Molecular cloning, expression, and characterization of mouse single-chain fragment variable antibody against *Citrus tristeza virus*

(Received: 01.08 .2006; Accepted: 02.09.2006)

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ABSTRACT

The sequences encoding the mouse heavy-chain (V_H) and light-chain (V_L) variable region genes were isolated by PCR and joined into a single-chain Fv (scFv) DNA by using a DNA linker encoding (Gly4Ser)$_3$ peptide. The scFv DNA fragment was cloned into the phagemid pCANTAB5E and expressed in Escherichia coli as a fusion protein with M13 phage p3 polypeptide and E tag. The scFv fusion protein was displayed on the surfaces of recombinant M13 phages in the presence of the helper phage M13K07. High-affinity scFv phage-bodies against citrus tristeza virus (CTV) were enriched through affinity selection on immobilized recombinant CTV coat protein preparations. The selected recombinant phages were used to infect Escherichia coli HB2151 for the production of soluble scFv antibodies. One selected clone in HB2151 secreted a soluble scFv antibody that detected CTV in extracts of infected citrus plants with a sensitivity comparable to that of a commercial monoclonal antibody. The nucleotide sequence of the light-chain and heavy-chain portions were closely related to other published scFv against CTV. The potential of this scFv was demonstrated in routine field testing using an inexpensive tissue print–ELISA.

Key words: *Cloning, expression, mouse single chain antibody, Tristeza virus.*

INTRODUCTION

Citrus tristeza virus (CTV), belonging to the Closteroviridae family, is one of the most destructive diseases of citrus worldwide (Wallace, 1956; Bar-Joseph *et al.*, 1979). CTV is a monopartite virus with flexuous-filamentous particles and a plus sense single stranded RNA genome of approximately 19.2 kb (Karasev *et al.,* 1995). CTV is semipersistently transmitted by different aphid species and different strains of CTV have been isolated and result in different syndrome including stem pitting, quick decline due to phloem necrosis, and tree death (Bar-Joseph *et al.,* 1989; Cambra *et al.,* 1995).

Quick and reliable CTV identification methods are needed. Serological methods play an important role in the detection and identification of CTV (Garnsey and Cambra, 1991; Bar-Joseph *et al*., 1997; Cambra *et al.,* 1993). Although widely used in routine diagnosis, polyclonal antisera are usually available in limited amounts and its specificity varies from batch to batch. Therefore, they are increasingly replaced by monoclonal antibodies (MAbs), because these antibodies can be produced indefinitely (Cambra *et al.,* 1990; Garnsey and Cambra, 1991). Several monoclonal antibodies against the CTV coat protein recognized the epitopic variations in the viral coat protein which are also related to

infection severity (Vela *et al.,* 1986 and 1988; Permar *et al.*, 1990). Recently, it has been shown that engineered antibodies and single chain fragment variable (scFv) antibody developed from a hybridoma cell line are useful in virus diagnoses (Fecker *et al.,* 1996; Toth *et al.*, 1999; Terrada *et al.,* 2000).

The technology of recombinant antibodies is fairly recent and offers a new approach for the detection of many pathogens. In combination with phage display, an extremely useful platform can be established for the production of specific antibodies (McCafferty *et al*., 1990; Hoogenboom *et al*., 1991; Griffiths *et al*., 1994; Vaughan *et al*., 1996). Furthermore, it has been evidenced that expression of scFv in transgenic plants can protect plants against virus attack (Tavladoraki *et al*., 1993; Fecker *et al*., 1997). Thus far, highly specific recombinant antibodies have already been produced against several important plant pathogens (Ziegler *et al*., 1995; Harper *et a*l., 1997; Susi *et al*., 1998; Griep *et al*., 2000) without the use of laboratory animals and time consuming immunization protocols.

In this study, we cloned the sequences encoding the mouse V_H and V_L domains and expressed them as a single chain fragment variable (scFv) antibody library in *Escherichia coli* (*E. coli*). A scFv antibody against CTV was selected from the M13 phage display library after panning on immobilized recombinant CTV coat protein preparation. This scFv antibody was compared with a commercial monoclonal antibody in field testing using tissue print–ELISA.

MATERIALS AND METHODS

Materials

Recombinant Phage Antibody System (RPAS), plasmid pCANTAB5E, *Escherichia coli* TG1 and HB2151, M13K07 helper phage, mouse anti-M13 antibody, mouse anti-E tag antibody, the *Sfi*I-*Not*I double-digested DNA encoding scFv against rabbit IgG, and the QuickPrep™ micro mRNA Purification Kit were obtained from Amersham Pharmacia Biotech (Piscataway, N.J.). Other molecular biology reagents used in this experiment, goat anti-mouse IgG peroxidase conjugate, and rabbit anti-mouse IgG alkaline phosphatase conjugate were purchased from Sigma Chemical Co. (St. Louis, Mo.). The SV Total RNA Isolation System was purchased from Promega (Madison, WI, USA). The oligo (dT) cellulose columns, *Not*I restriction enzyme, and PCR amplification primers were purchased from Gibco BRL (Gaithersburg, MD). The QIAEX gel extraction kit was from QIAGEN Inc. (Chatsworth, CA, USA). The restriction enzyme *Sfi*I was purchased from New England BioLabs (Beverly, Mass., USA). The CTV infected tissue (virus source) was kindly provided by Prof. Dr. Abou-Zeid, A. M. (Virus and Phytoplasma Research Department, Plant Pathology Research Institute, ARC, Giza).

