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Original Article

Construction of agro-infectious clones and screening of tomato cultivars for tolerance against tomato leaf curl Sudan virus and tomato yellow leaf curl virus

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Abstract

Tomato leaf curl disease is the most harmful disease of tomato caused by different begomovirus species mainly Tomato yellow leaf curl virus (TYLCV) and Tomato leaf curl Sudan virus (TLCSDV). Both are single-stranded DNA begomoviruses that cause leaf curling, stunting, vein banding, yellowing and results in significant crop losses in tomato in Saudi Arabia. In the first objective of this study, infectious clones of TLCSDV and TYLCV were constructed by cloning and subcloning of partial dimer genome containing two origins of replication on PstI-KpnI/KpnI sites in binary plasmid vector pGreenII 0000. To assess the functionality of agro-infectious clones, those were transformed into *Agrobacterium tumefaciens* GV3101 strain and infiltrated into the leaves of model host plant *Nicotiana benthamiana*. Three weeks post inoculation begomovirus symptoms were observed and infectivity of our infectious clones were confirmed by PCR using begomovirus diagnostic primers (AC1048 and AV494). In the second phase, 13 available commercial cultivars of tomato crop grown in open fields as well as greenhouse were inoculated with agro-infectious clones of TLCSDV and TYLCV to assess their tolerance against these important pathogens of tomato. The obtained results observed after 30 days of inoculation showed all cultivars were susceptible to both begomoviruses showing variable symptoms severity. These results were confirmed by PCR using AC1048 and AV494 primer. The significance of response of different cultivars against infection of TLCSDV and TYLCV were discussed.

Keywords: Tomato, TLCSDV, TYLCV, Agroinfiltration, Infectious clone, Cultivars, Virus resistance.

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Introduction

Begomoviruses are whitefly transmitted geminiviruses which only infect dicotyledonous plants. This group has significant economic importance since begomoviruses cause huge losses. Within the *Geminiviridae* family, the largest group are begomoviruses which contains bipartite viruses (two genomic components of approximately equal size known as DNA A and DNA-B) and monopartite viruses (one genomic component of around 2.8 kb) (Briddon et al., 2001; Stanley et al., 2005) (Figure 1). Five ORFs on the complementary-sense strand are encoded by the DNA-A component of bipartite and monopartite begomovirus genomes. These ORFs are referred to as (A)C1 to (A)C5 (or C1 to C5 in monopartite viruses). The replication enhancer protein, transcriptional activator protein, and Rep are encoded by (A) C1 through (A) C3. (A) C4 controls the host range, the intensity of the symptoms, and the virus's mobility and (A) C5 is not characterized yet (Jupin et al., 1994; Laufs et al., 1995; Wartig et al., 1997). Begomoviruses produce the V1 (Coat protein) and V2 genes, which are encoded by virion sense strands. But the AV2 is absent from the bipartite begomoviruses found in the New World. One gene, on the virion sense strand, the nuclear shuttle protein (BV1) and one on the complementary sense, the movement protein (BC1) are encoded by the DNA B component. Together, these two proteins help the virus spread throughout the plant's cells. With the exception of a ~200 nt length of sequence in the intergenic region known as the common region, the DNA-A and DNA-B components have very different sequences (Hanley-Bowdoin et al., 1999). Based on phylogenetic studies, begomoviruses are classified into two groups: The Old-World begomoviruses and the New World begomoviruses (Padidam et al., 1999; Paximadis et al., 1999). The genomes of begomoviruses from the Old and New Worlds differ in a number of ways. The majority of begomoviruses in the Old World are monopartite and mostly linked to satellite molecules; only a few are bipartite. In contrast, all begomoviruses in the New World are bipartite. Begomoviruses are primarily spread by the *Bemisia tabaci* (Gennadius) whitefly. With 500 species spread across 74 plant families, this species has an extremely broad host range (Cock, 1986), It is a vector for viruses belonging to the genera *Carlavirus* and *Closterovirus* as well as the families *Geminiviridae*, *Potyviridae*, and *Comoviridae*. When the virus spreads by circulative means, only the CP interacts with whitefly components; this makes the CP responsible for the specificity of geminivirus transmission from insect to plant. Whiteflies use their stylets to pierce plant tissue and identify the vascular tissue in order to feed on phloem sap (Pollard, 1955).

Tomato leaf curl disease (TLCD), mainly attributed to four begomovirus species, is the most destructive tomato disease: Tomato Leaf Curl Sudan Virus (TLCSDV), Tomato Yellow Leaf Curl Virus (TYLCV), Tomato leaf curl Palampur virus (ToLCPalV) Tomato leaf curl New Delhi virus (ToLCNDV). Since its discovery in Israel, TLCD caused widespread outbreaks on tomato plants in the Mediterranean region in the 1960s. From there, it expanded to the Middle East, Southeast Asia, East Asia, Africa, and several other nations and regions (Pico et al., 1996; Czosnek and Laterrot, 1997; Polston and Anderson, 1997; El-Din et al., 2004; Delatte et al., 2005; Bhyan et al., 2007).

