MOLECULAR CHARACTERIZATION OF WHITEFLY TRANSMITTED VIRUS DISEASES OF TOMATO IN KUWAIT

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ABSTRACT

Viral diseases cause significant economic losses in many crops. Proper disease diagnosis is essential for successful virus management and relies mainly on polymerase chain reaction (PCR) techniques. This activity aimed to adapt and optimize a rapid and very sensitive method for detection of TYLCV and other whitefly-transmitted viruses in tissues of tomato plants and in their vectors. This method allows detection of TYLCV in a large number of samples (leaves) from green house gown plants, and molecular characterization of virus isolates from different tomato growing regions in Kuwait and their comparison with other virus species detected in other countries. The activity started with optimizing both DNA extraction method and PCR on positive DNA sample and cloned TYLCV Tomato leaf samples were collected on monthly bases from October 2010 till January 2011. Collections were made from greenhouses farms in Wafra and Abdally. DNA was extracted from 100 collected infected tomato leaf samples and PCR detection was done on 50 collected samples by using two different primers. Data showed that TY1&TY2 primers were successful in detecting the TYLCV in samples collected in January, partially sequencing of the positive TYLCV done and the amplicon showed a new spp of TYLCV was detected and that whitefly infestation preceded viral infection. All tomato varieties studied were found to be susceptible to the virus. Practicing strict farm hygiene and control of whitefly were recommended by the research team. Future studies were suggested to be carried out in further study the molecular bases of TYLCV, its interaction with different tomato varieties and to complete sequencing the full length of the genome that was partially sequenced.

Key words: Virus detection, Tomato yellow leaf curl virus (TYLCV) , polymerase chain reaction (PCR)

INTRODUCTION

In Kuwait, TYLCV was reported as a major pest of tomato but it was not fully characterized at the molecular level. In addition to TYLCV, tomato may be susceptible to over 40 other viruses transmitted by whiteflies. The high economic losses induced by whitefly-transmitted viruses in Kuwait necessitates a rapid action for identification and molecular characterization of the virus species present in Kuwait in order to develop and recommend appropriate control strategies.

TYLCV was detected on tomato plants using two methods Accotto and Lohdi, since the aim of the project was to determine the best detection method. However, the project did not go in depth to sequencing level. The preliminary survey in previous project constitutes the first report in Kuwait of 15 out of the 18 viruses detected. TYLCV was also detected by PCR and sequencing of the amplicon which was a partial sequence of TYLCV. It is recommended to repeat the survey periodically, to confirm virus identification using molecular techniques.

LITERATURE REVIEW

The free trade of crops and ornamentals, plant propagation material and plant products coupled with the speed of long-distance transport has led to a concomitant spread of new plant pests and diseases. The worldwide spread of viral diseases, especially those transmitted by seed or insect vectors, is an example of the types of introduced pest problems that have caused severe yield losses, often reaching alarming levels. Therefore, viruses constitute complex and dynamically changing problems that should be monitored periodically in order to adapt disease management strategies to cope with the ever-changing viral complexes affecting field- or greenhouse-grown vegetables.

Viruses of Solanaceae

Solanaceous crops include plants like tomato, potato, eggplant and pepper. Like cucurbits, solanaceous crops may be infected by a large number of viruses. The tomato mosaic virus (ToMV), as well as the potato virus X (PVX) is

transmitted mechanically. Most of the other viruses infecting solanaceous crops are transmitted by aphids, whiteflies or thrips. A relatively small number is transmitted by nematodes (e.g., the tobacco rattle virus [TRV]) or by microorganisms (e.g., the potato mop-top virus [PMTV]).

Recently, severe epidemics of viruses transmitted by thrips (e.g., TSWV Tomato spotted wilt virus) and whiteflies have been reported. Some TSWV strains have even been able to infect recently released resistant varieties of plants.

The number of newly identified viruses is increasing every year. Viruses transmitted by whiteflies belong mainly to the genera Begomovirus (Family Geminiviridae) and Crinivirus (Family Closteroviridae).

