



**Full Length Article**

## **Association of Tomato Yellow Leaf Curl Virus and Chili Leaf Curl Virus with Leaf Curl Disease of Radish and the Synergistic Interaction on *Nicotiana benthamiana***

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### **Abstract**

Leaf yellowing and rolling with slight crinkling was observed in radish plants in Oman, indicative of a begomovirus infection. Viral sequences from radish were 99% identical to sequences of *Tomato yellow leaf curl virus* (TYLCV) and *Chilli leaf curl virus* (ChiLCV), from Oman. No beta- or alpha-satellites were detected. Infectious clones were developed and infectivity analyses of isolated viruses were done in a model host *Nicotiana benthamiana*. Both viruses, when inoculated separately, produced typical symptoms; however, symptom severity increased when these viruses were co-inoculated. Southern analyses confirmed that virus accumulation was high when both viruses were inoculated as compared to independent inoculation. Real-time qPCR analyses showed that copy number of both viruses was  $1 \times 10^6$  copies when *N. benthamiana* plants were inoculated with either TYLCV or ChiLCV; however, the copy number increased to  $1 \times 10^8$  copies when the two viruses were co-inoculated together in *N. benthamiana*. © 2017 Friends Science Publishers

**Keywords:** TYLCV; ChiLCV; *Begomovirus*; Radish; Mixed infection

### **Introduction**

Radish, (*Raphanus sativus*), which belongs to the family *Brassicaceae*, is one of the most important vegetable crops in Oman. Radish is usually grown along with companion plants such as cabbage, squash, carrot and lettuce. Recently field surveys have shown leaf curling and stunting of radish plants in Al-Batinah region in Oman. The symptoms are typical of begomovirus infection and the disease severity ranges from 50–80% in most of the farms.

Geminiviruses, a name given due to their twinned icosahedral particles, are single stranded DNA viruses. *Geminivirus* diseases have greatly affected the agriculture of Asia and Africa, where tomato yellow leaf curl disease, cassava mosaic disease, and maize streak disease have resulted in complete losses in infected fields (Legg and Fauquet, 2004; Shepherd *et al.*, 2010; Sattar *et al.*, 2013).

The *Geminiviridae* family is divided into seven genera, *Begomovirus*, *Mastervirus*, *Topcovirus*, *Curtovirus*, *Becurtovirus*, *Capulavirus*, *Eragrovirus* and *Turncurtovirus* (Varsani *et al.*, 2014). Begomoviruses are believed to have coevolved with their hosts over time; however, these viruses have become economically important pathogens only in the last two decades (Seal *et al.*, 2006). Begomoviruses native

to the New World (NW) have genomes consisting of two ssDNA genome components and are known as bipartite viruses. A bipartite genome is the genome that consists of both components together, referred to as DNA A and DNA B. Begomoviruses belonging to the Old World (OW), in contrast, have one or two genomic components, monopartite or bipartite genomes, respectively. Betasatellites are also known to encode silencing suppressors (Amin *et al.*, 2011). Betasatellites are required for the infectivity of some viruses (Briddon and Stanley, 2006). The role of alphasatellites is not fully understood; however, it has been shown that alphasatellites encode a suppressor that silences genes and can help to overcome the host defense response (Nawaz-ul-Rehman *et al.*, 2010).

Mixed infections and co-infections are common among geminiviruses in tropical and subtropical environments. A mixed infection potentially leads to geminivirus variability (Harrison *et al.*, 1997; Zhou *et al.*, 1997; Fondong *et al.*, 2000). Mixed infections also can affect symptoms, and virus accumulation, because they often result in synergistic interactions (Chakraborty *et al.*, 2008; Alves-Júnior *et al.*, 2009). The mechanism of the synergistic interaction of geminiviruses is not fully understood. However, it has been shown that the suppressor

activity of the geminiviral protein may play an important role in this. Different geminiviral proteins from different viruses may act as suppressors of gene silencing; their strength and mode of action may also differ like TrAP, C4, and V2 (Vanitharani *et al.*, 2004; Amin *et al.*, 2011). Synergism may lead to altered movement of the virus (Alves-Júnior *et al.*, 2009).