TYLCV has emerged as one of the most economically damaging plant virus in recent past and caused 100% crop losses in many regions (Yu et al., 2009). TLCSDV and TYLCV are mainly spread by the whitefly *Bemisia tabaci* and are members of the *Begomovirus* genus within the *Geminiviridae* family (Navot et al., 1991; Mazyad et al., 2007; Kil et al., 2016; Prasad et al., 2020; Li et al., 2022; Bupi et al., 2023). To date, the majority of this disease's research has been on pathogen identification and phylogenetic analysis. Since the domesticated tomato, *Solanum lycopersicum*, is susceptible to the virus and traditional treatments, breeding resistant cultivars is a useful method for disease prevention. In the breeding program, the inoculation method for determining plant resistance is especially crucial (Ye et al., 2009).

An efficient way to determine plant tolerance or resistance is to use infectious clone technology. The construction of an infectious clone has proven to be beneficial in identifying disease resistance in various plant viruses. In Saudi Arabia and other countries of Arabian Peninsula TLCSDV and TYLCV are most frequently linked to tomato leaf curl disease. It is now the biggest factor restricting tomato output worldwide and can result in significant loss. Begomoviruses are not transmitted mechanically therefore artificial methods of inoculation are very important to study the host range of begomoviruses and to evaluate the resistance source in different germplasms of the host plants. In this part of this research, the infectious clones of TLCSDV and

TYLCV in binary plasmid vector were constructed to be used for infectivity assay through *Agrobacterium* inoculation and infectious clones of these two viruses were used for screening of commercially available tomato cultivars for tolerance/resistance against these viruses.

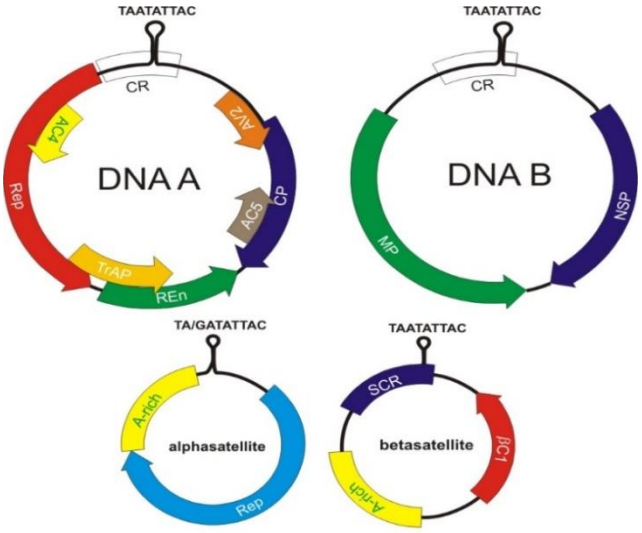

Figure-1. Genomic organization of Begomoviruses with satellites

Material and Methods

Sample collection, PCR detection and full genome amplification

Plant sample collection trips were organized during the growing seasons 2021 and 2022 to cover all major tomato growing cultivation regions in Saudi Arabia. These samples were collected from symptomatic and non-symptomatic plants in open fields and greenhouses. These samples were collected in separate plastic bags, labeled, and kept in ice bags until transported to Plant Virology lab. Total DNA was extracted from plant samples using Thermo Scientific Gene JET Plant DNA Purification Mini Kit and were subjected to PCR using a general diagnostic begomovirus primers (AC-1048: 5`-GGRTTDGARGCATGHGTACATG-3`and the reverse primer sequence AV-494: 5`-GCCYATRTAYAGRAAGCCMAG-3` (Wyatt and Brown, 1996). Full-length genome was amplified using PCR with universal begomovirus primers (Begomo F and Begomo R) [56]. Total reaction mixture of 25 ul was used for PCR reaction, using Thermo Scientific DreamTaq Green PCR Master Mix (2X). PCR products were analyzed using 1% agarose gel electrophoresis.

Construction of infectious clone

The amplified full-length genome was cloned in PCR cloning vector pGEMT-Easy vector (Promega, Madison, WI), and sequenced. The obtained sequence information of begomoviruses genomes were analyzed using BLAST program in NCBI Database. The clones were sequenced by Sanger sequencing technology.

Transformation of heat-shock competent E. coli cells

The ligation mixture (TLCSDV and TYLCV-pGEMT-Easy clones) was added to 200µl of thawed competent cells, carefully mixed, and allowed to cool down on ice for 30 minutes. In a water bath, the cells were heat shocked to 42°C. Cells were moved to ice and incubated for two minutes after one to two minutes. After adding 1 milliliter of LB liquid medium to each tube, the tubes were incubated for one hour at 37 degrees Celsius. Transformed cells were distributed over solid LB media plates, treated with the necessary antibiotics, and incubated for 16 hours at 37 °C in an incubator (Sambrook et al., 1989). The partial genomes of TLCSDV and TYLCV containing origin of replication were digested from TLCSDV-pGEMT-Easy and TYLCV-pGEMT-Easy clones with KpnI and HindIII and cloned into binary plasmid vector pGreenII 0000. Then the full-length monomer genome was digested with KpnI and ligated into 1.6ToLCSDV pGreenII 0000 and TYLCV- pGreenII 0000 to make partial dimer infectious clone. Clones' confirmation was done by restriction digestion analysis using KpnI and HindIII enzymes separately.