Begomoviruses include viruses that cause the tomato yellow leaf disease complex including several species and strains of tomato yellow leaf curl virus (TYLCV), tomato severe leaf curl virus (ToSLCV), tomato golden mottle virus (ToGMoV), tomato mild mottle virus (ToMiMoV), tomato yellow mottle virus (ToYMoV), pepper golden mosaic virus (PepGMV), tomato mosaic Havana virus (ToMHV), tomato leaf curl Sinaloa virus (ToLCSinV) and pepper huasteco yellow vein virus (PHYVV) (Nakhla et al., 2005). Other viruses were reported previously include ToLCSinV, tomato mottle virus (TMoV), tomato leaf crumple virus, and Sida golden mosaic virus (Rojas et al., 2000).

Criniviruses include tomato infectious chlorosis virus (TICV) and tomato chlorosis virus (ToCV) (Dovas et al., 2002; Abou-Jawdah et al., 2006).

Usually, nucleic-acid-based techniques are used to detect begomoviruses and criniviruses, because serological tests are not available, or are not available commercially, or because begomoviruses are serologically related and thus, the monoclonal antisera produced cross-react with some part of the other begomoviruses (Macintosh et al.,1992).

RESULTS AND DISCUSSION

Field observations carried out in this project for the period between October 2010 and January 2011 of tomato grown under protection and in open-fields indicated that the symptoms such as leaf yellowing, leaf cupping (Fig. 1), leaf curling, stunting of plants, were common. A total loss of the crop was also observed. Whitefly

infestation was very common on many crops in Wafra and Abdally. Tomato is transplanted in October, when heavy infestation with whitefly was recorded (Fig. 2). Wherever whitefly infestation was heavy early in the growing season, sever symptoms and losses of the tomato yield followed.

Data from this activity showed that TY1(+) &TY2(-) primers were successful in detecting the TYLCV in samples collected in January (Fig. 3), and partially sequencing of the positive TYLCV done and the amplicon showed a new spp of TYLCV was detected.

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001  CCCTGCTCGA TATCGTCGAA GTGCCCATCG
031  TAAAGTCCAG TCTTATGAGC AACGGGATGC
061  TATTAAAGGC ATGCTGGTAT TGTTCGTTGT
091  GTTAGTGATG TTACACGTGG ATCCGGAATT
121  ACCCACAGAG TGGGTAAGAG GTTCTGTGTT
151  AAATCCATAT ATTTTTTAGG TAAAGTCTGG
181  ATGGATGAAA ATATCAAGAA GCAGAATCAC
211  ACTAATCAGG TCATGTTCTT CTTGGTCCGT
241  GATAGAAGGC CCTATGGAAG CAGCCCAATG
271  GATTTTGGGC AGGTTTTTAA TATGTTCGAC
301  AATGAGCCCA GTACCGCAAC CGTGAAGAAT
321  GATCTCCGTG ATAGGTTTCA AGTGATGAGG
351  AAATTTCATG CTACAGTCAT TGGTGGACCA
381  TCTGGAATGA AGGAACAGGC TTTAGTTAAG
421  AGATTTTTTA AAATTAACAG TCATGTAACT
451  TATAATCATC AGGAAGCAGC CAAGTACGAG
381  AACCATACTG AAAACGCCTT GCTATTGTAT
310  ATGG
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The general conclusion of this project is that the method of Dellapotra (1983) method for DNA extraction and Accotto (2000) PCR detection were successful in detecting the TYLCV consistently. All of this is in addition to practicing strict hygiene to control the whitefly infestation in the greenhouse and adjacent fields. These data indicate that the TYLCV present in Kuwait belongs to a separate species from those reported in other countries, and hence, has been named tomato yellow leaf curl Kuwait virus (TYLCKWV).

