Jordan Journal of Agricultural Sciences, Volume **7**, No.**2** 2011

Complete Nucleotide Sequences and Construction of Infectious Clones of Two Jordanian Isolates of *Tomato Yellow Leaf Curl Virus*

Ayed M. Al-Abdallat*✉, Hmoud Al-Debei*, Muhannad Akash*, Samar Misbeh* and Anders Kvarnheden**

ABSTRACT

The complete nucleotide sequences of two Jordanian isolates of *Tomato Yellow Leaf Curl Virus* (TYLCV) were determined and compared with other reported TYLCV isolates. Sequence analysis revealed that one isolate belonged to the mild strain, TYLCV-Mld, while the other isolate belonged to the Israel strain, TYLCV-IL. Agrodrench inoculation of tomato (*Solanum lycopersicum* L.) plants with a 1.8-mer infectious clone of TYLCV-IL[JO:Ju:08] resulted in the development of severe symptoms, while tomato plants infected with a 1.8-mer infectious clone of the isolate TYLCV-Mld[JO:Ju:08] resulted in milder symptoms. The infectious clones can be an important tool for improved understanding of TYLCV-tomato interactions.

Keywords: Agrodrench, Begomovirus, *Solanum lycopersicum* L.

TYLCD: TomatoYellow Leaf Curl Disease, **TYLCV**: Tomato Yellow Leaf Curl Virus, **IR**: Intergenic Region, **ORFs**: Open Reading Frames, **CP**: Caot Protein, **REn**: Replication Enhancer protein, **Rep**: Replication-associated protein, **RCA**: Rolling Circle Amplification, **LB**: Luria –Bertani, **PCR**: Polymerase Chain Reaction.

INTRODUCTION

Tomato Yellow Leaf Curl Disease (TYLCD) is caused by *Tomato Yellow Curl Leaf Virus* (TYLCV) and closely related viruses. The disease was first reported in the Jordan valley in the 1930s (Cohen and Harpaz, 1964) and it is now a serious problem for cultivation of tomato (*Solanum lycopersicum*) worldwide. TYLCD is associated with outbreaks of the whitefly *Bemisia tabaci*, the insect vector that transmits the virus in a persistent and circulative manner (Cohen and Nitzany 1966). Symptoms of TYLCD are stunted tomato plants with small, chlorotic and curled up-margin leaves and premature drop of flowers.

In 1988, TYLCV was isolated, characterized and identified as a member of the family *Geminiviridae*, genus *Begomovirus* (Czosnek *et al.,* 1988). In the early 1990s, the TYLCV genome was sequenced and characterized (Navot *et al.,* 1991) and since then several TYLCV strains and isolates have been identified (Fauquet *et al.,* 2008). The TYLCV genome is a monopartite single-stranded DNA of approximately 2800 nucleotides encapsidated in geminate particles (Gronenborn, 2007; Navot *et al.,* 1991). The TYLCV genome has an intergenic region (IR) of approximately 300 nucleotides involved in regulating viral replication and transcription and encompassing six open reading frames (ORFs) encoding the coat protein (V1 or CP), V2, the replication-associated protein (C1 or Rep), the transcriptional activator protein (C2), the replication enhancer protein (C3 or REn) and the C4 protein (Gafni, 2003).

* Dept. of Horticulture and Crop Science, University of Jordan, Amman, Jordan.
** Dept. of Plant Biology and Forest Genetics, Uppsala Bio Center SLU, box SE-75007 Uppsala, Sweden.
Received on 3/4/2010 and Accepted for Publication on 12/8/2010.

Two different TYLCV strains, TYLCV-IL and TYLCV-Mld, have been isolated from a maintained culture (Cohen and Nitzany, 1966) and have been found to share high DNA sequence identity, except for the IR and the 5´ end of the *Rep* gene (Antignus and Cohen, 1994; Navot *et al.*, 1991). Due to a recombination event, the TYLCV-IL genome contains a portion of the *Rep* 5´ end and IR from tomato-infecting Begomoviruses originating from India, while the rest of its genome is almost identical to TYLCV-Mld (Harrison and Robinson, 1999; Navas-Castillo *et al.*, 2000). Although TYLCV-Mld produces milder disease symptoms than TYLCV-IL when used to infect TY2, a TYLCV-resistant tomato cultivar (Antignus and Cohen, 1994), some reports describe indistinguishable disease symptoms between the two strains (Lefeuvre *et al.*, 2007; Navas-Castillo *et al.*, 1997). Many TYLCV isolates showing similarity to these two strains have been identified worldwide (reviewed in Czosnek and Laterrot 1997), including the Dominican Republic (Salati *et al.*, 2002), USA (Polston *et al.*, 1999), Spain (Navas-Castillo *et al.*, 1997), Egypt (Nakhla *et al.*, 1993) and Turkey (Köklü *et al.*, 2006). Phylogenetic analysis revealed that these isolates occur into two major clades, one clade including TYLCV-IL isolates and the other including TYLCV-Mld isolates (Fauquet *et al.*, 2008).

In Jordan, TYLCD was first reported in 1978 (Makkouk, 1978), but there are speculations for its existence since the 1940's. TYLCV has been reported from many locations in Jordan and molecular evidence indicates the spread of TYLCV in different tomato-cultivating areas (Anfoka *et al.*, 2005; Anfoka *et al.*, 2008). Recently, new TYLCV strains were detected in many production areas where they are rapidly spreading and they have become well established (Anfoka *et al.*, 2008). Nowadays, TYLCD is the most important constraint upon tomato production in open fields, particularly in the Jordan Valley and the highland cultivated areas. Additionally, the management of the TYLCD in tomato production under greenhouse conditions is very difficult and expensive (Lapidot and Friedmann, 2002). The control of TYLCD is focused mainly on the vector control and based on insecticide treatments and/or physical barriers usage. In Jordan, many chemical, physical, biological and cultural methods have been adapted to reduce the abundance of *Bemisia tabaci* populations on tomato and hence to suppress the viral infection with TYLCV (Nazer and Sharaf, 1982).