Transformation of infectious clone in Agrobacterium tumefaciens GV3101

To construct agro-infectious clone of TLCSDV and TYLCV, monomer clone was digested with restriction enzymes KpnI/PstI to release a fragment of 1.6 Kb. The fragment of 1.6 Kb carrying one origins of replication was cloned in binary plasmid vector pGreen II 0000, to produce 1.6 TLCSDV pgreen. The confirmed partial dimer clones of pG-TLCSDV and pG-TYLCV were transformed into *A. tumefaciens* GV1303 using electroporation technique (ECM 600, BTX, U.S.A). The transformed cells were spread on LB agar plate with kanamycin (K) (50µg/mL) and rifampicin (R) (50µg/mL) antibiotics as selection marker and incubated on 28℃ for 48 hours. After 2 days agrobacterium colonies appeared

on plate and further colony PCR was done using virus specific primers for confirmation of transformation. Confirmed colonies are further cultured in LB broth with appropriate antibiotics as selection marker and grown in shaker incubator on 28°C for 48 hours. Then agrobacterium culture was stored as glycerol stock on -80°C. In order to salvage bacterial cultures from glycerol stocks, just a little of the culture was streaked on solid growth media plates containing the appropriate antibiotics and kept at an appropriate temperature.

Agroinfiltration for ToLCSDV and TYLCV-pGreenII 0000

The inoculum was prepared by growing transformed Agrobacterium colony in LB broth with appropriate antibiotics as selection marker. The culture was incubated in shaker on 28°C with vigorous shaking for 2 days. After centrifugation of agrobacterium cells, pellet was resuspended in 10mM $MgCl_2$ and 100µM acetosyringone for activation of inoculum. Activated agrobacterium inoculums harboring agro-infectious clones of pG-TLCSDV and pG-TYLCV were infiltrated into 15 *N. benthamiana* plants (6 plants with each virus clones and 3 mock control plants). Mock plants were inoculated with agrobacterium harboring empty pGreen vector. Three leaves from each plant were infiltrated, and each leaf received approximately the same amount of inoculum. The culture's final OD600 was modified to range from 0.1 to 0.5 before being utilized for agroinfiltration, using a needleless syringe, completely grown tomato leaves that were three to four weeks older were chosen for infiltration. The infiltrated part of all leaves and systemic leaves was harvested after 30 days infiltration and used for downstream analysis of infectivity.

PCR confirmation of infectivity with gene specific primers

Isolated DNA from the systemic leaves of agroinfiltrated *N. benthamiana* plants were used as template to perform PCR using gene specific primers to further confirm the infectivity of our infectious clones in the model host plants. C1 gene primers were used for TLCSVD-C1 and C1 and CP gene primers were used for TYLCV. A PCR was conducted in a reaction volume (25 µL) using Thermo Scientific Dream Taq Green PCR Master mix and thermal cycle program was performed using the following thermal cycle 1 cycle at 95°C for 7 min, 30 cycles at 95°C for 30 sec, at 53°C for 45 sec, at 72°C for 1min and 1 cycle at 72°C for 5 min.

Source of *N. benthamiana* and *S. lycopersicum* germplasms and infectivity assay by PCR

For infectivity analysis of begomovirus in model host *N. benthamiana* and original host tomato, an agro-infectious clone was constructed by cloning partial dimer of full-length genome of begomovirus in binary plasmid vector. The partial dimer clone carried two origins of replication and in between these origins of replication complete monomer genome of begomovirus was replicated and circularized to make virus infectious in host cells. Thirteen commonly grown tomato cultivars grown in open fields and greenhouses were obtained from seed market of Riyadh, Saudi Arabia namely: Newton, Quaresma, Dafnis, JV 1S, Jamilah, Seraj, Mawal, Baikonour, Mulla F1, Dusmo F1, Meghina F1, Tone Guitar, and Titanic. Tomato seedlings of these varieties were inoculated with activated inoculum of *Agrobacterium* harboring infectious clone of TLCSDV and TYLCV. After 3-5 true leaves of each cultivar, inoculation was done using needleless syringes and were kept in insect free chambers with controlled environment. All cultivars showed typical disease symptoms of tomato leaf curl disease after 3-4 weeks of inoculation in their systemic leaves. The symptomatic systemic leaves were photographed and harvested for infectivity analysis by PCR. Total DNA was extracted from systemic leaves of agro-inoculated and controlled tomato plants after 30 days of agroinfiltration using Thermo Scientific Gene JET Plant DNA Purification Mini Kit as recommended protocol. Confirmation was done by PCR using AC 1048 and AV 494 primers, (Wyatt and Brown, 1996). The schematic diagram shows the whole methodology in figure 2.