According to the different host range studies of TYLCV, various plant species are susceptible to TYLCV. In Oman the screening of vegetable crops other than tomato and pepper for the presence of begomoviruses indicates the widespread presence of these viruses in different crops. Tomatoes and many other major vegetable crops grown in Oman are susceptible to TYLCV-OM, and disease incidences may reach up to 100% during summer and fall seasons. The incidences of disease are increasing in new crops due to the emergence of recently isolated new recombinants of TYLCV-OM from Oman (Al-Shihi *et al.*, 2014). During a field survey in February 2014, in Albatinah region of Oman, leaf yellowing and rolling with slight crinkling was observed in radish plants, which are indicative of a begomovirus infection. Our results show that the leaf curl disease of radish in Oman is caused by a mixed infection of TYLCV-OM and ChiLCV-OM. We also show that there is a synergistic interaction of these viruses, which results in severe infection and high virus accumulation. The implications of these results are discussed.

## Materials and Methods

### Sample Collection and DNA Extraction

During the winter season of 2014, radish plants growing in the fields of Al-Batinah region showed leaf yellowing and rolling with slight crinkling (Fig. 1), suggestive of begomovirus infection. Leaf samples were collected from four infected radish crops and one non-symptomatic radish plant and the total nucleic acid was extracted using the CTAB method (Doyle and Doyle, 1990).

### PCR, Cloning and Sequencing of Virus Components

Degenerate primers were designed and used to amplify the CP gene (Ammara *et al.*, 2015), which was used to conduct diagnostic PCR of all infected and healthy plants. The PCR profile used in the thermal cycler was as explained by Ammara *et al.* (2015).

The PCR-positive samples were used for the amplification of circular DNA molecules by rolling-circle amplification (RCA). Digestion of RCA products was done using *Pst I* and *Xba I*. The 2.8 kb fragments were further cloned in plasmid vector pUC19. A Gene JET Plasmid Miniprep Kit (Fermentas, Vilnius, Lithuania) was used to purify putative full-length clones followed by sequencing the cloned insert both orientations (Macrogen Inc. South Korea).

### Sequence Analysis

All sequences were compared with sequences available in the GenBank database using BLAST (Altschul *et al.*, 1990). Multiple sequence alignments and phylogenetic analysis were carried out as described by Ammara *et al.* (2015).

### Inoculation

A partial tandem repeat (PTR) of the begomovirus clone Rad-1/TYLCV-OM (HG969261) was prepared for an *Agrobacterium*-mediated inoculation in a binary vector pCambia1301. A fragment of ~1.9kb with the intergenic region was released from the pUC19-Rad-1 clone by using *Pst I* and *EcoR I* restriction enzymes. This fragment was ligated into the *Pst I/EcoR I* site of the binary pCAMBIA-1301 vector to get partial dimer of TYLCV-OM. A monomer of the Rad-1 clone was released from the pUC19-Rad-1 by digestion with *Pst I* and was ligated head to tail in a partial dimer of Rad-1 linearized by digestion with *Pst I* to produce PTR. The orientation of the PTR was confirmed by the digestion with *EcoR I* to produce a genome length fragment. Similarly a partial dimer construct of Rad-2 (HG969262) was also produced. The clone Rad-2/ChiLCV-OM was digested with *BamH I* and *Sac I* and the released fragment was ligated into the binary vector pCambia 1301. The remaining steps were as described by Ammara *et al.* (2015).

### Southern Blot Hybridization

Upper leaf samples were collected from all inoculated plants, from which extracted DNA was resolved by electrophoresis. For all TYLCV-OM and TYLCV-OM/ChLCV-OM inoculated plants, three replicates from each treatment were selected for analysis and ChiLCV-OM inoculated plants were used as hybridization control in addition to negative control. Similarly for ChiLCV-OM and TYLCV-OM/ChiLCV-OM inoculated plants, three replicates from each treatment were analyzed in addition to TYLCV-OM inoculated plant as a hybridization control. Nucleic acids were transferred to nylon membranes (Roche GmbH, Germany). Blots were labeled and then probed with a ~650bp fragment from the CP region of the clone Rad-1/TYLCV-OM, or a ~600 bp fragment from the Rep region of the clone Rad-2/ChLCV-OM (probes were prepared using PCR based probes Kit using the clones of each virus).