To gain better understanding of the nature of TYLCD in Jordan, two TYLCV isolates, TYLCV-IL[JO:Ju:08] and TYLCV-Mld[JO:Ju:08], were cloned using the method of rolling circle amplification (RCA). The complete nucleotide sequences of the two TYLCV isolates were determined, analyzed and compared to other reported TYLCV isolates. Additionally, two infectious clones for the two Jordanian TYLCV isolates were constructed and tested on tomato using an agroinfiltration method. Furthermore, symptom development in inoculated tomato plants using the two infectious clones was monitored and analyzed.

MATERIALS AND METHODS

Cloning and Sequence Analysis of Viral Genomes

Leaves showing severe TYLCD symptoms were collected in 2008 from a greenhouse-grown tomato plant at Jubiha Agricultural Research Station, University of Jordan. Total DNA was extracted from the collected leaves using a CTAB method (Doyle and Doyle, 1987). The full-length DNA of TYLCV was amplified by RCA (Inoue-Nagata *et al.*, 2004) using bacteriophage *Phi29* DNA polymerase (New England BioLabs) as follows: 1 µg of extracted total DNA was added to 5 µl of dNTPs (100 µM) and 5 µl random hexamers (2 pmol/µl). The mixture was heated to 95 ºC for 5 minutes, chilled on ice and combined with 5 µl of reaction buffer plus 5 units of *Phi29* DNA polymerase, then distilled water was added to

a final volume of 50 µl. The reaction mixture was incubated for 18 h at 30 ºC, followed by inactivation of the enzyme at 65 ºC for 10 minutes. The amplified DNA, containing tandem repeats of the TYLCV genome was separated in a 1% agarose gel. For cloning the full-length TYLCV genome, 1 µg of the amplified DNA was digested with the restriction enzyme *Nco*I and the resulting ~2.8 kb DNA fragment was inserted into the same restriction site of the plasmid pCAMBIA1380 (Cambia, Canberra, Australia) to produce the pTY[JO:Ju]X clones. To verify the identity of the TYLCV isolate, several pTY[JO:Ju]X clones were sequenced and analyzed using restriction enzymes cut at unique restriction sites for the TYLCV-Mld strain. A positive clone showing high identity to TYLCV-Mld was named pTY[JO:Ju]-Mld, while a clone showing high identity to TYLCV-IL was named pTY[JO:Ju]. The TYLCV genomes cloned in pTY[JO:Ju] and pTY[JO:Ju]-Mld were named TYLCV-IL[JO:Ju:08] (GenBank accession no. GQ861426) and TYLCV-Mld[JO:Ju:08] (GenBank accession no. GQ861427), respectively.

Sequence and Phylogenetic Analysis

Phylogenetic analysis was carried out using Mega 4 (Tamura *et al.,* 2007). The complete genome sequences of the two isolates from this study, together with TYLCV sequences from GenBank, were aligned using the Clustal W algorithm in MEGA 4. The alignment was used to calculate distance matrices for neighbour-joining analysis with the Kimura two-parameter model. Bootstrap analysis with 1000 replicates was performed to test the robustness of the internal branches.

Construction of Infectious Clones

An infectious clone of TYLCV-IL[JO:Ju:08] was constructed using a 1.8-mer tandem repeat of the TYLCV-IL[JO:Ju:08] genome. For this purpose, a DNA fragment of 2.35 kb was cut out from pTY[JO:Ju] using *Eco*RI and *Nco*I and cloned into the plasmid pCAMBIA1380 to create pBTY[JO:Ju]P. Then, the complete genome of TYLCV-IL[JO:Ju:08] was excised from pTY[JO:Ju] using *Nco*I and ligated to *Nco*I-linearized pBTY[JO:Ju]P yielding pBTY[JO:Ju], a binary plasmid containing a 1.8-mer of the TYLCV-IL[JO:Ju:08] genome. Similar cloning strategy was used to produce a 1.8-mer infectious clone of TYLCV-Mld[JO:Ju:08] using the pTY[JO:Ju]-Mld plasmid. Both infectious clones contain two copies of IR to ensure successful infection.

Leaf Agroinfiltration Inoculation

To inoculate tomato plants with TYLCV-IL[JO:Ju:08] and TYLCV-Mld[JO:Ju:08], a leaf agroinfiltration method was used. For this purpose, the *Agrobacterium tumefaciens* strain GV3101 was transformed by electroporation with the plasmids pCAMBIA1380 (negative control), pBTY[JO:Ju] or pBTY[JO:Ju]-Mld. Bacteria were grown for 24 hours at 28 ºC in Luria-Bertani (LB) media supplemented with appropriate antibiotics. Bacterial cells were harvested by centrifugation and resuspended into *A. tumefaciens* inoculation buffer (10 mM $MgCl_2$, 10 mM MES pH 5.6, 150 µM acetosyringone) to a final OD_{600} of 1.0 and shaken for 4 h at 28 ºC. The bacterial suspension was infiltrated into the lower side of leaves of 3-weeks old TYLCV-susceptible tomato "line 102" using a 1 ml needleless syringe. Inoculated plants were grown in a greenhouse under mesh cover to avoid vector insects. Inoculated plants were observed for development of TYLCD symptoms for eight weeks post-inoculation.