Mice Immunization

The recombinant CTV/CP, used as an immunogen in the immunization experiment, was expressed in *E. coli* and purified as a 6x-His fusion protein by Ni-NTA resin affinity columns as described by Bar-Joseph *et al.* (1997); Sequeira and Nolasco (2002); and Wahle *et al.* (1999). The immunization schedule was carried out on six BALB/c-mice females of 10 to12 weeks old as described by Harlow and Lane, (1988) and Zola, (1995). For primary immunization, 20 mg (100-300 µl) of CTV expressed as a 6x-His fusion protein were emulsified in complete Freund's adjuvant (CFA) and injected intraperitoneally (IP) for at day zero. For secondary immunizations, the same amount of immunogen was emulsified in incomplete Freund's adjuvant (IFA) and administered IP at

days 14, 28, and 42. The mice with appropriate immune response were sacrificed by cervical dislocation. The abdominal skin of the animal was wetted with 70% alcohol and then cut with sterile scissors to expose spleen. Spleens were removed using sterile forceps and placed in a sterile mortar for homogenization in liquid nitrogen for mRNA isolation.

Preparation of mRNA

Total RNAs were prepared from 3 different spleens of immunized mice by using the SV Total RNA Isolation System. The mRNA was purified by affinity chromatography using the QuickPrep™ micro mRNA Purification System and oligo (dT)-cellulose column according to the instructions of the manufacturers.

Synthesis of cDNA, amplification of V_H and V_L regions, and assembly of scFv DNA

First-strand cDNA was synthesized from mRNA template by using the first-strand cDNA synthesis kit (Pharmacia Biotech) with either random hexadeoxyribonucleotides [pd(N)6] primers or oligo dT or the specific minus-sense primer. The variable regions of heavy chain (V_H) and light chain (Vκ) were amplified from first-strand cDNA by using PCR primers (Table 1) provided by Amersham Pharmacia Biotech (Piscataway, N.J.) and *Taq* DNA polymerase with 30 cycles of PCR (1 cycle is 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C). A 93-bp DNA linker containing a sequence encoding a short flexible peptide, $(Gly4Ser)_3$, was also amplified with primers LNK(+) and LNK(-) shown in Table (1). After gel purification of the amplified V_H and V_L genes with a QIAEX gel extraction kit (QIAGEN Inc.), the scFv genes were assembled by overlap PCR as described in the Recombinant Phage Antibody System (RPAS) manual provided by Amersham Pharmacia Biotech (Piscataway, N.J.). The assembled scFv products were reamplified with restriction site-tagged primers (VH1S (+) and an equimolar amount of VK1 (-), VK2N (-), VK3N (-), and VK4N (-) mix or RS Primers Mix from Pharmacia Biotech) to append an *Sfi*I site on the 5` end and a *Not*I site on the 3` end of the scFv DNA. The scFv DNA products were digested with *Sfi*I and *Not*I restriction enzymes, agarose gel-purified, and ligated into the phage-display vector pCANTAB5-E (Amersham Pharmacia biotech) that had been cut with the same restriction enzymes. All molecular biology procedures were carried out in accordance with the standard protocols described by Sambrook *et al.* (1989).

ScFv-phage Rescue

The ligat products of scFv and pCANTAB5-E were used to transform competent cells of *E. coli* TG1 (amber suppressor strain, *supE*). Transformed cells were plated on SOB medium containing 100 µg of ampicillin per ml and 2% glucose and incubated at 30°C overnight. Colonies were pooled and infected with M13K07 helper phage in 2XYT medium containing 100 ug of ampicillin per ml and 50 ug of kanamycin per ml to rescue the phagemid with its scFv gene inserts and to display scFv fusion protein on the surfaces of the recombinant phages as described in the Recombinant Phage Antibody System (RPAS) manual provided by Amersham Pharmacia Biotech (Piscataway, N.J.). The recombinant phages were prepared from the supernatant by polyethylene glycol (PEG)/NaCl precipitation.

Affinity Selection

Culture plates were coated with recombinant CTV coat protein at the concentration of 80 µg/ml in coating buffer (0.05 mol/L $NaHCO_3$, pH 9.6). The plate was blocked with 3% BSA for 2 hours and the concentrated phages (about 5 x 10^{10} PFU) were added to the well of the plate, incubated

Table (1): Primers used to amplify the mouse V_H and V_L regions.