### Real-time Quantitative PCR

Full-length TYLCV-OM clone (Acc. No. HG969261) and ChLCV-OM clone (Acc. No. HG969262) was quantified by Nano Drop 8000 spectrophotometer (Thermo Fisher Scientific, USA) and used to prepare serial dilutions for standard curve analysis. The standard concentration  $1 \times 10^9$  copies of virus was prepared with suitable amount of

plasmid DNA and 10-fold diluted to prepare five serial dilutions. Each serial dilution was spiked with 10 ng/uL of healthy tomato plant genomic DNA developed by tissue culture to get the same background as in inoculated plants. The threshold cycle (Ct) value of three replicates of each standard-dilution and the log of total DNA in each sample were used to obtain the standard curves by linear regression analysis.

The upper leaf samples from all inoculated plants were analyzed by qPCR for the relative quantification of virus in each sample. For all TYLCV-OM and TYLCV-OM/ChiLCV-OM inoculated plants, five replicates from each treatment were selected for analysis and ChiLCV-OM inoculated plants were used as hybridization control in addition to negative control. Similarly for ChiLCV-OM and TYLCV-OM/ChiLCV-OM inoculated plants, five replicates from each treatment were analyzed in addition to TYLCV-OM inoculated plants as a hybridization control.

The copy number of viruses was calculated as follows. Primer pairs UTF/TYR amplified a 244nt product specific to TYLCV-OM and UCF/ChR amplified a 197nt product specific to ChiLCV-OM. To standardize and validate qPCR results, an internal control EF1 (EF1-F 5' TACTGGTGGTTTTGAAGCTG 3', EF1-R 5' AACCTCCTTCACGATTTTCATCATA 3') was designed and used. Data analyses (reaction efficiency, Standard curve, melt curve analysis) was performed (Ammara *et al.*, 2015).

## Results

### Evidence of Begomovirus Infection

Total genomic DNA was extracted from radish crops showing begomovirus like symptoms and it was subjected to PCR using primers FD-CP382 and RD-CP1038. A fragment of 650 bp was yielded. This was the first indication of the presence of begomoviruses in symptomatic radish plants. No amplification was detected in radish crops that showed no begomovirus like symptoms. No betasatellite or alpha satellite were detected in any of the samples, suggesting that no satellite molecule is involved in leaf curl disease of radish in Oman.

### Cloning and Sequencing

The PCR positive samples were used for further amplification and the cloning of full length viral genomes were amplified by rolling circle amplification using phi 29 polymerase enzyme (Haible *et al.*, 2006). Restriction of the RCA products with *Pst* I and *Xba* I resulted in a 2.8 Kb fragment indicative of a full-length viral genome. Seven samples were randomly selected for cloning. Rad-1 and Rad-2 clones were isolated from the first sample; Rad-3 and Rad-5 were cloned from the second sample, whereas Rad-4, Rad-6, and Rad-7 were cloned from the third sample. All seven clones, apparently bearing full-length genomes,

were selected for DNA sequencing by primer walking. DNA sequences were assembled in a contig using the SeqMan software. The origin and features of TYLCV-OM and ChiLCV-OM are described in Table 1.

### Sequence Analysis

Analysis of the sequences isolated from radish showed that four sequences, Rad-1, Rad-5, Rad-6, and Rad-7, share 98-99% sequence identity to TYLCV KW1 isolate, Accession no. JN604484, whereas the other three isolates, Rad-2, Rad-3, and Rad-4 showed 99% sequence identity to ChiLCV isolate, Accession no. KF229719. The pairwise comparison of all 7 isolates by using Muscle algorithm showed a 80-81% nucleotide identity between two species, and more than a 99% identity among isolates of the same species. These results confirmed that leaf curl disease of radish in Oman is caused by the mix infection of TYLCV-OM and ChiLCV-OM.

### Phylogenetic Analyses

A phylogenetic tree shows that the viral sequences from this study clustered with TYLCV-OM and ChiLCV-OM sequences. ChiLCV isolated from radish falls in the same clade with the previously detected ChiLCV-OM strain, while the TYLCV sequences from radish are in the same clade with TYLCV-OM (Fig. 2).