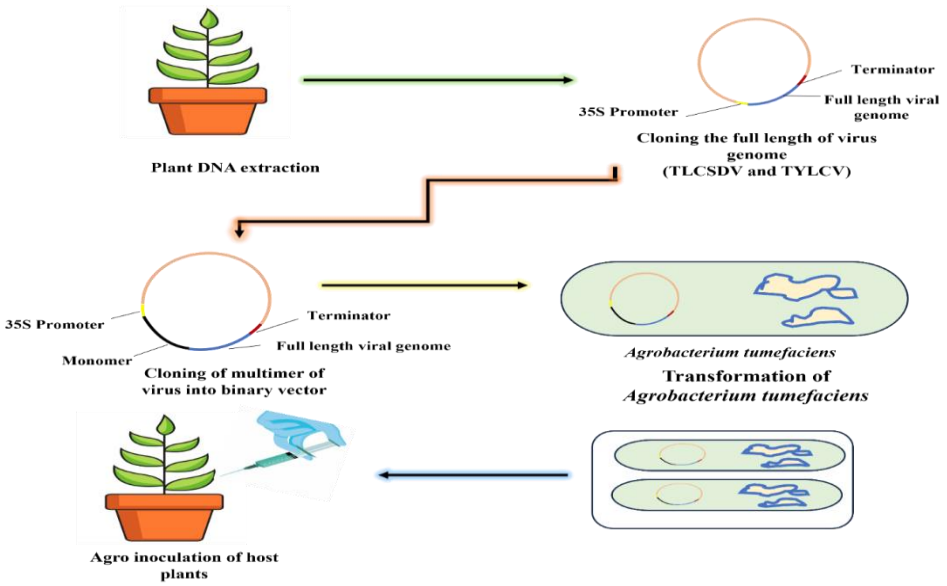

Figure-2. Summary of the methodology involved in construction of agro-infectious clones of TYLCV and ToLCSV.

Results

PCR diagnosis, full genome amplification and construction of infectious clone of TLCSDV

The obtained PCR results using general diagnostic begomovirus primers (AC1048/AV494) showed the presence of specific amplified DNA fragments at approx. 550 bp comparing with Hyper Ladder™ 50bp. Begomo F&R primers successfully amplified the full genome at approx. 2.8 kb of both these viruses which were then used in the construction of agro-infectious clones. These clones were sequenced and identified as TOLCSDV and TYLCV. Sequencing of the amplified PCR product revealed the presence of two begomovirus species, TLCSDV-RIY-SA-8-21 clone (Accession number, OR724726) and TYLCV-RIY-SA-22 clone (Accession number, OR724727).

Infectivity analysis by Agro infiltration of *N. benthamiana* plants

Infectious clones of TLCSDV and TYLCV were recovered from A. tumefaciens GV3101 glycerol stocks. A. tumefaciens GV3101 harboring infectious clones of pG-TLCSDV and pG-TYLCV grown in 2ml LB-KR medium. The obtained results of inoculated seedlings with the infectious clones of TLCSDV and TYLCV showed typical symptoms of downward leaf curling, vein swelling and stunted growth in 10-14 days post-inoculation while the negative controls showed no symptoms (Figure 3). Symptomatic leaves were harvested and confirmed with PCR using AC 1048 and AV 494 primers. Detection of begomovirus positive samples of *N. benthamiana* plants using degenerate primers yielded approx. 550 bp size (Figure 4).

PCR Confirmation of Infectivity with gene specific primers

The PCR results of gene specific primers with C1 gene for TLCSVD-C1, C1 and CP genes for TYLCV showed specific fragments at 1.1 kb, 750 bp and 1.1 kb, respectively. These results revealed the infectivity of our infectious clones in the model host plants. However, only one sample was positive with TYLCV using C1 and C represents the control (Figure 5).

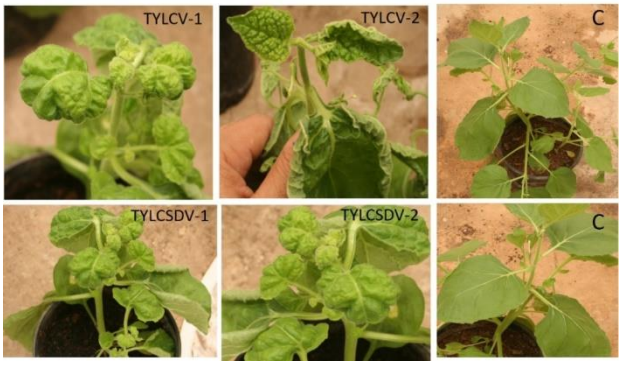

Figure-3. *N. benthamiana* plants agroinfiltrated with infectious clones of pG-TYLCV (TYLCV-1 and TYLCV-2) showing symptoms and pG-TLCSDV (TLCSDV-1 and TLCSDV -2) showing symptoms after 10-14 dpi respectively. Uninoculated plants as a control (C) showing no symptoms.