Viral DNA Detection Using PCR

To confirm the presence of TYLCV-IL[JO:Ju:08] and TYLCV-Mld[JO:Ju:08] in infected plants, polymerase chain reaction (PCR) was run using two sets

of isolate-specific primers. The two sets of specific primers were designed based on sequence differences between the two strains in the 5' part of the *Rep* gene (Lefeuvre *et al.,* 2007). TYLCV-Mld[JO:Ju:08] was detected using the primers TYMF (5'-AAGCGCTTCCAAATAAATTG-3') and TYMR (5'-TACTAATTCTTTAATGATTC-3'), which amplify a 450-bp DNA fragment specific for TYLCV-Mld[JO:Ju:08]. For the detection of TYLCV-IL[JO:Ju:08], the specific primers TYSF (5'-CGTTTATTTAAAATATATGCC-3') and TYSR (5'-GGAAACTCCAAAATCAATGA-3') were used to amplify a 336-bp DNA fragment.

RESULTS

Cloning of Jordanian TYLCV Full-length Clones

Genomic DNA extracted from a tomato plant showing severe TYLCD symptoms was used in an RCA reaction for cloning the full-length genome of TYLCV (Fig. 1). Restriction digestion with unique enzymes and sequence analysis revealed the presence of two different TYLCV genotypes in the infected tomato plant (data not shown). The complete nucleotide sequences of both genotypes were determined, and they were named TYLCV-IL[JO:Ju:08] and TYLCV-Mld[JO:Ju:08]. The genome organization of the two Jordanian isolates was similar to previously reported TYLCV genomes (Fig. 2a; Antignus and Cohen, 1994; Navot *et al.,* 1991). Both genomes contained the typical IR as well as six open reading frames encoding proteins with a molecular mass greater than 10 kDa similar to the previously reported TYLCV genomes (Fig. 2; Gronenborn, 2007).

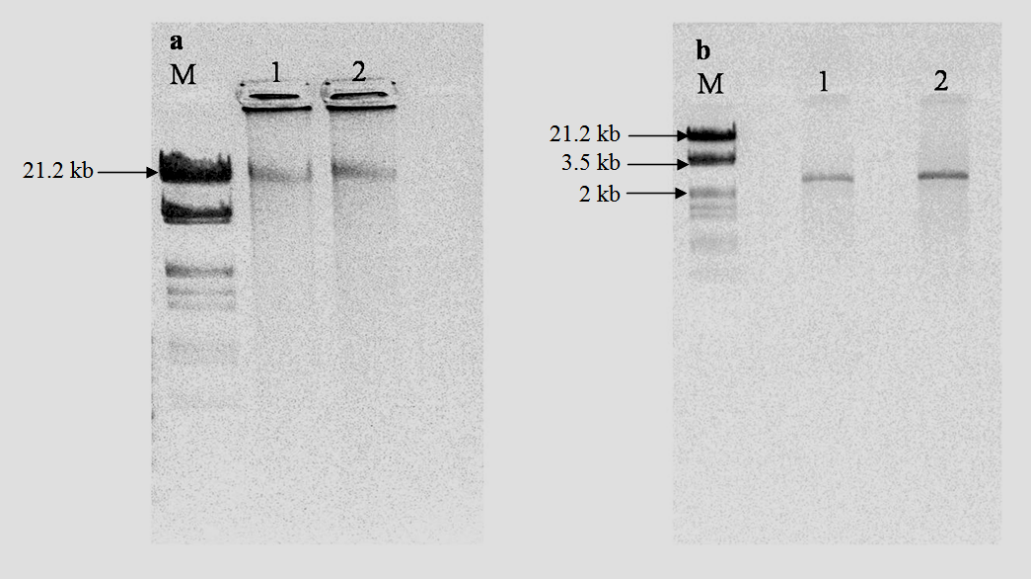

Figure 1: Cloning of the full length genomes of TYLCV-IL[JO:Ju:08] and TYLCV-Mld[JO:Ju:08]. (a) Agarose gel electrophoresis analysis of amplified undigested TYLCV DNA (lanes 1 and 2) generated by rolling circle amplification. (b) Amplified TYLCV DNA products digested with *Nco*I (lanes 1 and 2). M: Lambda *Eco*RI-*Hin*dIII DNA marker.