### Synergism between TYLCV and ChiLCV

All infectivity studies were done with the model plant *N. benthamiana*. The viruses were highly infectious to *N. benthamiana*, with all inoculated plants developing symptoms within 13–18 days post inoculation (dpi). The typical symptoms of plants inoculated with the ChiLCV-OM isolate Rad-2 alone consisted of an upward curling of the young leaves and a stunting in the growth of the plants (Fig. 3, panel A). The infectivity of the TYLCV-OM isolate Rad-1 was also assessed in *N. benthamiana* plants. The symptoms of TYLCV-OM in *N. benthamiana* consisted of a downward leaf curling, leaf crumpling and a stunting in the growth of the plants (Fig. 3, panel B). The *N. benthamiana* plants inoculated with both TYLCV-OM Rad-1 and ChiLCV-OM Rad-2 isolates in this study showed severe chlorosis along the edges of leaves, which was associated with leaf curling, crumpling, and yellowing (Fig. 3, panel C). Another major observation was the onset of symptoms; in case of TYLCV-OM Rad-1 and ChiLCV-OM Rad-2 inoculations, symptom appeared at 18-21 dpi. However, in case of TYLCV-OM Rad-1/ChiLCV-OM Rad-2 symptoms appeared at 13-15 dpi (Table 2). The combined effect of both viruses has bidirectionally enhanced symptoms and symptoms appeared earlier than individual viruses. Necrosis developed in the veins of older symptomatic leaves. A detail of the infectivity analyses along with the symptoms and severity scale is provided in Table 2.

**Table 1:** Origins and features of two *Begomovirus* species, the *Tomato yellow leaf curl virus* (TYLCV) and the *Chili leaf curl virus* (ChiLCV) isolated from a radish crop from the Al-Batinah region of Oman

Isolate	Isolate descriptor	Begomovirus species	Origin	Accession No.	Size (nt)	Position of genes (coordinates)/ No. of amino acids					
						Rep	TrAP	REn	C4	V1	V2
Rad 1	OM-Br-Rad1-14	TYLCV	Barka	HG969261	2776	1535-2608 /357	1213-1620 /135	1068-1472 /134	2158-2451 /97	295-1071 /258	135-485 /116
Rad 2	OM-Br-Rad2-14	ChLCV	Barka	HG969262	2761	1531-2616 /361	1224-1628 /134	1079-1483 /134	2166-2459 /97	309-1082 /257	149-514 /121
Rad 3	OM-Br-Rad3-14	ChLCV	Barka	HG969263	2761	1531-2616 /361	1224-1628 /134	1079-1483 /134	2166-2459 /97	309-1082 /257	149-514 /121
Rad 4	OM-Br-Rad4-14	ChLCV	Barka	HG969264	2761	1531-2616 /361	1224-1628 /134	1079-1483 /134	2166-2459 /97	309-1082 /257	149-514 121
Rad 5	OM-Br-Rad5-14	TYLCV	Barka	HG969270	2777	1536-2609 /357	1214-1621 /135	1069-1473 /134	2159-2452 /97	296-1072 /258	136-486 /116
Rad 6	OM-Br-Rad6-14	TYLCV	Barka	HG969271	2804	1538-2611 /357	1216-1623 /135	1071-1475 /134	2161-2454 /97	298-1074 /258	138-488 /116
Rad 7	OM-Br-Rad7-14	TYLCV	Barka	HG969272	2784	1543-2616 /357	1221-1628 /135	1076-1480 /134	2166-2459 /97	303-1079 /258	137-493 /118

**Table 2:** Infectivity data of *N. benthamiana* inoculated with the TYLCV-OM Rad-1, the ChiLCV-OM Rad-2, and the combination of TYLCV-OM Rad-1/ChiLCV-OM Rad-2

Inoculum	Experiment	Plants showed symptom/Plants inoculated	Onset of symptoms	Symptom	Severity
TYLCV-OM	I	4/5	18	Y	1
	II	5/5	20	C	2
	III	5/5	18	Y	2
	IV	4/5	19	C	1
ChiLCV-OM	I	5/5	18	C	2
	II	4/5	21	C	1
	III	5/5	18	RNG	1
	IV	5/5	19	C	2
TYLCVOM+ChiLCV-OM	I	5/5	15	Y	2
	II	5/5	13	C+Y	3
	III	5/5	14	S	3
	IV	5/5	13	C+S	3