ID	Nucleotide Sequence (5'—3')
SCFVS+	ATTGGCCCAGCCGGCCATG
VH1S+	CATGCCATGACTCGCGGCCCAGCCGGCCATGGCCSAGGTSMARCTGCAGSAGTCWGG
VH1+	AGGTSMARCTGCAGSAGTCWGG
VH1-	TGAGGAGACGGTGACCGTGGTGCCTTGGCCCC
LNK+	ACGGTCACCGTCTCCTCAGGTGGAGGC
LNK-	AGTGAGCTGGATGTCCGATCCGCCACC
VK1+	GACATCCAGCTCACTCAGTCTCCA
VK1-	CCGTTTGATTTCCAGCTTGGTGCC
VK1N-	GAGTCATTCTGCGGCCGCCCGTTTGATTTCCAGCTTGGTGCC
VK2-	CCGTTTTATTTCCAGCTTGGTCCC
VK2N-	GAGTCATTCTGCGGCCGCCCGTTTTATTTCCAGCTTGGTCCC
VK3-	CCGTTTTATTTCCAACTTTGTCCC
VK3N-	GAGTCATTCTGCGGCCGCCCGTTTTATTTCCAACTTTGTCCC
VK4-	CCGTTTCAGCTCCAGCTTGGTCCC
VK4N-	GAGTCATTCTGCGGCCGCCCGTTTCAGCTCCAGCTTGGTCCC
SCFVN-	TCATTCTGCGGCCGCCCGT

K=G or T; M=A or C; S=C or G; R=A or G; W=A or T

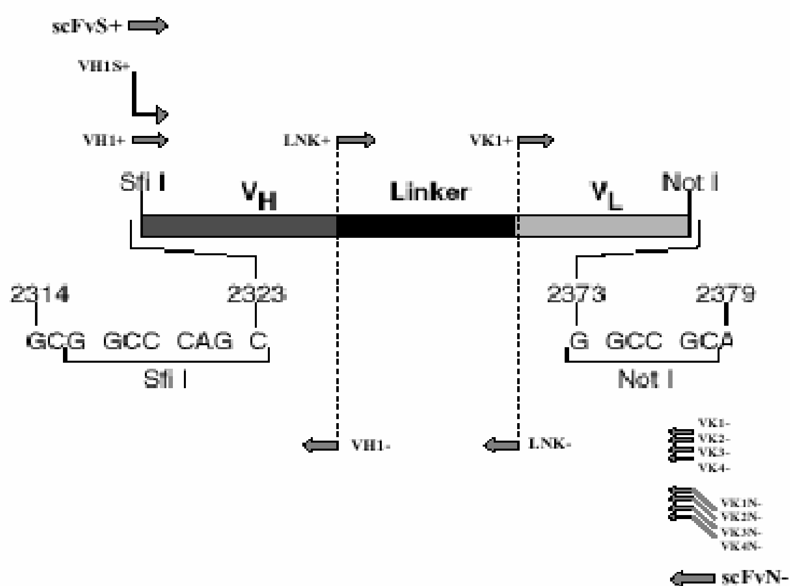

Fig. (1): Primer Strategy used to amplify the mouse scFv antibody repertoire: The locations of the primers and the restriction sites are shown in the diagram.

at the room temperature for 90 min. The plates were washed 10 times with PBST and the bound phage was eluted by the 0.1M of triethylamine and neutralized with 1M Tris buffer (pH 7.4). These CTV-specific phages were used to reinfect *E. coli* TG1 cells for subsequent rounds of selection. The affinity of recombinant phage-bodies was tested with enzyme-linked immunosorbent assay (ELISA) after each round of selection.

Soluble scFv

Recombinant phages from selected clones were used to infect *E. coli* HB2151 for production of soluble scFv antibodies as described by the manufacturer of the Recombinant Phage Antibody System (RPAS). The infected cells were cultured in SB medium (35 g of tryptone per liter, 20 g of yeast extract per liter, 5 g of NaCl per liter) supplemented with 100 µg of ampicillin per ml at 30ºC with 250 rpm shaking until they reached an A_{600} of 0.5. To induce expression, IPTG was added to 1 mM final concentration, and the cells were incubated on a shaker at 30ºC overnight. The supernatants containing the extracellular soluble scFv antibodies were separated from the cell pellets by centrifugation at 1,500 x*g* for 15 min and filtered through a 0.45 um-pore-size filter.

ELISA

The affinities of recombinant phage-bodies or soluble scFv antibodies were tested with enzyme-linked immunosorbent assay (ELISA) in microtiter plates coated with 1 µg/well of recombinant CTV-CP antigen Blocking was performed with 3 % bovine serum albumin (BSA) at 37ºC for 2 h according to standard procedures. The plates were washed with PBS buffer, and 100 µl of alkaline phosphatase/anti-E tag conjugate (soluble scFv) or mouse anti-M13 antibody followed by anti-mouse IgG alkaline phosphatase conjugate ((phage-bodies or phage-ELISA) in PBS buffer containing 1 % BSA) were added, and incubated at 37 ºC for 1 h.