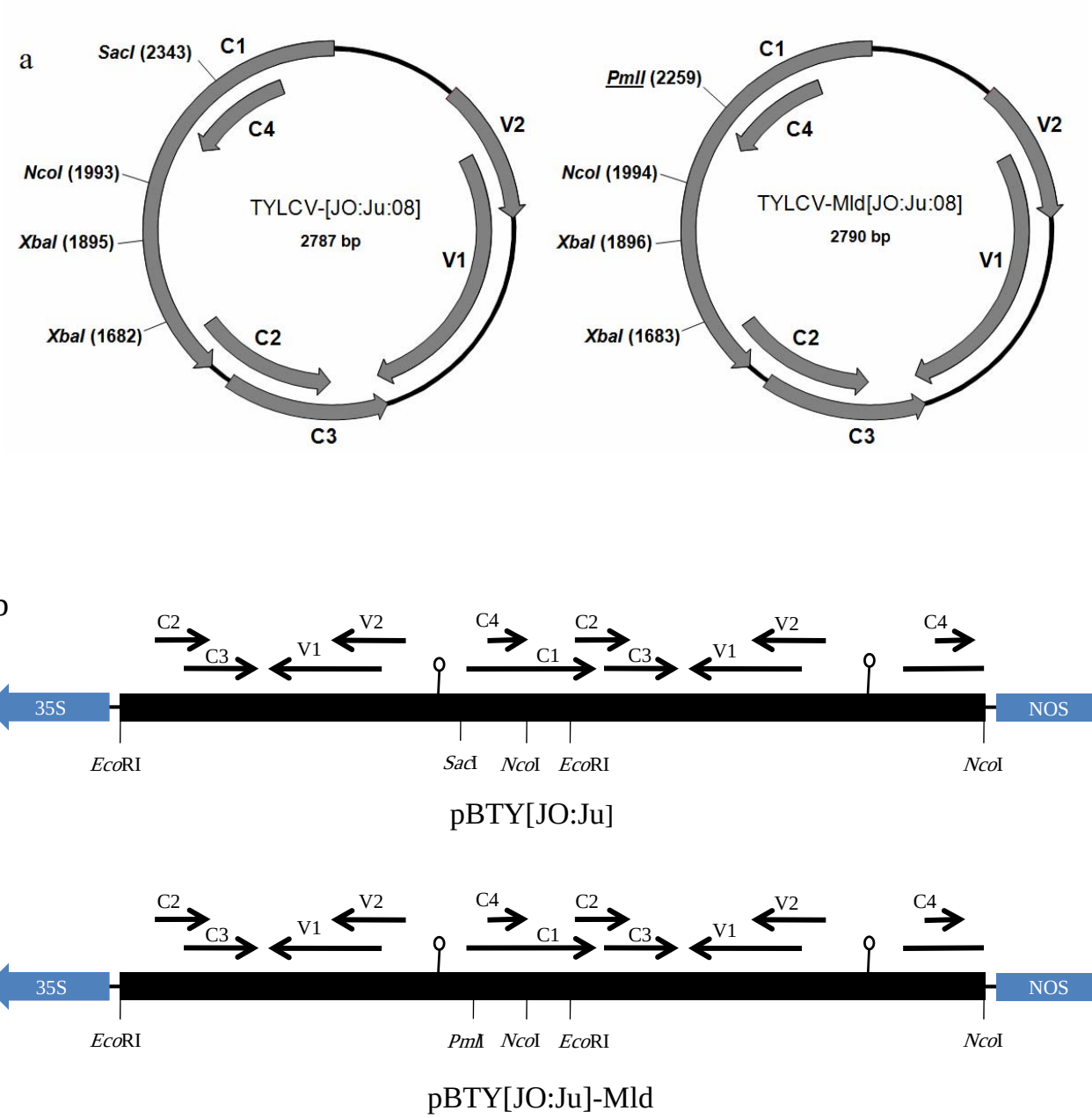

Figure 2: Genome organization of TYLCV-IL[JO:Ju:08] and TYLCV-Mld[JO:Ju:08]. Gray arrows show the positions of the open reading frames and the position of selected restriction endonuclease cleavage sites. The unique *Pml*I restriction site in TYLCV-Mld[JO:Ju:08] and *Sac*I site in TYLCV-IL[JO:Ju:08] used to discriminate between the two isolates are underlined.

Sequence and Phylogenetic Analysis

The complete genome sequence of TYLCV-Mld[JO:Ju:08] shared 97-98% similarity with TYLCV-Mld[IL:93] (Antignus and Cohen, 1994) and other isolates of the mild strain. In contrast, the other sequenced genome, TYLCV-IL[JO:Ju:08] showed the highest nucleotide similarity at 97-98% to TYLCV-IL[IL:Reo:86] (Navot *et al.*, 1991) and other isolates of the Israel strain. Phylogenetic analysis with genome sequences of known TYLCV strains and closely related species (Fig. 3) confirmed the strain identification of TYLCV-Mld[JO:Ju:08] and TYLCV-IL[JO:Ju:08]. Within the two strains, no strict grouping could be found according to geographic origin or host species.