Severity scale: 0=No symptom, 1=slight leaf curl and yellowing, 2=Mild leaf curl, yellowing, 3=severe leaf curl, yellowing and stunting Y=Yellowing, C=Curling, S=Stunting, RNG=Reduced new growths

The Southern blot analysis shows the presence of the DNA forms that are typical of geminivirus replication in symptomatic plants (Fig. 4). The TYLCV-OM Rad-1 was used as a probe and results are shown in Fig. 4 panel AI. Our results confirm that TYLCV-OM alone, and also in combination with ChiLCV-OM, are able to replicate in *N. benthamiana* plants. When the same blot was probed with the ChiLCV-OM-Rad-2 probe, a similar intensity band was observed (Fig 4, panel AII). These results prove that both viruses can replicate singly. Southern blot analyses of the plants inoculated with TYLCV-OM Rad-1 and ChiLCV-OM Rad-2 showed a high accumulation of both viruses (Fig 4, panels AI and AII).

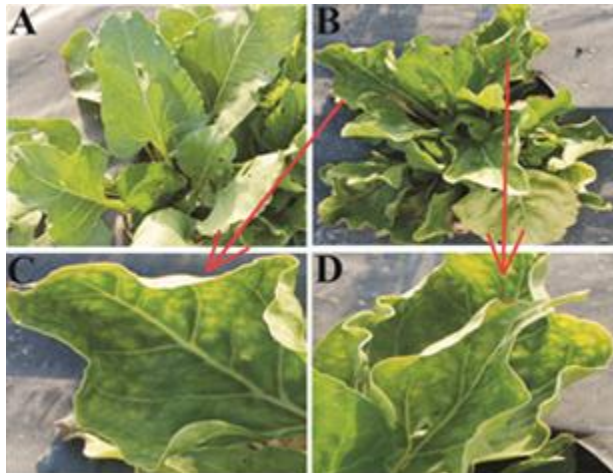
Real time quantitative PCR (qPCR) was performed to calculate the copy number of TYLCV-OM Rad-1 and ChiLCV-OM Rad-2 alone, as well as in mixed infections, in *N. benthamiana*. qPCR analyses showed that in the case of the TYLCV-OM Rad -1 inoculation, the average copy number of the virus is  $1 \times 10^6$  copies in infected plants (Fig. 4 panel BI), while almost the same copy number  $1 \times 10^6$  was observed in *N. benthamiana* plants inoculated with the ChiLCV-OM Rad-2 (Fig 4. panel BII). However, *N.*

*benthamiana* plants inoculated with the TYLCV-OM Rad-1/ChLCV-OM Rad-2 combination showed a significant increase in the copy number of both viruses: up to  $1 \times 10^8$  copies of each virus (Fig. 4. Panel BIII). Both Southern and qPCR analyses confirmed that number of TYLCV-OM virus copies or titer increased upon a mixed infection, and thus indicate that the TYLCV-OM Rad-1 and the ChLCV-OM Rad-2 have a synergistic interaction.

All attempts to infect radish with the ChiLCV-OM Rad-2 in the presence and absence of the TYLCV-OM Rad-1 were unsuccessful. Three different strains of agrobacterium (LBA 4404, GV 3101 and AGL1) were used to inoculate radish plants. All three strains were not able to produce symptoms with the possible clones combination isolated from radish. However, symptoms were observed in *N. benthamiana*, suggested that virus is infectious however; the method of inoculation is not suitable for radish.

## Discussion

In this study we have shown that radish plants in Oman are infected by two viruses, TYLCV-OM and ChiLCV-OM.

