAGCTTGCCCCAATCGAAG-TGAAAGTAATG--TTAAAGA 460
GSP19           GGCCCAAGCAAGAACTGCTTCCGCCTGCCC------------------------------ 419
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Differential Display and the Up-and Down-Regulated Genes in Inoculated Cultivars, Sequence and Sequence Analysis

Results indicated the presence of up-regulated genes in the inoculated plant samples compared to control (Figure 4). A large number of such unique bands that are only present in inoculated host plants or whose expression increased post inoculation were purified. The major observation was an increase in the expression of the actin genes in inoculated plants. Differential host plant defense gene expression was higher in number in case of Elisa cultivar and relatively lower in case of the cultivar G.S. Six different bands from the three cultivars were further sequenced. Genes (Egh6def, Egh7def and Egh8def) were amplified by Actin 1-f specific primer from Elisa, Super and G.S cultivars, respectively. But the other three genes; Egh9def, Egh10def and Egh11def were amplified by Actin 1-r primer from the three inoculated cultivars (Table 1).

Phylogentic Relationship Among Suspected *L.esculentum* Defense Genes

It is shown that some suspected defense genes cDNA has sequence homology to that of the other previously described defense genes in *L.esculentum*. The Egh6def, Egh7def, Egh8def, Egh9def, Egh10def and Egh11def genes were found in the same cluster with the Actine genes from different *L.esculentum* cultivars with homology between 95-86% (Figure 5). Egh12def, Egh13def, Egh14def and Egh15def genes have homology of (< 91%) to resistance genes under traits from *L.esculentum* and (< 92%) with cluster which have genes enhancing disease resistance and ABA 8'-hydroxylase gene in *L. esculentum*, while Egh16def and Egh17def genes have 96% homology to genes enhancing disease resistance in *L. esculentum.*