SDS-PAGE and Western blot analysis

Supernatants containing the extracellular soluble scFv antibodies secreted from *E. coli* HB2151 were precipitated with trichloroacetic acid (TCA) at a final concentration of 10% on ice for 20 min. After centrifugation in a microcentrifuge at full speed for 10 min, the pellet was resuspended in 15 ml of 0.5 M Tris buffer (pH 8.0), heated for 5 min at 95°C after the addition of 5 ml of 4X loading buffer, and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12% SDS-PAGE) in a Biometra Electrophoresis System according to the procedure of Laemmli (1970). After separation, the protein bands in one gel were transferred to a nitrocellulose membrane (Schleicher & Schuell, Keene, N.H.). The transblotted membrane was probed with anti-E tag antibody (about 8 µg/ml in PBS-OVA containing 0.05% Tween 20) and then incubated first with rabbit anti-mouse IgG-alkaline phosphatase conjugate (Sigma Chemical Company) as described by the manufacturer's instructions and then with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate (BCIP/NBT) substrate for alkaline phosphatase until the protein bands have reached the desired intensity. The reaction was stopped by washing the membrane in deionized water for several minutes. The membrane was air dried on a filter paper and photographed.

Nucleotide Sequence Analysis

Phagemid DNAs from the clones producing scFv antibody against CTV were isolated from *E. coli* HB2151 by alkaline lysis (Sambrook *et al*., 1989). V_H and V_K DNA

portions in were sequenced on both strands with the dye terminator chemistry and using pCANTAB5 sequence primers (Amersham Pharmacia Biotech). All automated sequencing was performed at the Sequencing Facility of the Plant Pathology Resaerch Institute, ARC, (Giza).

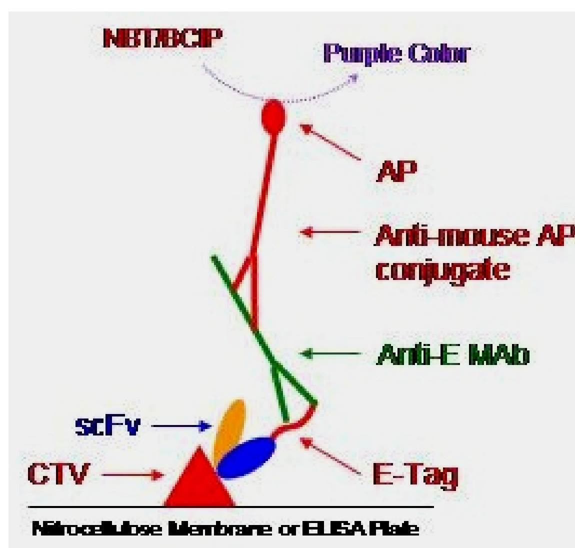

Fig. (2): Tissue Print ELISA: The anti-CTV scFv was tested in field examinations on tissue prints of fresh citrus stems collected from Gaafareia region (Abou Hammad, El-Sharkeia Governorate). Membranes were incubated with anti-CTV scFv purified from E. coli and/or the 3DF1 monoclonal antibody (INGENASA, Madrid, Spain), or a non-specific scFv antibody against rabbit IgG (as a negative control) and tested according to the CTV detection procedure described by Garnsey et al. (1993), and Cambra et al. (1999).

The printed membranes were blocked by incubation in 3% bovine serum albumin in distilled water overnight. Tissue print ELISA was performed by incubation of the membranes with antibodies for 2 hours at room temperature. After washing, membranes were sequentially incubated with anti-E-Tag antibodies for 2 hours at room temperature. Finally, the membranes were incubated with AP-conjugated anti-mouse immunoglobulins (Sigma, Aldrich) at 4ºC overnight. After washing, nitro blue tetrazolium and 5-bromo-4-chloro-3indolyl phosphate (Sigma) was used as substrates. The processed membranes were washed in water, dried and examined under a binocular microscope at 5 to 50x magnification.

RESULTS

PCR amplification

The V_H and V_K cDNAs were amplified by PCR and assembled into scFv DNA product (Fig. 1). The amplification of V_H generated the expected 340-bp fragment, while the amplification of V_K generated a major DNA fragment with the expected length (about 324 bp).

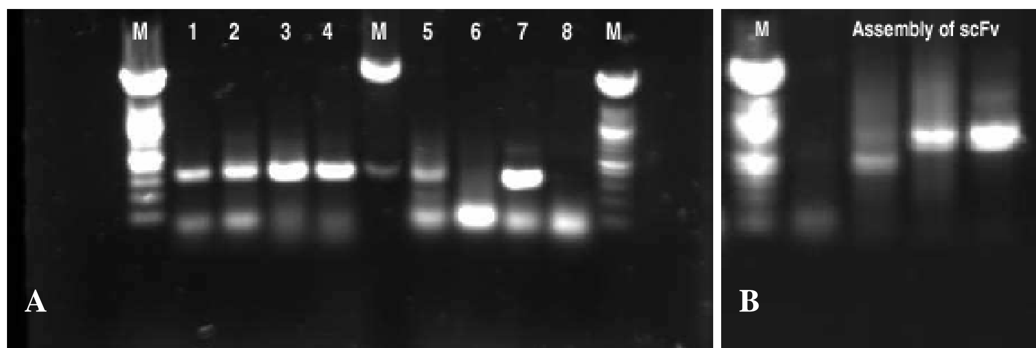

Fig. (3): (A) Amplification of F_v genes from splenocytes mRNA by RT-PCR. Lane M: Molecular weight marker. Lanes 1, 2, 3, and 4: V_H, ~340 bp. Lanes 5, 6, and 7: V_L, ~325 bp. (B) Assembly of scFv DNA by fusion PCR or PCR-mediated splicing. The assembled scFvs had a size of ~750 bp (arrow).