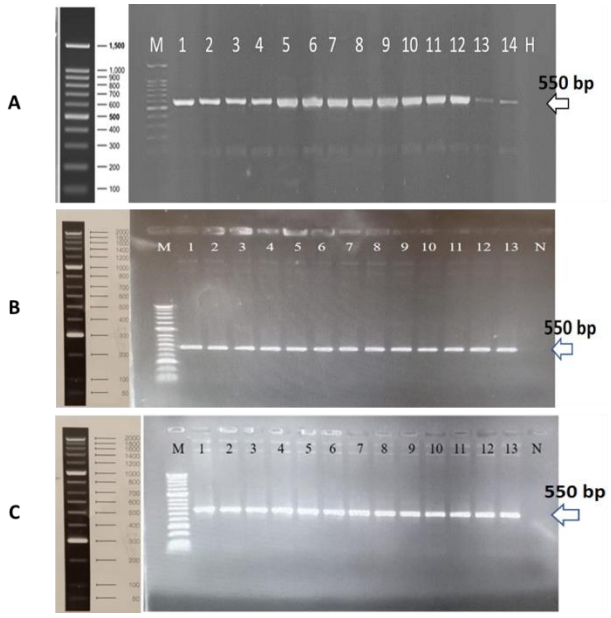

Figure-4. Panel A represents PCR detection of TLCSDV (Lanes 1-7) and TYLCV (Lanes 8-14) from systemic leaves of agroinfiltrated *N. benthamiana* seedlings using the same degenerate begomovirus primer.

Lane-M: 100 bp marker (Genedirex), Lane H is a healthy control plant. Panel B represents PCR amplification of TLCSDV genome fragment using begomovirus universal diagnostic primers (AC1048F and AV494R). M showing HyperLadder™ 50bp (Meridian Bioscience Inc.). All varieties from lane 1-13 showed positive results; 1: BAIKONOUR, 2: MULLA F1, 3: DUSMO F1, 4: MEGHNA F1, 5: TONE GUITAR, 6: NEWTON, 7: QUARESMA, 8: DAFNIS, 9: JV1S, 10: MAWAL, 11: JAMILAH, 12: SERAJ, and 13: TITANIC. N: is negative control. Panel C represents PCR amplification of TYLCV genome fragment using begomovirus universal diagnostic primers (AC1048F and AV494R). Panel M showing HyperLadder™ 50bp (Meridian Bioscience Inc.), All varieties from lane 1-13 showed positive results; 1-BAIKONOUR, 2-MULLA F1, 3-DUSMO F1, 4-MEGHNA F1, 5-TONE GUITAR, 6-NEWTON, 7-QUARESMA, 8-DAFNIS, 9-JV1S, 10-MAWAL, 11-JAMILAH, 12-SERAJ, and 13-TITANIC and N is negative control.

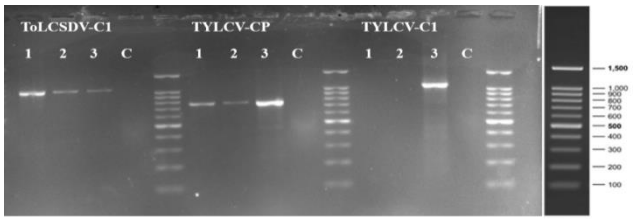

Figure-5. PCR detection of TLCSDV and TYLCV using gene specific primers.

First panel showing TLCSDV positive samples (1 ,2 ,3) using C1 primers, second panel is showing CP gene amplification of TYLCV positive samples (1, 2, 3), and third panel has TYLCV-C1 gene amplification of positive sample (3) whereas, C represents healthy control sample in each panel.

Infectivity assay of TLCSDV

After 30 days post inoculation, the symptoms were observed and recorded. All thirteen varieties were susceptible to TLCSDV and showed variety of symptoms including leaf curling, yellow spots on the leaves, leaf crumpling, severe leaf curling (observed in one variety NEWTON) and vein swelling (Figure 6). Among 13 varieties three (NEWTON, SERAJ, TITANIC) showed 100% infectivity. All other varieties showed more than 80% infectivity of TLCSDV (Table 1). The infectivity of the infectious clone of TLCSDV was further analyzed by PCR using universal begomovirus diagnostic primers (AC1048F and AV494R). The obtained results of PCR showed that all verities were positive with TLCSDV, and the DNA fragments were visible at 550 bp (Figure 4 B).