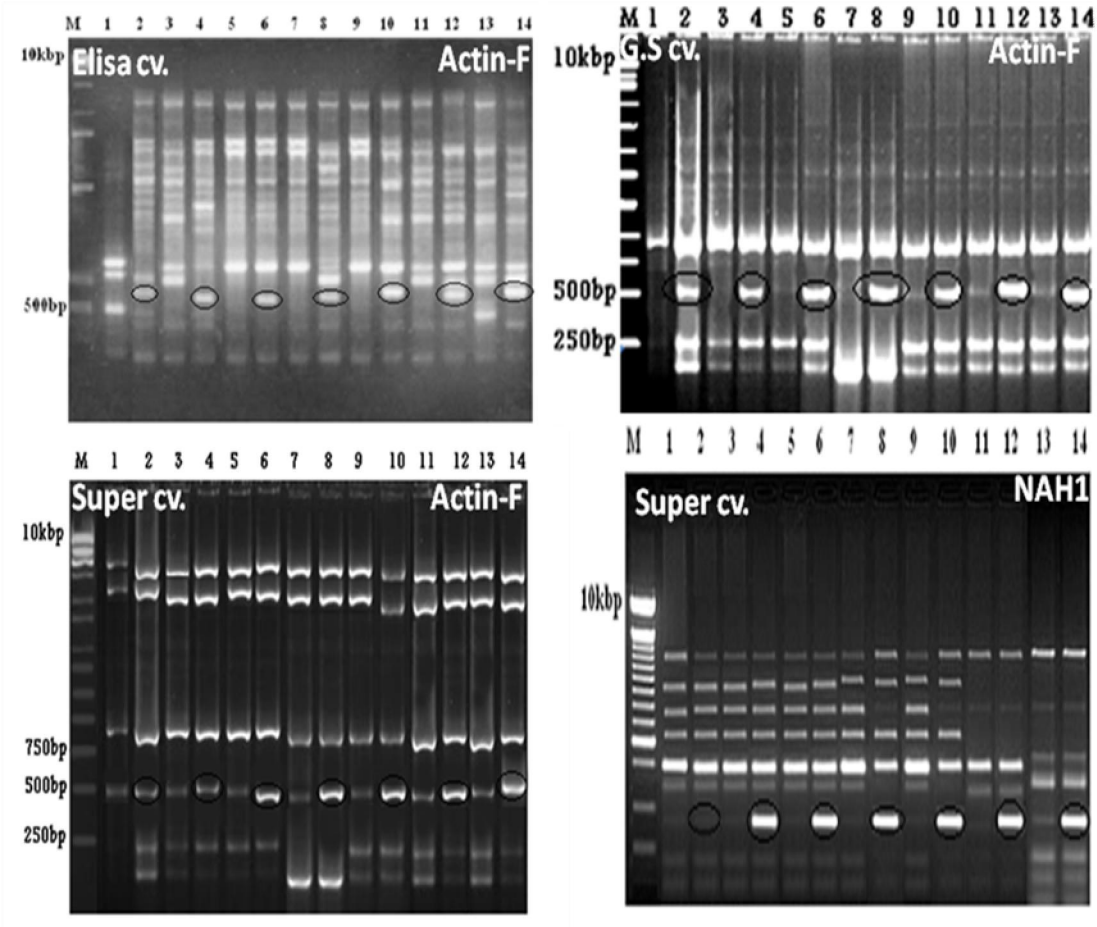

Figure 4. Representative of Differential display for the TBSV inoculated *L.esculentum cvs*; Elisa, G.S and Supper. The DD-PCR was carried out using different arbitrary primers. Lane M: Molecular weight standard 1 Kb plus DNA ladder (Sib Enzyme); Lanes respectively: 1. control 1 day post inoculation (dpi), 2.TBSVEgh inoculated 1 dpi, 3. control 2 dpi, 4. TBSVEgh inoculated 2 dpi, 5. control 7 dpi, 6. TBSVEgh inoculated 7 dpi, 7. control 14 dpi, 8. TBSVEgh inoculated 14 dpi, 9. control 21 dpi, 10. TBSVEgh inoculated 21dpi, 11. control 28 dpi, 12. TBSVEgh inoculated 28 dpi, 13. control 35 dpi, 14. TBSVEgh inoculated 35 dpi.

Table 1. P19 protein gene sequences

Clone name	Length (bp)	Source of isolation	Accession number	Primers
P19 gene of TBSV Egh	846	Purified TBS Egh isolate	GQ206146.1	P19 universal
P19 Egh1 gene	780	Tomato cultivar Elisa	FJ712197.1	P19 universal
P19 Egh2 gene	778	Tomato cultivar GS	FJ712198.1	P19 universal
P19 Egh3 gene	778	Tomato cultivar Super	FJ712199.1	P19 universal
Egh6def	468	Tomato cultivar Elisa *	FJ712202.1	Actine-1 forward
Egh7def	460	Tomato cultivar Super *	FJ712203.1	Actine-1 forward
Egh8def	462	Tomato cultivar GS *	FJ712204.1	Actine-1 forward
Egh9def	465	Tomato cultivar Elisa **	FJ712205.1	Actine-1 reverse
Egh10def	306	Tomato cultivar Super **	FJ712206.1	Actine-1 reverse
Egh11def	355	Tomato cultivar GS **	FJ712207.1	Actine-1 reverse