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MATERIALS AND METHODS

1-Sample Collection

The field survey started in October2010. Farms were selected in two major production areas, namely, the Wafra and Abdally agricultural districts. Each farm was visited four times during the growing season, i.e., from October 2010 to January 2011 for

tomato. A total of 100 plant samples (50 plant samples in duplicates) were collected and tested. Sampling of tomato leaves was taken place as four visits per the growing season of tomato plants. As follows (Oct 2010 first month of planting tomato plants in the field, Nov 2010 before flowering, Dec 2010 after flowering, Jan 2011 fruiting).

2-DNA extraction

Dellaporta method for total genomic extraction was used in this activity (Dellaporta et al.,1983).

50mg of leave tissues were grinded with 700µl of extraction buffer I and 5µl of -mercapto ethanol, then 50µl of 20% SDS were added and the solution was vortexted then incubated at 65°C for 10 min followed by vortexing again. 200µl of 3M potassium acetate pH.5 were added and vortexed. The solution then was centrifuged at 25,000 xg for 20 min, and then the supernatant was transferred to a clean tube containing 500µl isopropanol and mixed well. The solution was incubated for 30 min at -20°C. Centrifugation was carried out for 15 min at 20,000 xg, then the supernatant was gently poured and the DNA pellet was lightly dried for 10 min. The DNA pellet was redissolved with 300µl of 50mM Tris and 10mM EDTA, pH.8. Followed by centrifugation for 10 min at 20,000 xg to remove the insoluble debris. The resultant supernatant was transferred to a new eppendorff tube and treated with 30µl of 3M sodium acetate and 600µl of cold isopropanol. Later, the solution was centrifuged for 10 min at 20,000 xg and the supernatant was discarded. The remaining pellet was washed with 500µl of 70% ethanol by spinning for 10 min at 20,000 xg and the pellet was dried. At the end, the pellet was resuspended in 70µl of TE buffer pH.8 and stored at -20°C for further analysis.

Extraction Buffer I:

* 100 mM of tris hydroxymethyl amino methane hydrochloride (tris-HCl) pH8,
* 50 mM of ethylene diaminetetraacetic acid (EDTA),
* 500 mM of sodium chloride (NaCl),
* 10 mM B-mercaptoethanol.

3- PCR Tests:

Two pairs of primers were used in PCR protocol to detect several genes in TYLCV spp: replicase gene, intergenic region and partly coat protein gene. The primers were: TY1(+) and TY2(-) (Accotto et al., 2000), the second primer pair used was TYC1R and TYC1F. Optimizations were done with both pairs using several variations, namely: gradient concentration of MgCl2(2mM, 2.5mM, 3mM and 3.5mM into final concentration), different Tm (annealing temperatures) were used with each primer pair 55, 57 and 60 °C. All the optimizations were carried out using positive controls and all the results showed that the optimum annealing temperature using TY1 and TY2 was 57°C and the best MgCl2 concentration was 2.5mM, so that these conditions with that pair of primers were used to run PCR tests on all DNA samples extracted from tomato leaves . The same optimization tests were done using the primer pair TYC1R and TYC1F, but none of these tests was successful. there for that pair of primer wasn't used to detect TYLCV spp in our DNA samples.

Detection of TYLCV spp. in tomato by Accotto PCR method (Accotto et al., 2000).

• Oligonucleotide primer sequences: ot TY1 (+): 5' GCC CAT GTA (T/C) C G (A/G) AAG CC 3'

TY2 (-): 5' GG (A/G) TTA GA (A/G) GCA TG (A/C) GTA C

PCR Amplification Protocols. For TYLCV/TYLCSV PCR test in plants, the PCR reaction mix was prepared in a 1.5 ml microfuge tube.