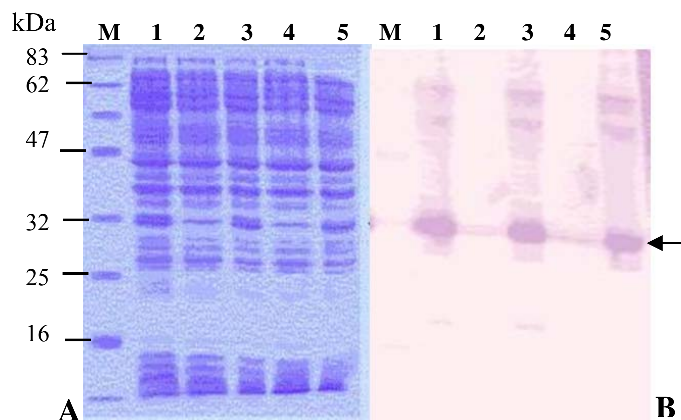

Fig. (4): SDS-PAGE (A) and Western blot (B) analysis of proteins detected by an anti-E tag antibody. The TCA- precipitated material from induced (Lanes 1, 3, and 5) and two non-induced (2, and 4) E. coli clones were subjected to 12% SDS-PAGE and Western blotting. As expected, the detectable scFv anti-CTV (positive band) has a molecular weight of about 32 kDa (arrow).

Synthesis of scFv by fusion PCR

The amplified V_H and V_k genes were linked together with a DNA fragment encoding a flexible peptide $(Gly4Ser)_3$ via fusion PCR to synthesize the scFv genes. The assembled V_H-linker-V_K scFvs, with a size of ~750 bp (Fig. 1), were purified from agarose gels and cloned into the phagemid vector pCANTAB-5E through restriction enzyme sites *Sfi* I and *Not* I.

Biopanning for selection of functional scFv from the libraries

After five rounds of affinity selection on CTV/CP coated plates, a clear enrichment for scFv clones expressing CTV binding was observed. Twenty single colonies secreting phage scFv antibodies were tested for binding target antigens in phage-ELISA. Some of these clones gave absorbance values similar to those of the negative control wells coated with healthy plant sap or BSA, but one clone gave

values two to three times greater than the background was selected (anti-CTV scFv).

Expression of scFv antibodies in *E. coli*

Specific expression of soluble scFv was confirmed by SDS-PAGE and Western blot analysis. The positive clones containing pCANTAB-scFv constructs were used to infect HB2151 cells for expressing a soluble scFv. The *lac*-promoter allows induction of expression of scFv by IPTG. The expression of the scFv from pCANTAB5-scFv after induction with 1 mM IPTG is demonstrated in Figure (2). A protein of about 32 kDa (calculated size is 28.4 kDa) can be seen on the stained gels. No band of the same size is observed in the non-induced controls. The scFv proteins were detected by Western blot analysis (Figure 2B) using anti-E-Tag antibodies, specific for the E-tag peptide that is fused to the C terminus of the scFv. No significant protein expression was observed in the control non-induced cells.

Sequence analysis of the anti-CTV scFv

A clone producing anti-CTV scFv with the highest affinity for CTV/CP was chosen for DNA sequencing. The DNA sequence and the deduced amino acid sequence of this anti-CTV scFv are shown in Figure (3A). The nucleotide sequence of the heavy-chain and light-chain portions of the anti-CTV scFv were closely related to other published scFv against CTV (Figure 3B). Nucleotide sequence alignment with other published scFv sequences indicated that the clone had several unique same-sense base substitutions. Only one base substitution resulted in a different amino acid (mis-sense) where glutamic acid (E) has been replaced by glutamine (Q) in FW1 (Figure 3B).