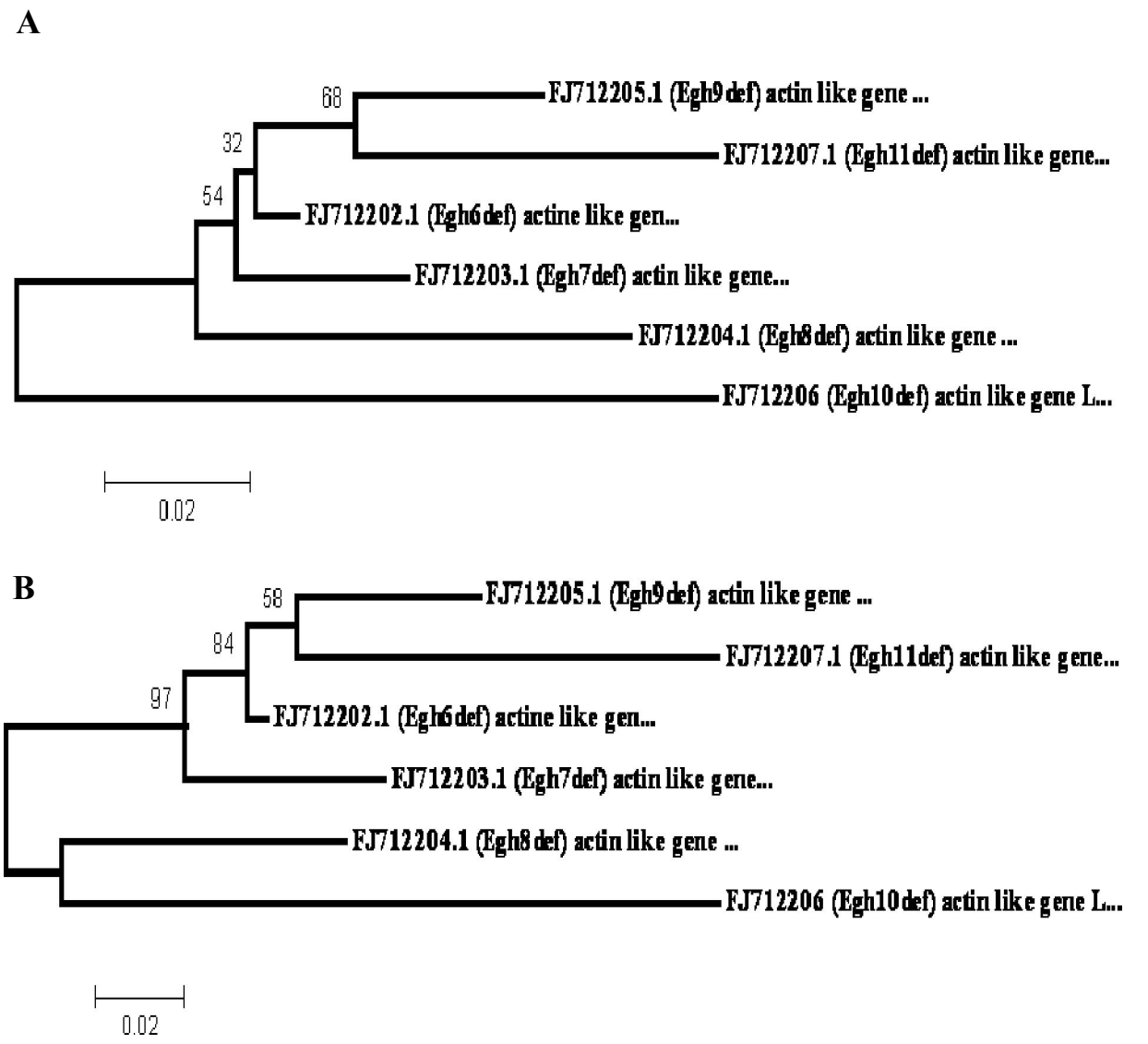

Figure 5. Phylogentic tree for the amplified Actin genes from the three *L.esculentum cvs* inoculated with TBSV Egyptian isolate. (A) phylogeny based on the DNA nucleotide sequence, (B) phylogeny based on the DNA nucleotide sequence.

4. Discussions

Based on symptomatology and serological identification *Tomat bushy stunt virus* was detected in 7 different governorates in Egypt, the TBSV Egh isolate was purified and characterized (Hafez et al., 2010). Characteristic symptoms were detected 7 dpi. It has been reported that TBSV-Ch, TBSV-Nf, TBSV-P or TBSV-S isolates, show characteristic symptoms, however, are not enough to delineate different strains of the virus.

Primers were designed from the available sequence data in the GenBank for cloning and sequencing of the genome of the Egyptian P19 TBSV Egh. Results indicated that the sequence of the P19 gene coding for the 19KDa protein occupied the same cluster with JA6 and type isolate with a 99% homology. The P19 gene on ORF4 encodes viral movement protein that is necessary for cell to-cell movement and symptom determination on certain host plants (Scholthof et al., 1995) from nt 3844 to nt 4413. Based on comparison of 3'-end sequences 96 to 95% divergences was found between different TBSV isolates, it showed a less degree of homology with other isolates namely Nipplefruit, Statice and Cherry strains included in the comparison. Pairwise amino acid sequence comparison on the other hand also indicated more homology to Lettuce JA6 isolates and

Cherry strains. Accordingly TBSV Egh isolate may be regarded as a new strain (Hafez et al., 2010).