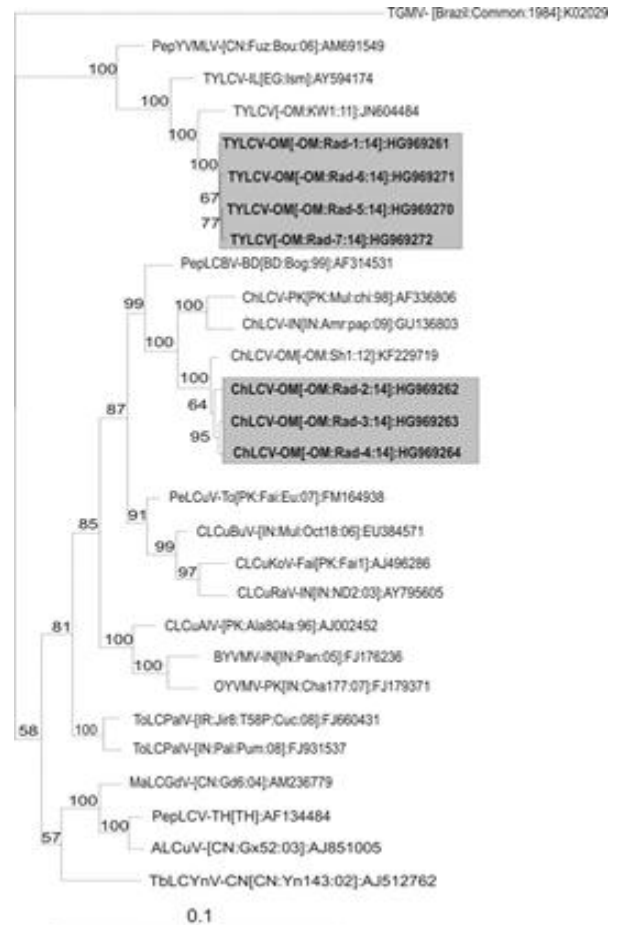


**Fig. 1:** (A) A healthy radish plant with no visible symptoms of begomoviruses (B) A radish plant showing yellowing and curling symptoms. Panels C and D are the close up images of the radish plant in panel B

Both viruses have a synergistic interaction, which results in a severe phenotype in a model system. These findings indicate an increased host range of TYLCV-OM in Oman. This is the first report of the leaf curl disease of radish caused by a mixed infection of TYLCV-OM and ChLCV-OM.

*Geminiviruses* have become a potential threat to agriculture due to the emergence of new recombinants, especially in tropical and subtropical countries (Zhou *et al.*, 1998; Moffat, 1999). The emergence of new recombinant begomoviruses has recently been found to be associated with the destructive diseases of most economically important crops around the globe (Padidam *et al.*, 1999; Amin *et al.*, 2006). This group of viruses has a high ability for genomic variation independently from their host; however, at the same time, they reveal great geographical dependence. The presence of a diverse begomovirus species in one region helps to exchange their genetic material by recombination and to emerge as outbreaks in formerly unaffected crops (Saunders *et al.*, 2001; 2002). The results presented here show that radish is the host of two different species of begomovirus, TYLCV-OM and ChLCV-OM, and thus can provide a platform for these viruses to recombine, which may lead to new strains of the viruses. A detailed study is warranted in order to understand all the factors that play important roles in the synergistic interaction of TYLCV-OM and ChLCV-OM

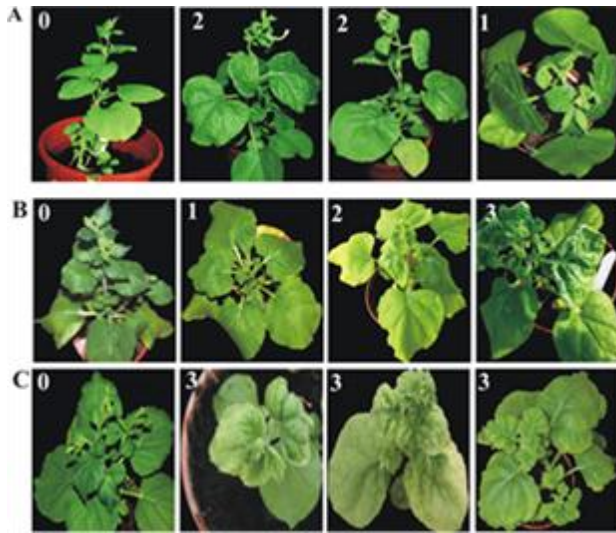
Several horticultural crops that form a major dietary component in these areas were introduced during colonial rule in Asia and Africa. These introduced crops are often found to be highly susceptible to the most prevalent begomoviruses, where they were introduced. Tomato, chilies, cassava, cotton and tobacco are among the crops affected by numerous begomoviruses.