Tissue Printing ELISA

The results obtained using the anti-CTV scFv and 3DF1 mAb showed that all healthy tissues gave negative signals (Figure 4 A-C). The patterns of tissue prints obtained by using the scFv were essentially similar to those obtained by using the 3DF1 mAb. In general, the tissues infected with T302 CTV strain gave positive and strong signals with either the anti-CTV scFv or the 3DF1 mAb. Tissues infected with T397-P CTV strain gave stronger positive signals with the anti-CTV scFv, as compared to the 3DF1 mAb. On the other hand, tissues infected with T388 CTV strain gave stronger signals with the 3DF1 mAb, as compared to the anti-CTV scFv antibody. Using both of the scFv anti-CTV and the 3DF1 mAb in the same reaction improved the detectability of T397-P and T388 CTV strains to a greater extent (Figure 4C). In the negative control reactions using a non-specific scFv (scFv against rabbit IgG), all samples did not show any substrate precipitation (not shown). The results of field testing using the scFv anti-CTV and 3DF1 mAb are shown in Figure (4D). The immunoprint in Figure (4D) shows the results of testing 96 samples representing 32 citrus trees with 3 replicas for each tree (row). No positive signals were detected in the field samples, while all positive and negative controls printed on the membranes gave the correct positive and negative signals, respectively. Positive controls reacted strongly with the antibody mixture (Figure 4D). The scFv anti-CTV antibody was able to show the localization of the virus in phloem tissues. Figure 4 (E and F) show magnified details of positive reactions using the anti-CTV scFv antibody. These results show that anti-CTV scFv is endowed with an affinity for the CTV coat-protein comparable to that of the commercially available monoclonal antibody. This work demonstrates the feasibility of the approach and the potential applications to the detection of *citrus tristeza virus*.

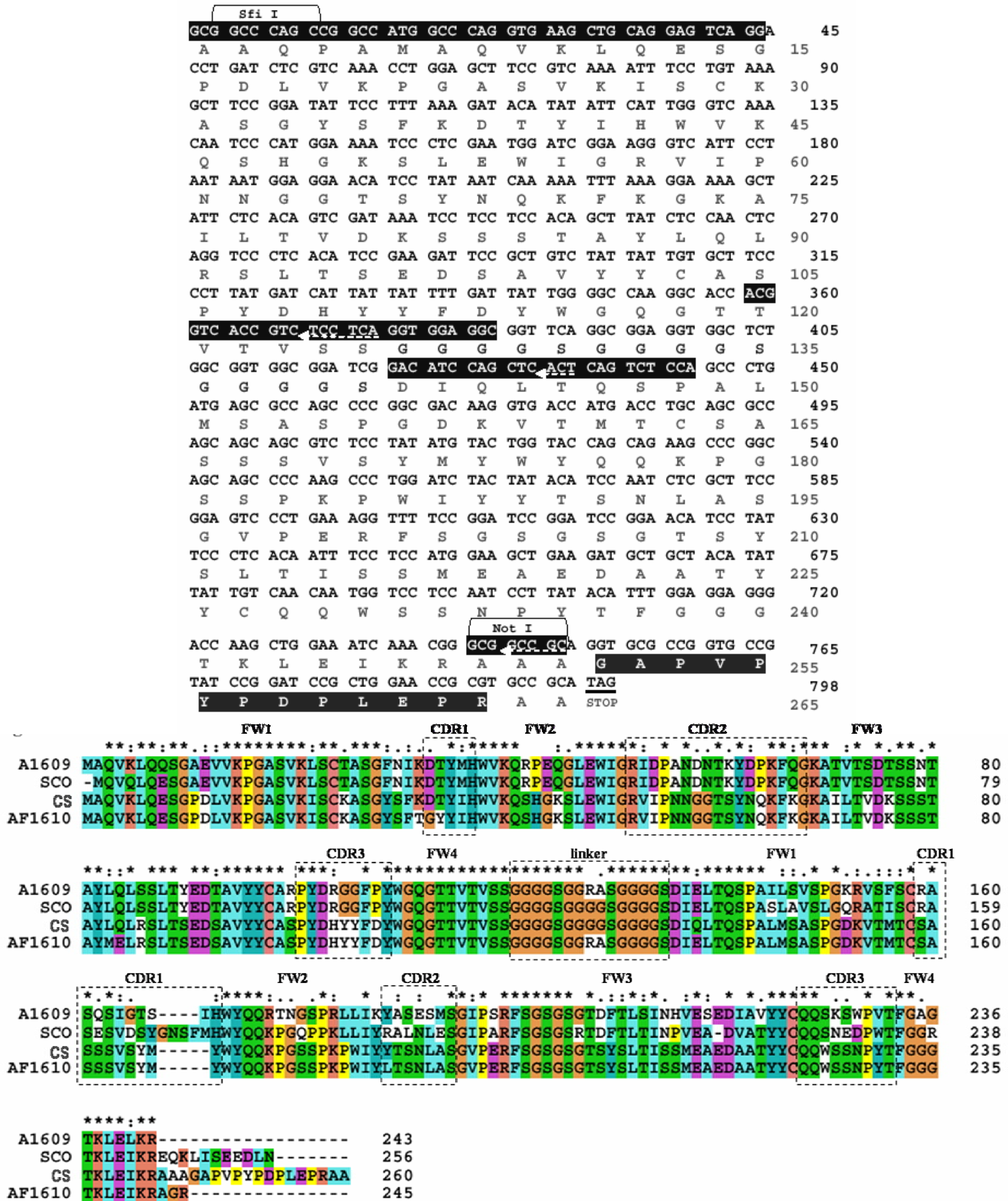

Fig. (5): (A) Nucleotide and deduced aa sequences of anti-CTV scFv. The positions of restriction sites for SfiI and NotI enzymes and primers are shown in reverse color. (B) Amino acid sequence alignment between the anti-CTV scFv CS (Current Study), A1609 (locus AF162709), SCO (locus SCO278109), and AF1610 (locus AF162710). CDR and linker sequences are boxed. Framework (FW) sequences and conserved sequences (*) are indicated.