Increase in the expression of P19 gene compared to the increase of time post inoculation among Cultivars G.S, Super and Elisa respectively was detected. Each RT-PCR product gave a band of about 780bp which is different from the product of RT-PCR made to purified viral RNA of TBSV Egh which is about 850bp. Results indicated that sequence of P19 Egh1 and P19 Egh3 from *cv*. Elisa and Super respectively had additional nucleotide sequences which showed homology to the enhancing part with several defense genes of *L.esculentum* previously described. This confirms an interaction between TBSV P19 gene and host proteins which resulted in recombination of the mRNA of P19 gene in both cultivars Elisa and Super (Baulcombe and Molnar, 2004; Zamore, 2004; Burgyan, 2006). A deletion in P19 Egh2 was also detected further confirming genetic divergence due to virus host interaction. Many plant viruses have adopted strategies for evading or directly suppressing the host cell's RNA silencing pathway (Zamore, 2004; Burgyan, 2006). Tombusviruses have adapted the RNA silencing pathway through the production of a 19KDa protein (P19) that acts as a siRNA inhibitor (Scholthof, 2006; Omarov et al., 2007). The P19 protein acts as a dimer and binds to the minor groove of siRNA duplexes (Ye et al., 2003). The P19 dimer appears to block silencing by directly binding siRNA and sequestering it from the RNA silencing pathway machinery (Ye et al., 2003; Burgyan, 2006). TBSV P19 has the possibility of the interference with host in the assays (Molnar et al., 2005; Wang and Nagy, 2008) as the P19 is known to be a suppressor of VIGS (Roth et al., 2004).

A large number of disease resistance genes has been cloned and sequenced in recent years, and the proteins that they encode are structurally related. Most resistance genes encode modular, cytosolic proteins that possess a central nucleotide-binding site (NBS) linked to a C-terminal leucine-rich repeat (LRR) domain (Dangl and Jones, 2001). Differential display (DD) is a technique that may allow rapid, accurate and sensitive detection of altered gene expression. *L. esculentum* Mill has been examained by Wei et al (2000), using Differential Display-Polymerase Chain Reaction (DD-PCR). The DD-PCR is used for screening induced genes in the TBSV Egh infected *L.esculentum* cultivars at different dpi in comparison to control mock-inoculated plants. Such procedure allowed the detection of synthesize of a large number of genes which are suspected to be defense genes transcribed and translated in response to viral infection. The DD-PCR by using actin-f and actin-r primers proved that the actin gene was more expressed in infected plants of different cultivars more than the control plants. This rate of expression increased with the increase of time post inoculation. Such genes were also different for different cultivars. Cultivar Elisa showed expression of more up-regulated genes than cultivar Super and no detected defense genes in cultivar G.S. Similar results were obtained by Zhao and Liu (2002) who induced the plant immune system by abiscisic acid (ABA) in salt stressed plants and observed a regulating salt-responsive gene expression in roots of the examained plants. According to the *in vitro* translation products and phylogenetic relationships we found that similarity was observed between the suspected defense genes compared to other known defense genes in *L.esculentum* but with percentage less than 95%. This may indicate that the suspected defense genes against TBSV Egh are *de novo* genes. According to previous results, cultivar Elisa is a more resistant cultivar to TBSV Egh. RNA silencing which is a common strategy shared by eukaryotic organisms to regulate gene expression, and also operates as a defense mechanism against invasive nucleic acids such as viral transcripts (Asselbergh et al., 2008; Scholthof and Alvarado, 2009).

Expression of P19 increased in *L.esculentum* cultivars infected with TBSVEgh but also many conformational changes occurred along the gene when the virus interacted with the host plant cells. The P19 gene activates the plant defense system, which renders some of these genes to express themselves such as Actine- 1 gene and ABA 8'-hydroxylase gene. It is clear that both genes' expression increased with increasing the inoculation time. P19 isolated from infected plant tissues by TBSV, had deleted regions when compared with P19 isolated from purified virus preparations.

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References

1. Culver JN, Padmanabhan, MS. Virus-induced disease: Altering host physiology one interaction at a time. Annu Rev Phytopathol 2007;45:221-43.
2. Ahlquist P, Noueiry AO, Lee WM, Kushner DB, Dye BT. Host factors in positive-strand RNA virus genome replication. J Virol 2003;77:8181-6.
3. Sanfacon H. Replication of positive-strand RNA viruses in plants: contact points between plant and virus components. Can J Bot 2005;83:1529-49.