Infectivity assay of TYLCV

The plants symptoms were recorded and photographed after 30 days of inoculation. All thirteen varieties were susceptible to TYLCV and showed variety of symptoms including leaf yellowing, leaf curling, yellow spots on the leaves, leaf crumpling, severe leaf curling (observed in three varieties variety DUSMO F1, NEWTON, JAMILA) and vein swelling (Figure 7). The infectivity analysis of the infectious clone of TYLCV using PCR showed all the varieties were positive (Figure 4 C). Among 13 varieties three (NEWTON, SERAJ, TITANIC) showed 100% infectivity. All other varieties showed more than 80% infectivity of TYLCV (Table 2)

Table-1. Infectivity and symptoms of 13 tomato varieties by agro-inoculation TLCSDV at 30 DPI

No.	Variety name	Total Number of plants inoculated	No. of plants infected	Infectivity Percentage	Symptoms*
1	BAIKONOUR	15	12	80	LCr, YS
2	MULLA F1	15	12	80	LCr, YS
3	DUSMO F1	15	13	86	LC, YS, VS
4	MEGHNA F1	15	14	93	LCr, YS, VS
5	TONE GUITAR	15	13	86	LCr, YS
6	NEWTON	15	15	100	SLC, YS, VS
7	QUARESMA	15	12	80	LC, LCr, YS
8	DAFNIS	15	13	86	LCr, YS, VS
9	JV 1S	15	12	80	LCr, YS
10	MAWAL	15	14	93	LC, LCr, YS
11	JAMILAH	15	13	86	LCr, YS
12	SERAJ	15	15	100	LC, LCr, YS
13	TITANIC	15	15	100	LCr, YS, VS

*LC= Leaf curling, SLC= Severe leaf curling, LCr= Leaf crumpling, YS= Yellow spots, VS= Vein swelling

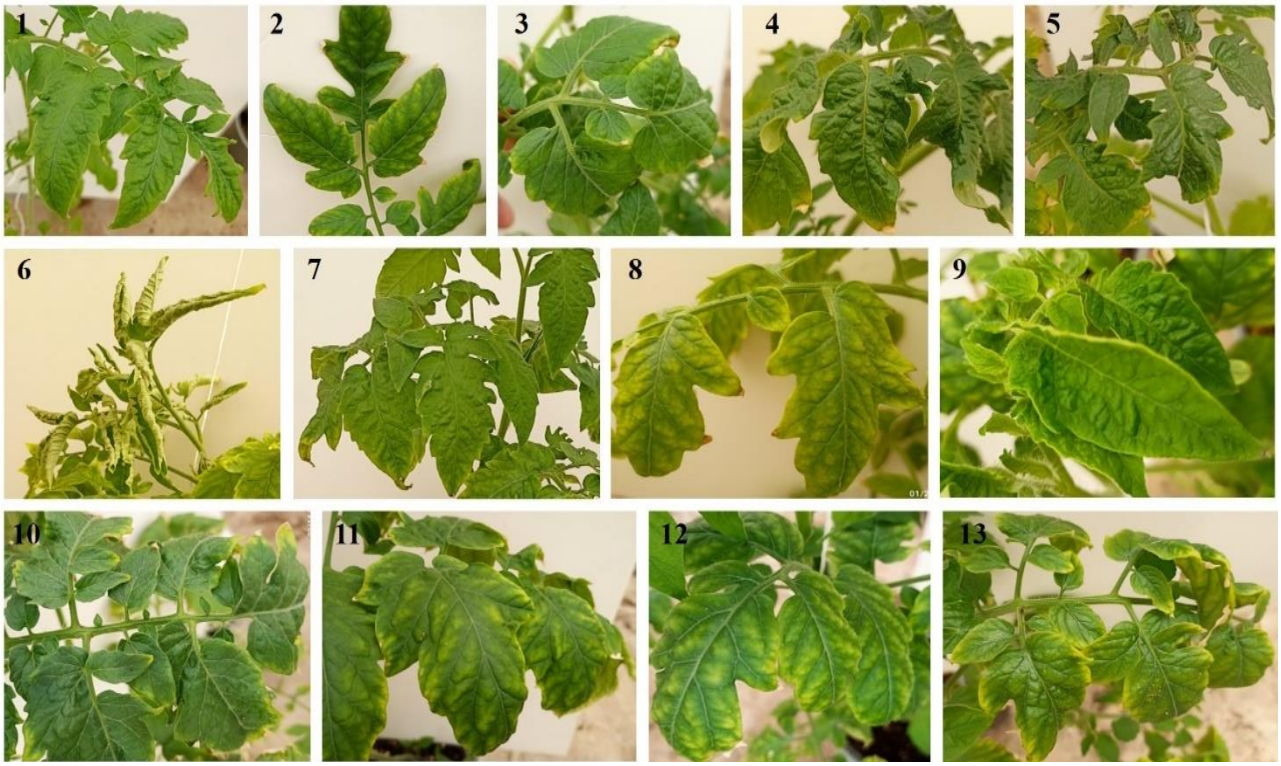

Figure-6. Symptoms development of tomato leaf curl disease after inoculation with TLCSDV in 13 varieties of tomato plants, 1: BAIKONOUR, 2: MULLA F1, 3: DUSMO F1, 4: MEGHNA F1, 5: TONE GUITAR, 6: NEWTON, 7: QUARESMA, 8: DAFNIS, 9: JV1S, 10: MAWAL, 11: JAMILAH, 12: SERAJ, and 13: TITANIC N: is negative control.