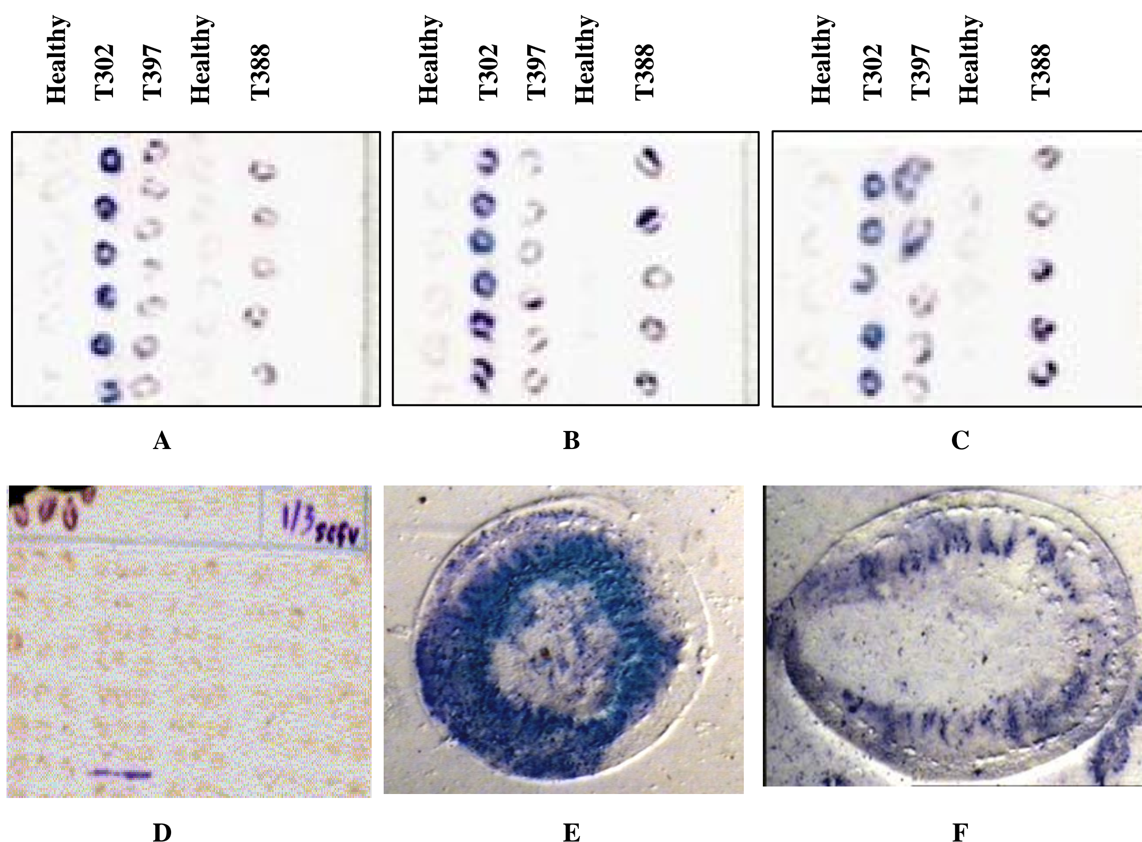

Fig. (6): Immunoprints of transversal sections of plant materials. From left to right, each column of tissue prints refers to healthy tissue, tissue infected by T302, tissue infected by T397-P, healthy tissue, and tissue infected by T388. Immunoprints were incubated with scFv anti-CTV (A), 3DF1 mAb (B), and scFv anti-CTV and 3DF1 mAb (C). A tissue print showing representative results of a field testing using the scFv anti-CTV and 3DF1 mAb is shown in (D). In this tissue print, all tested samples (32 trees x 3 replicas per tree = 96 samples) showed negative signals, while positive (upper left) and negative (upper right) controls showed the expected positive and negative signals, respectively. Magnified details of positive reactions using the scFv anti-CTV antibody, illustrating the localization of the virus in the phloem tissue, are shown in (E and F).

DISCUSSION

Methodologies to produce recombinant specific antibodies in bacteria have been introduced in the early 1990s (McCafferty *et al.,* 1990; Hoogenboom *et al.,* 1991). Simple manipulation and versatile use made single-chain antibody fragment (scFv) the most popular recombinant antibody format. The scFv consists of only variable regions of heavy and light chains connected with a linker sequence. Elucidation of the molecular structure and sequence of immunoglobulins has made it possible to develop immuno-globulin-specific oligonucleotide primers and to use them in conjunction with polymerase

chain reaction (PCR) techniques to clone antibody fragments for generating recombinant antibodies. This scFv antibody retains the specificity of the intact antibody, and is encoded by a single gene and expressed as a single polypeptide chain, and can be generated from either hybridomas or phage display or ribosomal display libraries. In this study, a scFv cDNA library was prepared from the V genes of mice spleens immunized with the CTV coat protein expressed as a fusion product of histidine-tagged protein and purified by Ni-NTA resin affinity columns (Wahle *et al.,* 1999). The antibody fragments were readily expressed in *E. coli* as a single chain fragment variable (scFv) antibody library. Fully active soluble scFv were produced in *Escherichia coli* at high yields.