PCR Reaction Components (kept on ice) Final Concentrations:

• 2 mM MgCl
• 0.2 mM dNTPs
• 0.4 µm (final concn) primer TY1
• 0.4 µm (final concn) primer TY2
• 0.04-U µl-1 Taq DNA polymerase
• Total 25 µl

For further reactions, the quantity of each component for the required number of reactions (25 µl per sample aliquot) was calculated, and 1 more sample, than needed for the pipetting was made up. For PCR after mixing the components, 25 µl of PCR reaction mix was transferred with 1 µl of nucleic acid DNA to a 0.2-ml Eppendorf tube. Procedures (OEPP/EPPO, p. 324) are as follows:

• Place the tubes in the thermal cycler heating block.
• Run the following programme on the thermal cycler.
• Denaturation of template: 1 min at 94°C.
• Followed by 40 cycles of 30 s at 94°C, 1 min at 57°C, and 1 min at 72°C; followed by a final extension step of 10 min at 72°C.
• Remove tubes from the thermal cycler.
• Analyse 25 µl of PCR product on a 1 % agarose gel or store tubes at - 20°C, until analysis is performed.

The same protocol was adopted when using TYC1R and TYC1F primer pair.

• Oligonucleotide primer sequences:

-TYC1F: GGGCCTAGAGACCTGGCCCAC

-TYC1R: CCGGTAATATTATACGGATGGC

Interpretation of the PCR Test Result. If an amplicon of the expected size of 580 bp were present, the remaining 20 ul of PCR product was kept for futher studies. The test is positive if the 540-bp fragment (TYLCV spp/other gemini virus) is detected, and the fragment is identical with the positive control isolate(s).

Fig. 2. vHeavy whitefly infestation on tomato leaves.

Fig.1 Cupping of the upper leaves on a tomato plant.

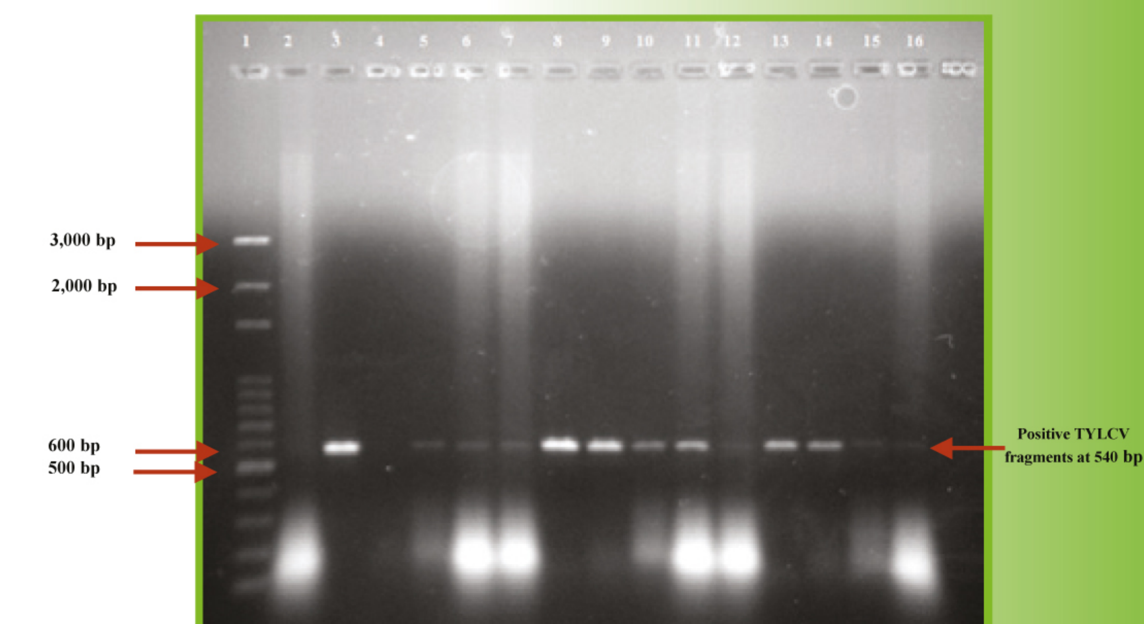

Fig. 3. PCR conditions using TY1(+), TY2(-) primer pair.