Table-2. Infectivity and symptoms of 13 tomato varieties by agro-inoculation TYLCV at 30 DPI

No.	Variety name	Total Number of plants inoculated	No. of plants infected	Infectivity Percentage	Symptoms*
1	BAIKONOUR	15	13	86	LY, LC
2	MULLA F1	15	14	93	LY, LCr
3	DUSMO F1	15	13	86	SLC, VS
4	MEGHNA F1	15	13	86	LY, LCr
5	TONE GUITAR	15	13	86	LY, LCr
6	NEWTON	15	15	100	SLC, LY, VS
7	QUARESMA	15	12	80	LY, LCr, YS
8	DAFNIS	15	14	93	LY, LCr
9	JV15	15	14	93	LC, YS
10	MAWAL	15	14	93	LY, LCr
11	JAMILAH	15	13	86	SLC, YS,
12	SERAJ	15	15	100	LCr, YS
13	TITANIC	15	15	100	LCr, YS, VS

*LY= Leaf yellowing, LC= Leaf curling, SLC= Severe leaf curling, LCr= Leaf crumpling, YS= Yellow spots,

Figure-7. Symptoms development of tomato leaf curl disease in 13 varieties of tomato plants with TYLCV. 1: BAIKONOUR, 2: MULLA F1, 3: DUSMO F1, 4: MEGHNA F1, 5: TONE GUITAR, 6: NEWTON, 7: QUARESMA, 8: DAFNIS, 9: JV1S, 10: MAWAL, 11: JAMILAH, 12: SERAJ, and 13: TITANIC.

Discussion

The process of constructing an infectious clone enables molecular functional investigation of the viruses and their biological functions in pathogenesis, replication, and transmission (Pico et al., 1996; Trenado et al., 2011). To prevent a genetically unstable viral population or a virus population which is unable to transmit mechanically, it is required to produce an inoculum that may be utilized for resistance screening by making infectious clones that carry wild-type viral genomes. This also removes the requirement for the virus to be maintained and spread within plants. Infectious clones also give an alternative to in-effective inoculation processes such as grafting, and diseases transferred via insect vectors (Brewer et al., 2018). Without the necessity for sustained plant transgenesis, it is possible to modify infectious clones to accomplish virus-induced gene silencing and to create expression vectors for the plant that can be employed in plant pathology and research on plant gene functions (Lange et al., 2013). In addition to this, the capacity to recreate a fully functional and transferable virus will encourage reckless usage of infectious clones that might harm the environment. These hazards may result from their innate pathogenicity as well as any introduced genetic changes (Brewer et al., 2018).

The creation of infectious clones for Begomovirus has made it possible to learn a lot of information. Through the artificial alteration of Begomovirus genomes, new insights into the interactions between the virus and host cells have been described. These manipulations include site-directed mutagenesis, deletion or insertion, and reorganization. Begomovirus infectious clones are also vital for screening resistant strains. Since the cloned genome is easily manipulable, infectious clones of plant viruses are essential tools in molecular virology research. Developing an infectious clone makes it feasible to characterize a virus (Moury et al., 2004). Restriction endonucleases have been used in the construction of DNA constructs and virus clones in order to produce compatible ends. This strategy was not successful due to the existence or lack of restriction sites in the viral genome and vector sequences.

To get around these restrictions, plant virologists have used a variety of cloning techniques, including overlap-
based techniques, yeast homologous recombination, Gibson assembly, and Golden Gate cloning. The main drawbacks of using restriction enzymes are removed by overlap-based techniques, which are adaptable and flexible. Infectious clones can be assembled quickly and effectively using overlap-based cloning. The PCR-Gibson Assembly methodology was shown to be a straightforward method for producing infectious clones of Bean Golden Mosaic Virus (Ferro et al., 2019). Begomovirus infectious clones require dimeric structures with two replication origins for each genomic component. The virus's genome was successfully assembled in an isothermal reaction using a single-step GA method. The formation of infectious clones in begomoviruses has not yet been observed to occur through Golden Gate cloning or yeast homologous recombination; this may be because the genomes of these viruses are small, and the genome can be cloned rather easily. The creation of artificial and functional genomes has shown the potential of synthetic biology. Specifically, it has been demonstrated that assembling synthetic replicon from a virus genome is a successful de novo synthesis technique. There have been reports of several infectious clones derived from plant viral genomes that were created completely by de novo synthesis and assembly (Cooper, 2014; Lovato et al., 2014; Pasin et al., 2019). The conserved sequences of Begomovirus enable it to amplify the whole genome, including all of plant DNA, using its degenerate “universal” primers. A method RCA–NGS (rolling circle amplification–next generation sequencing) was presented that allowed the assembly of 19 full genome begomoviruses and one alphasatellite, as well as the investigation of the diversity of begomoviruses in North America (Bornancini et al., 2020). The accuracy and stability of begomoviral sequences will be confirmed by next-generation sequencing technologies by contrasting them with those obtained through traditional techniques like PCR and RCA.