The scFv fusion protein readily detected CTV/CP in ELISA, phage-ELISA, and in tissue prints of citrus plants infected with three CTV strains (positive controls). The tests with the scFv fusion proteins gave similar results to those obtained with a commercial monoclonal antibody. Furthermore, the scFv-fusion protein successfully located the virus in the vascular cells of citrus leaf sections. These observations indicated that the fragments adopted the proper folding and retained the same binding reactivity as compared with monoclonal antibodies. The results of DNA sequencing further supported this conclusion. DNA coding for the anti-CTV scFv was automatically sequenced to check the assembly in the final vectors and the correct coding regions. The inferred amino acid sequence belongs to the immunoglobulin class and the product of translation was expected to adopt proper folding and to have biological activity, due to the presence of the amino acids involved in correct folding in the right positions (Chothia *et al.,* 1985; Chothia and Lesk, 1987; Lesk and Tramontano, 1992; Foote and Winter, 1992; Knappik and Pluckthun, 1995).

This work demonstrates that library-derived clones secreting recombinant antibodies fragments represent a valuable tool for inexpensive diagnosis of citrus tristeza virus (CTV), the most economically important virus disease of citrus. These *in vitro*–generated recombinant antibodies combine several advantages over traditional antibodies. They can be generated in a short time and from only a minimum amount of protein or peptide. The approach used in this study represents a promising procedure suitable for immunodiagnosis of plant viruses and allows for the production of unlimited supply of highly specific and cheap antibody fragments.

ACKNOWLEDGMENTS

This research was supported by the Plant Pathology Research Institute, Agriculture Research Center, Giza., Egypt. We thank Prof. Dr. Abou-Zeid, A. M. for providing the CTV isolate (infected tissues) and we also thank Prof. Dr. Cambra, M. for providing the 3DF1 mAb and the nitrocellulose membranes printed with the CTV isolates T302, T379-P, and T388.

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الملخص العربي

الكلونة الجزيئية و التعبير الجينى والتوصيف الجزيئى للسلسة الأحادية المتغيرة للأجسام المضادة المنتجة داخل فئران التجارب ضد فيروس التدهور السريع فى الموالح

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تم عزل و اكثار قطعة من التتابعات النيوكليوتيدية المشفرة للسلسلة الثقيلة و السلسلة الخفيفة للأجسام المضادة المنتجة داخل فئران التجارب باستخدام تفاعل البلمرة المتسلسل ثم التحام هذه التتابعات بواسطة رابطة (DNA linker) يشفر للأحماض الأمينية (جليسين-٤-سيرين)٣ لتصبح التركيبة على هيئة سلسلة أحادية متغيرة من التابعات النيوكليوتيدية تعرف باسم (scFv) ثم كلونة هذه التتابعات داخل فاجميد يسمى pCANTA B5E حيث تم التعبير البروتينى عنها على هيئة بروتين مدمج مع التتابع (E) و التابع (P3) لجين الغلاف البروتينى للفاج M13 داخل بكتيريا القولون (TG1) حيث تقوم الأجسام المضادة لفيروس التدهور السريع فى الموالح (CTV) بعمل عرض ظاهرى على سطح الفاج الخارجى وذلك فى وجود الفاج المساعد M13KO7. و من هذه المكتبة الجينية للفاج تم اكثار الأجسام المضادة للفيروس و المرتبطة بجسم الفاج عن طريق اختبار قوة تفاعلها مع الغلاف البروتينى للفيروس المعاد الألتحام فى تكنيك الأليزا. و هذه الأجسام المضادة للفيروس التى تم اختيارها تستخدم فى عدوى بكتيريا الأيشيريشيا كولاى من النوع HB2151 لأنتاج الأجسام المضادة للفيروس فى صورة ذائبة. و قد استطاعت أحد التتابعات المكلونة للأجسام المضادة المنتجة بالمكتبة الجينية فى بكتيريا HB2151 أن تكشف عن فيروس التدهور السريع فى الموالح CTV فى عصير نباتات الموالح المصابة بالفيروس بحساسية عالية عند مقارنتها بالأجسام المضادة الأحادية التخصص للفيروس والمتاحة بالأسواق. وقد اثبتت النتائج أن كلا التتابعين (السلسلة الخفيفة و الثقيلة) ذو تشابه وثيق مع تلك التتابعات المنشورة

للأجسام المضادة لفيروس CTV. وقد أوضحت هذه الأجسام المضادة المنتجة خلال الدراسة مدى قدرتها فى الكشف الروتينى عن فيروس CTV فى الحقل باستخدام تقنية طبع الأنسجة المناعى الغير مكلف.