4. Nagy PD. Yeast as a model host to explore plant virus-host interactions. Annu Rev Phytopathol 2008;46:217-42.
5. Martelli GP, Russo M, Rubbino M. Tomato bushy stunt virus, Aab Descriptions of Plant Viruses. Publisher: AAB. 2011;382.
6. Scholthof KBG, Scholthof HB, Jackson AO. The tomato bushy stunt virus replicase proteins are coordinately expressed and membrane associated. Virology 1995;208:365-9.
7. Turina M, Omarov R, Murphy JF, Bazaldua-Hernandez C, Desvoyes B, Scholthof HB. A newly identified role for Tomato bushy stunt virus P19 in short distance spread. Mol Plant Pathol 2003;4:67-72.
8. Lakatos L, Szittya G, Silhavy D, Burgyan J. Molecular mechanism of RNA silencing suppression mediated by p19 protein of tombusviruses. EMBO J 2004;23:876-84.
9. Voinnet O, Rivas S, Mestre P, Baulcombe D. An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. Plant J 2003;33:949-56.
10. Qiu W, Park JW, Scholthof HB. Tombusvirus P19-mediated suppression of virus-induced gene silencing is controlled by genetic and dosage features that influence pathogenicity. Mol Plant–Microbe Interact 2002;15:269-80.
11. Kost B, Chua NH. The plant cytoskeleton: Vacuoles and cell walls make the difference. Cell 2002;108:9-12.
12. Mathur J., Hu¨ lskamp M. Microtubules and microfilaments in cell morphogenesis in higher plants. Curr Biol 2002;12:R669-76.
13. Miller DD, de Ruijter NCA, Bisseling T, Emons AMC. The role of actin in root hair morphogenesis: Studies with lipochito-oligosaccharide as a growth stimulator and cytochalasin as an actin perturbing drug. Plant J 1999;17:141-54.
14. Gilliland LU, Pawloski LC, Kandasamy MK, Meagher RB. Arabidopsis actin gene ACT7 plays an essential role in germination and root growth. Plant J 2003;33:319-28.
15. Kandasamy MK, McKinney EC, Meagher RB. Functional nonequivalency of actin isovariants in Arabidopsis. Mol Biol Cell 2002;13:251-61.
16. Hafez EE, Saber G, Fattouh FA. *Tomato Bushy Stunt Virus* (TBSV) infecting *Lycopersicon esculentum*. Z Naturforsch 2010;65c:619-26.
17. Baulcombe DC, Molnar A. Crystal structure of p19-a universal suppressor of RNA silencing. Trends Biochem Sci 2004;29:279-81.
18. Zamore PD. Plant RNAi: how a viral silencing suppressor inactivates siRNA. Curr Biol 2004;14:R198-200.
19. Burgyán J. Virus induced RNA silencing and suppression: defense and counter defense. J Plant Pathol 2006;88:233-44.
20. Scholthof HB. The Tombusvirus-encoded P19: from irrelevance to elegance. Nat Rev Microbiol 2006;4:405-11.
21. Omarov RT, Ciomperlik JJ, Scholthof HB. RNAi-associated ssRNA-specific ribonucleases in Tombusvirus P19 mutant-infected plants and evidence for a discrete siRNAcontaining effector complex. Proc Natl Acad Sci USA 2007;104:1714-9.
22. Ye K, Malinina L, Patel DJ. Recognition of small interfering RNA by a viral suppressor of RNA silencing. Nature 2003;426:874-8.
23. Molnar A, Csorba T, Lakatos L, Varallyay E, Lacomme C, Burgyan J. Plant virus-derived small interfering RNAs originate predominantly from highly structured single stranded viral RNAs. J Virol 2005;79:7812-8.
24. Wang R, Nagy P. Tomato bushy stunt virus co-opts the RNA-binding function of a host metabolic enzyme for viral genomic RNA synthesis. Cell Host Microbe 2008;3:178-87.
25. Roth BM, Pruss GJ, Vance VB. Plant viral suppressors of RNA silencing. Virus Res 2004;102:97-108.
26. Dangl JL, Jones JD. Plant-pathogens and integrated defense responses to infection. Nature 2001;411:826-33.
27. Zhao G, Liu J. Isolation of a cotton RGP gene: a homology of reversibly glycosylated polypeptide highly expressed during fiber development. Biochim Biophys acta 2002;1574:370-4.
28. Asselbergh B, Achuo AE, Höfte M, Gijsegem FV. Abscisic acid deficiency leads to rapid activation of tomato defense responses upon infection with *Erwinia chrysanthemi*. Mol Plant Pathol 2008;9:11-24.
29. Scholthof HB, Alvarado V. Plant responses against invasive nucleic acids: RNA silencing and its suppression by plant viral pathogens. Semin Cell Dev Biol 2009;20:1032-40.

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