Plant viruses have been utilized to create infectious clones through cell-free cloning procedures (Fakhfakh et al., 1996; Jailani et al., 2017). Through in vitro amplification, uncloned genome copies can be created, and the resulting products can be utilized to inoculate plants through rubbing or a biologist's method. In general, this approach is inappropriate for reverse genetic research (Youssef et al., 2011). Plant virology can now be studied in novel ways thanks to the finding that Agrobacterium is capable of infecting

plants with infectious clones. Agro-inoculation offers the most effective and widely applicable technique for transferring DNA or RNA viruses to plants. By employing synthetic biology techniques, its potential is significantly enhanced (Roy et al., 2019; Khan et al., 2020). Viral vectors, biomaterial sources, and nanotechnologies tools are all possible to generate by engineering full-length infectious clones to be adapted for use in industry (Cooper, 2014).

Phyto-pathogenic viruses are a significant global restriction on crop yields. The low crop output in Saudi Arabia can be partially explained by the regrettable fact that the kingdom, like all other Middle Eastern nations, harbors individuals of nearly every taxonomic group of viruses that infect plants. However, emerging nations in tropical and subtropical regions suffer the greatest losses and hardships. There are several reasons for this, including the increased diversity and prevalence of plant-infecting viruses of agricultural importance, the more hospitable environment for the virus's vectors, the farmers' being unable to afford the costly chemical control agents, and frequently their lack of the necessary educational background and training. The most common of these viruses are begomoviruses, which have become prevalent diseases on a number of commercially significant crops, including papaya, tomato, chili, eggplant, and cucurbits. For the purpose of identifying and categorizing begomoviruses, the entire nucleotide sequence of DNA A is enough. More than 90% sequence identity distinguishes viruses as strains of the same virus, but DNA sequence identity of less than 89% distinguishes viruses as new species (Fauquet et al., 2003).

In recent years, the technique of inoculating plants with *Agrobacterium* to determine their level of viral resistance has been established. The virus can replicate and spread after inoculating a plant, at which point it can cause symptoms in the plant. The poliovirus was the target of the method's first effective application (Ahlquist et al., 1984). Numerous contagious plant virus clones have been successfully developed since then (Zhang et al., 2009; Zhang et al., 2010). The infectious clones of the TYLCV Shanghai isolate and the Papaya leaf curl China virus were constructed by Pratap et al. and Malik et al., respectively. Additionally, the ToLCPalV and the tomato leaf curl virus (TYLCV) of eggplant were developed as well (Malik et al., 2011; Pratap et al., 2011). The method can induce symptoms was proved. In our study we have constructed partial dimer infectious clones of Saudi Arabian isolate TLCSDV and TYLCV in the binary vector and inoculated those infectious clones through agroinfiltration into 13 different genotypes of tomato commercially available to the farmers for cultivation in open field and greenhouses. Our results showed that all commercially available genotypes are susceptible to these two-dominating tomato begomoviruses and more than 80% of inoculated plants of all cultivars showed symptoms. In a similar study, (Jin et al., 2011) TYLCV Tianjin isolate infectious clone was created, and tomato plant was vaccinated. After around a month, the symptoms began appearing. This investigation supported our findings that symptoms of full bloom appear at 30 DPI (Zhan et al., 1991; Haley et al., 1992). Previous studies state that the TYLCV outbreak primarily affected tropical and subtropical areas (Valizadeh et al., 2011).

Conclusion

For begomoviruses, the availability of infectious clones has been shown to be an effective molecular tool. This method makes it easier to genetically screen germplasm for virus resistance and permits the functional analysis of a viral gene and its biological characteristics. Utilizing Agrobacterium tumefaciens and its binary vectors, agro-inoculation of viral clones is an economical and extremely successful way to assess the infectivity of begomoviruses. Thus, virologists employ it extensively. To date, the procedures for creating an infectious full-length clone construct have been stream-lined, and ongoing kit and methodology advances have made a deeper knowledge of viral infectivity possible.

In this study, our results showed that all commercially available germplasms of tomato crop in Saudi Arabia are susceptible to TLCSDV and TYLCV showing variable symptoms of tomato leaf curl disease. It is need of the hour to use advanced biotechnological approaches to develop resistant crops against begomoviruses to safeguard agriculture and to uplift the economy of farmers and ultimately to boost the economy of the country.

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Contribution of Authors

Amer MA & Khalid Z: Created and designed experiments for this study, data analysis and manuscript drafting and editing
Hussain K: Data analysis and manuscript drafting
Amir M, Zaman & M Al-Shahwan IM: Critically reviewed and edited the article for significant intellectual content
Al-Saleh MA: Created and designed the experiments for this study, collected the samples and conducted experiments in the laboratory

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