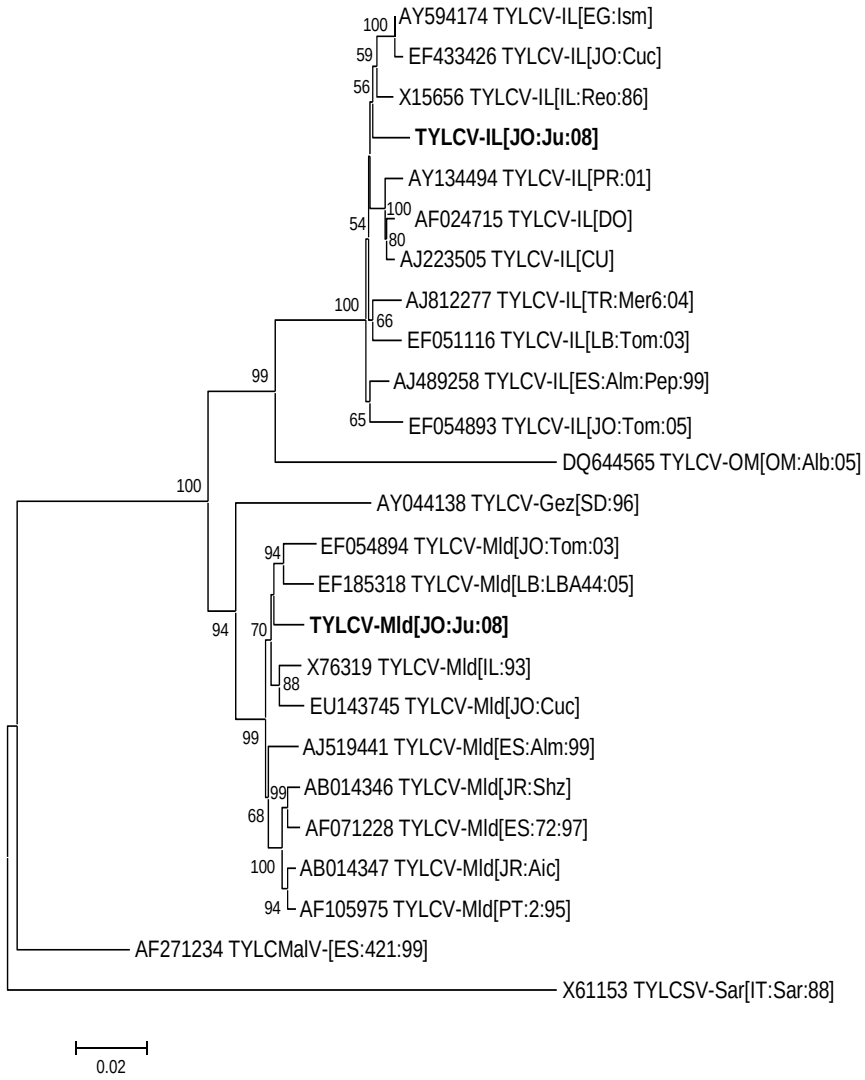

Figure 3: Neighbour-joining analysis showing predicted relationships between isolates of *Tomato Yellow Leaf Curl Virus* (TYLCV), *Tomato Yellow Leaf Curl Malaga Virus* (TYLCMalV) and *Tomato Yellow Leaf Curl Sardinia Virus* (TYLCSV), based on complete genomic nucleotide sequences. The two Jordanian TYLCV isolates determined in this study are indicated in bold. The accession numbers for the sequences are shown next to the isolate names. Horizontal lines are in proportion to the number of nucleotide differences between branch nodes. Numbers represent bootstrap values out of 1000 replicates. Only bootstrap values higher than 50% are shown.

Infection Tests

To test the pathogenecity of both genotypes, 1.8-mer genome copies for TYLCV-IL[JO:Ju:08] and TYLCV-Mld[JO:Ju:08] were cloned into the binary vector

pCAMBIA1380 to produce the infectious clones pTY[JO:Ju] and pTY[JO:Ju]-Mld, respectively (Fig. 2b). The infectious clones and the empty binary plasmid pCAMPIA1380 (negative control) were transformed into *A. tumefaciens* strain GV3101 and the transformed cells were used for agrodrench inoculation experiments. Four weeks after inoculation, typical symptoms of TYLCD with leaf curling and yellowing were observed in 13 out of 15 of the tomato plants inoculated with the infectious clone pTY[JO:Ju]-Mld and 11 out of 15 of the plants inoculated with pTY[JO:Ju] (Fig. 4b and c), while the control plants showed no symptoms (Fig. 4a). Eight weeks after inoculation, tomato plants inoculated with pTY[JO:Ju]-Mld showed mild TYLCD symptoms or a recovery phenotype, which was characterized by mild leaf curling, slightly chlorotic leaf margins and continuing growth (Fig. 4e). At the same time, tomato plants inoculated with pTY[JO:Ju] continued to display severe stunting, leaf curling and yellowing (Fig. 4f).

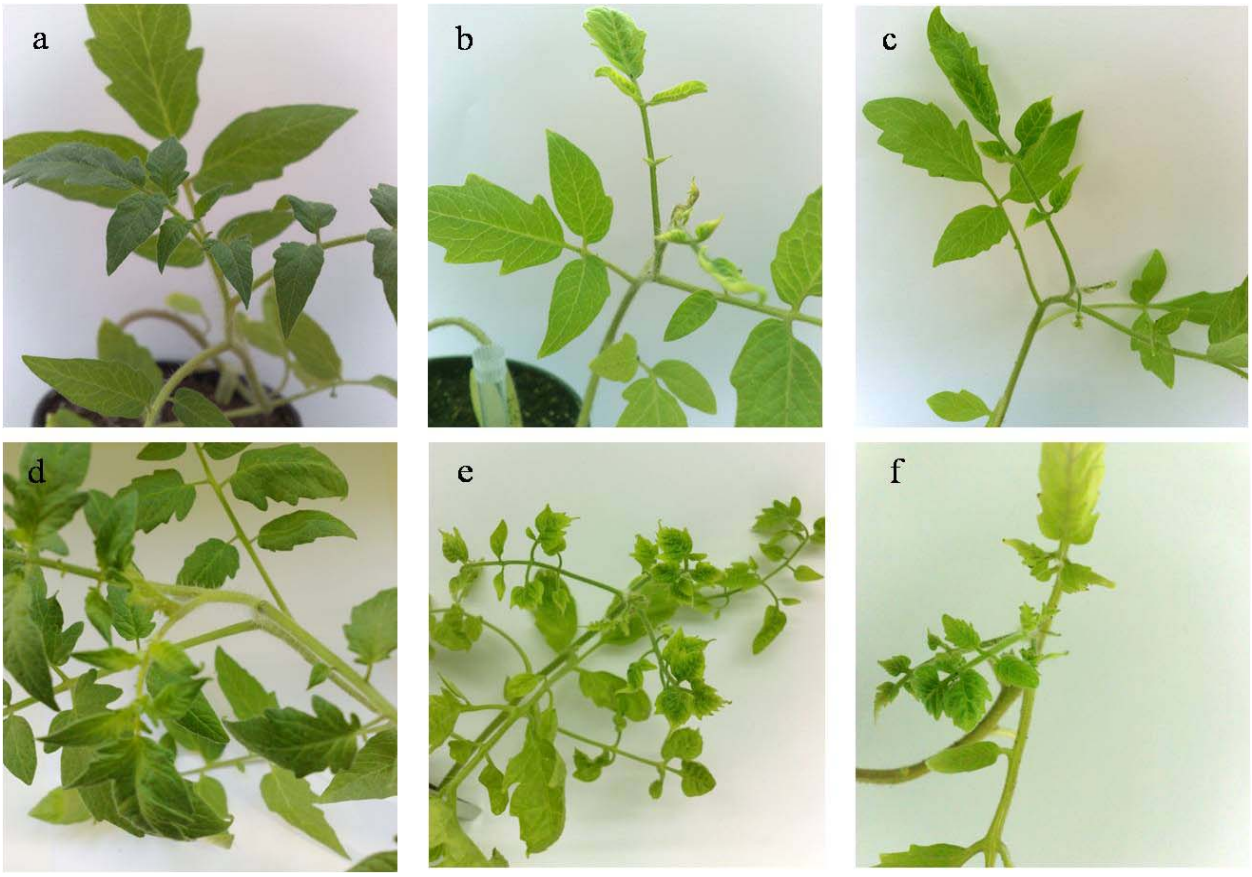

Figure 4: TYLCV-IL[JO:Ju:08] and TYLCV-Mld[JO:Ju:08] infections in plants of the susceptible tomato "line 102" via agrodrench inoculation using the infectious clones pBTY[JO:Ju] and pBTY[JO:Ju]-Mld, respectively. Tomato plants 4 weeks after inoculation with pCambia1380 (a), the infectious clone pBTY[JO:Ju]-Mld (b) and the infectious clone pBTY[JO:Ju] (c). Tomato plants 8 weeks after inoculation with pCambia1380 (d), pBTY[JO:Ju]-Mld (e) and pBTY[JO:Ju] (f).

To confirm the pathogenecity of the Jordanian TYLCV isolates after leaf agroinfiltration inoculation, PCR was carried out using two sets of isolate-specific primers and DNA extracts from tomato plants inoculated with the infectious clones or the empty binary plasmid. No amplification products were observed in PCR product using specific primers for TYLCV-Mld and DNA extracted from plants either inoculated with empty vector or the pTY[JO:Ju] infectious clone (Fig. 5). However, an amplification product of 336 bp was observed in PCRs using the specific primers for TYLCV-Mld and DNA extracted from plants inoculated with the pTY[JO:Ju]-Mld infectious clone (Fig. 5a). Similarly, the specific amplification product for TYLCV-IL was obtained from tomato plants inoculated with the infectious clone pTY[JO:Ju] (Fig. 5b). No PCR products for TYLCV-IL/TYLCV-Mld or disease symptoms were observed in tomato plants inoculated with *A. tumefaciens* containing the empty binary vector (Fig. 5).

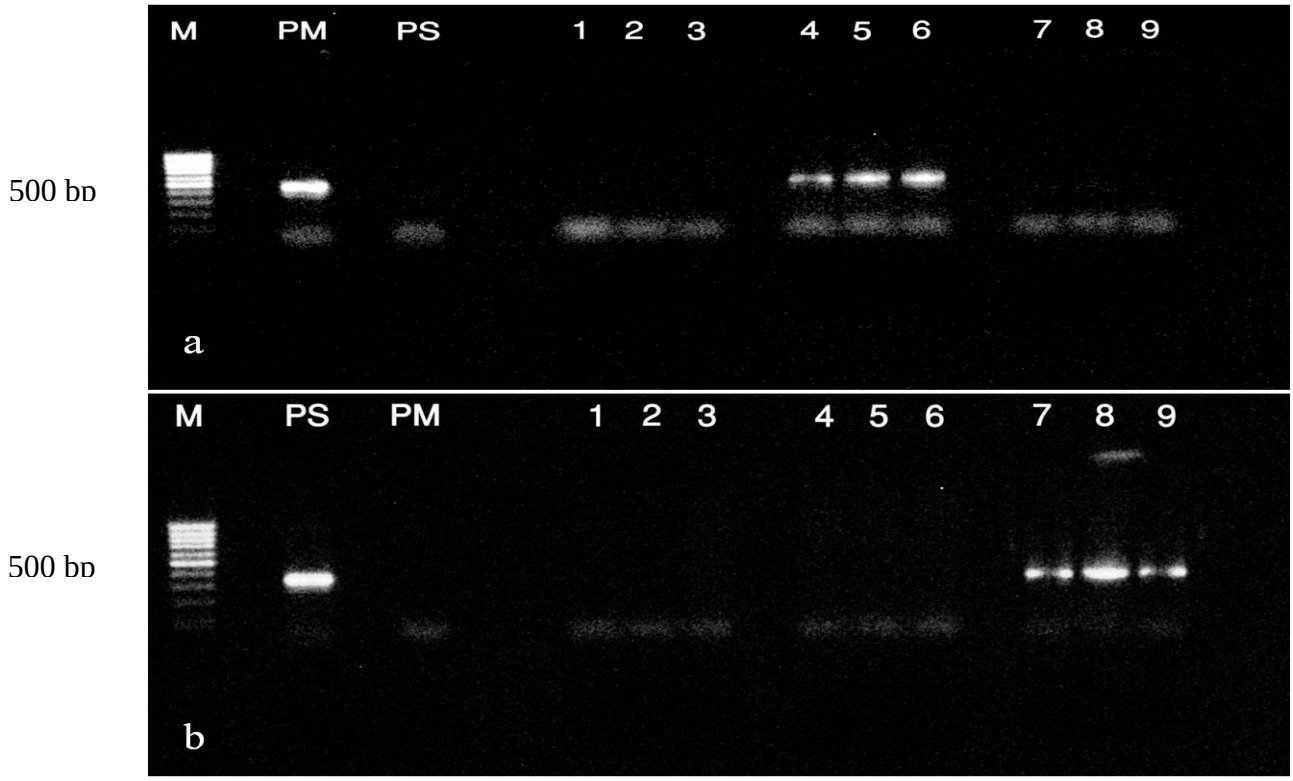

Figure 5: Detection of TYLCV DNA in tomato plants inoculated with TYLCV-IL[JO:Ju:08] and TYLCV-Mld[JO:Ju:08] using the infectious clones pBTY[JO:Ju] and pBTY[JO:Ju]-Mld, respectively. Agarose gel showing PCR products amplified from tomato DNA extracts using the primer pair TYMF/TYMR (a) and the primer pair TYSF/TYSR (b). Lanes 1-3: plants inoculated with pCAMBIA1380; Lanes 4-6: plants inoculated with pBTY[JO:Ju]-Mld; Lanes 7-9: plants inoculated with pBTY[JO:Ju]. PM: 50 ng of the pBTY[JO:Ju]-Mld plasmid was used as a DNA template in the PCR reaction. PS: 50 ng of pBTY[JO:Ju]plasmid was used as a DNA template in the PCR reaction. M: Low range DNA marker (Fermentas).

REFERENCES

Anfoka, G.H., M. Abhary and M.K. Nakhla. 2005. Molecular identification of species of the tomato yellow leaf curl virus complex in Jordan. ***Journal of Plant Pathology***. 87: 65-70.

Anfoka, G., M.Abhary, F. Haj Ahmad, A.F. Hussein, A. Rezk, F. Akad, Y. Abou-Jawdah, M. Lapidot, F. Vidavski, M.K. Nakhla, H. Sobh, H. Atamian, L. Cohen, I. Sobol, H. Mazyad, D.P. Maxwell and H. Czosnek. 2008. Survey of tomato yellow leaf curl disease-associated viruses in the eastern Mediterranean basin. ***Journal of Plant Pathology***. 90: 313-322.

Anfoka, G., F. Haj Ahmad, M. Abhary and A. Hussein. 2009. Detection and molecular characterization of viruses associated with tomato yellow leaf curl disease in cucurbit crops in Jordan. ***Plant Pathology*** 58: 754-762.

Antignus, Y. and S. Cohen. 1994. Complete nucleotide sequence of an infectious clone of a mild isolate of tomato yellow leaf curl virus (TYLCV). ***Phytopathology***. 84: 707-712.

Bananej, K., Kheyr-Pour, A., Salekdeh, Gh. H. and Ahoonmanesh, A. 2004. Complete nucleotide sequence of Iranian tomato yellow leaf curl virus isolate: further evidence for natural recombination amongst begomoviruses. ***Archives of Virology.*** 149:1435-1443.

Cohen, S. and I. Harpaz. 1964. Periodic rather than continual acquisition of a new tomato virus by its vector, the tobacco whitefly (*Bemisia tabaci* Gennadius). ***Entomologia Experimentalis et Applicata***. 7: 155-166.

Cohen, S. and F.E. Nitzany. 1966. Transmission and host range of the tomato yellow leaf curl virus. ***Phytopathology***. 56: 1127-1131.

Czosnek, H. and H. Laterrot. 1997. A worldwide survey of tomato yellow leaf curl viruses. ***Archives of Virology***. 142: 1391-1406.

Czosnek, H., R. Ber, Y. Antignus, S. Cohen, N. Navot and D. Zamir. 1988. Isolation of the tomato yellow leaf curl virus-a geminivirus. ***Phytopathology***. 78: 508-512.

Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissue. ***Phytochemical Bulletin***. 19: 11-15.

Fauquet, C.M., R.W. Briddon, J.K. Brown, E. Moriones, J. Stanley, M. Zerbini and X. Zhou. 2008. Geminivirus strain demarcation and nomenclature. ***Archives of Virology.*** 153: 783-821.

Gafni, Y. 2003. *Tomato yellow leaf curl virus*, the intracellular dynamics of a plant DNA virus. ***Molecular Plant Pathology***. 4: 9-15.

Gronenborn, B. 2007. *Tomato yellow leaf curl virus*, genome and function of its proteins. In, Tomato yellow leaf curl virus disease, edited by H. Czosnek, Springer, The Netherlands, 329-342.

Harrison, B.D. and D.J. Robinson. 1999. Natural genomic and antigenic variation in whitefly transmitted geminiviruses (begomoviruses). ***Annual Review of Phytopathology***. 37: 369-398.

Inoue-Nagata, A.K., L.C. Albuquerque, W.B. Rocha and T. Nagata. 2004. A simple method for cloning the complete begomovirus genome using the bacteriophage *phi29* DNA polymerase. ***Journal of Virological Methods.*** 116: 209-211.

Köklü, G., A. Rojas and A. Kvarnheden. 2006. Molecular identification and the complete nucleotide sequence of a *tomato yellow leaf curl virus* isolate from Turkey. ***Journal of Plant Pathology***. 88: 61-66.

Lapidot, M. and M. Friedmann. 2002. Breeding for resistance to whitefly-transmitted geminiviruses. ***Ann. Appl. Biol***. 140:109-127.

Lapidot, M. 2007. Screening for TYLCV-resistant plants using whitefly-mediated inoculation. In, Tomato yellow leaf curl virus disease, edited by H. Czosnek, Springer, The Netherlands, 329-342.

Lefeuvre, P., M. Hoareau, H. Delatte, B. Reynaud and J.-M. Lett. 2007. A multiplex PCR method discriminating between the TYLCV and TYLCV-Mld clades of *tomato*

yellow leaf curl virus. ***Journal of Virological Methods***. 144: 165-168.

Makkouk, K., M. 1978. A study on tomato viruses in the Jordan valley with special emphasis on *tomato yellow leaf curl*. ***Plant Disease Reporter***. 62: 259-262.

Morilla, G., D. Janssen, S. García-Andrés, E. Moriones, I.M.Cuadrado and E. R. Bejarano. 2005. Pepper (*Capsicum annuum*) is a dead-end host for *tomato yellow leaf curl virus*. ***Phytopathology***. 95: 1089-1097.

Nakhla, M.K., H.M. Mazyad and D.P. Maxwell. 1993. Molecular characterization of four tomato yellow leaf curl virus isolates from Egypt and development of diagnostic methods. ***Phytopathologia Mediterranea***. 32: 163-173.

Navas-Castillo, J., S. Sánchez-Campos, J.A. Díaz, E. Sáez-Alonso and E. Moriones. 1997. First report of tomato yellow leaf curl virus-Is in Spain: coexistence of two different geminiviruses in the same epidemic outbreak. ***Plant Disease.*** 81: 1461.

Navas-Castillo, J., S. Sánchez-Campos, E. Noris, D. Louro, G.P. Accotto and E. Moriones. 2000. Natural recombination between *tomato yellow leaf curl virus*-Is and *Tomato leaf curl virus*. ***Journal of General Virology***. 81: 2797-2801.

Navot, N., E. Pichersky, M. Zeidan, D. Zamir and H. Czosnek. 1991. *Tomato yellow leaf curl virus*: a whitefly-transmitted geminivirus with a single genomic component. ***Virology***. 185: 151-161.

Nazer, I. and Sharaf, N., 1982. Control of the immature stages of the tobacco whitefly *(Bemisia tabaci* Genn.) by certain insecticides on tomatoes in the Jordan Valley. ***Dirasat***. 9: 183-190.

Padidam, M., S. Sawyer and C.M. Fauquet. 1999. Possible emergence of new geminiviruses by frequent recombination. ***Virology***. 265: 218-225.

Polston, J. E., L. Cohen, T.A. Sherwood, R. Ben-Joseph and M. Lapidot. 2006. *Capsicum* Species: symptomless hosts and reservoirs of tomato yellow leaf curl virus. ***Phytopathology***. 96: 447-452.

Polston, J. E., R.J. McGovern and L.G. Brown. 1999. Introduction of tomato yellow leaf curl virus in Florida and implications for the spread of this and other geminiviruses of tomato. ***Plant Disease***. 83: 984-988.

Salati, R., M.K. Nahkla, M.R. Rojas, P. Guzman, J. Jaquez, D. Maxwell and R.L. Gilbertson. 2002. Tomato yellow leaf curl virus in the Dominican Republic: characterization of an infectious clone, virus monitoring in whiteflies and identification of reservoir hosts. ***Phytopathology***. 92: 487-496.

Tamura, K., J. Dudley, M. Nei and S. Kumar. 2007. *MEGA4*: molecular evolutionary genetics analysis (MEGA) software version 4.0. ***Molecular Biology and Evolution.*** 24: 1596-1599.

تحليل المتتاليات النيكليوتيدية الكاملة و بناء نسخ معدية لعزلتين أردنيتين من فيروس مرض تجعد و اصفرار أوراق البندورة

عايد العبدلات، حمود الدبعي*، مهند عكش*، سمر مصبح*، أندرس كفارنهيدن***

ملخـص

تم في هذه الدراسة تحديد المتتاليات النيكليوتيدية الكاملة لعزلتين أردنيتين من فيروس مرض تجعد و اصفرار أوراق البندورة. عند مقارنة المتتاليات النيكليوتيدية لكلتا العزلتين مع عزلات معروفة سابقاً، وجد أن إحدى العزلتين تنتمي إلى سلالة TYLCV-Mld، بينما انتمت العزلة الأخرى إلى سلالة TYLCV-IL. كما تم بناء نسخ معدية من كلتا العزلتين و من ثم اختبارهما على نباتات بندورة حساسة للإصابة بالفيروس باستخدام طريقة Agrodrench. عند مقارنة الأعراض، وجد أن النسخة المعدية من العزلة TYLCV-Mld[JO:Ju:08] قد أحدثت أعراض إصابة خفيفة عند مقارنتها مع النسخة المعدية من العزلة TYLCV-IL[JO:Ju:08]. كلتا العزلتين سيتم استخدامهما لاحقاً في أبحاث متعلقة بدراسة التفاعلات بين نبات البندورة و فيروس مرض تجعد و اصفرار أوراق البندورة.

الكلمات الدالة:.Agrodrench, Begomovirus, *Solanum lycopersicum* L

* قسم البستنة والمحاصيل، كلية الزراعة، الجامعة الأردنية، عمان، الأردن.

** قسم بيولوجيا النبات وجينية الغابات، أوبسالا، السويد.

تاريخ استلام البحث 2010/4/13 وتاريخ قبوله 2010/8/12.