**Fig. 2:** Dendrogram is based on the alignments of selected begomovirus sequences. Branches are arbitrary, and the values at the nodes indicate their percentage bootstrap values (1000 replicates). The begomovirus tree was arbitrarily rooted on the DNA A component of the *Tomato golden mosaic virus* (TGMV), a distantly related *Begomovirus*. In the virus tree, the positions of the TYLCV-OM, ToLCABV, and ChLCV-OM strains are indicated. The sequences produced as part of this study are in the shaded square

Therefore, the introduction of susceptible crops has resulted in the mobilization of indigenous begomoviruses. It seems that radish in Oman is also behaving similarly, as it is a host of two distinct species of viruses, TYLCV-OM and ChLCV-OM. The introduction of resistant varieties in an endemic area on the other hand, may select for resistance, breaking the strains (Amin *et al.*, 2006).

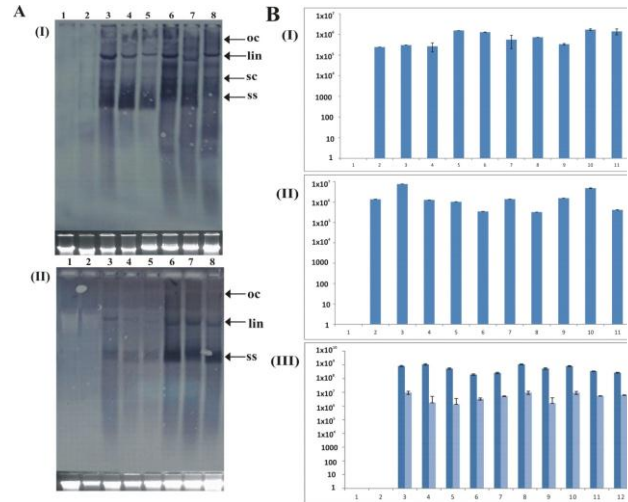
The increase in the symptom severity and the accumulation of viral DNA can be correlated by a synergism between the two geminiviruses infecting tomato crops. Recently, we have detected *Tomato leaf curl Barka virus* (ToLCBrV), which is the recombinant between *Tomato leaf curl Oman virus* (ToLCOMV) and *Croton yellow vein virus* (CrYVV) (Al-Shihi *et al.*, 2014). These



**Fig. 3:** The symptoms induced by the TYLCV-OM and the ChLCV-OM clones in the *N. benthamiana* plants. The plants in panel I are healthy non-inoculated *N. benthamiana* plants. (A) *N. benthamiana* plants inoculated with an agro-infectious clone of the TYLCV-OM. (B) *N. benthamiana* plants inoculated with an agro-infectious clone of the ChLCV-OM. (C) *N. benthamiana* plants inoculated with an agro-infectious clone of the combination of both the TYLCV-OM and the ChLCV-OM. Photographs were taken at 30 dpi. Severity scale: 0=No symptom, 1=slight leaf curl and yellowing, 2=Mild leaf curl, yellowing, 3=severe leaf curl, yellowing and stunting

viruses share same vector (whitefly) and thus the chance of the transmission of co-existing viruses increases, as the synergism among the virus species increases the amount of both viruses in the systemically infected leaves. As a result, crops which are infected by more than one virus species or by mixed infection, have a potential source of inoculum for both viruses, and whiteflies (Chakraborty *et al.*, 2008). Our findings here have shown that radish in Oman is such a host that can harbor two viruses and thus can act as a reservoir of viruses.

Plants respond to viruses by triggering the RNAi pathway, which is a natural defense response to silence invading viruses (Baulcombe, 2004). However, the viruses, as a counter defense, possess genes that can code proteins to suppress the RNAi pathway. These proteins are referred to as suppressors of gene silencing. Usually viruses encode multiple suppressors that can silence the RNAi pathway at multiple places (Vanitharani *et al.*, 2004; Amin *et al.*, 2011). In synergistic interactions, these suppressors of gene silencing play an important role. In such interactions a suppressor encoded by one virus can be used by the other virus, and thus result in its increase in accumulation. The V2 protein of the TYLCV- CN (china) has been shown to be a



**Fig. 4:** Panel A southern detection of (I) ChLCV-OM and (II) TYLCV-OM in inoculated *N. benthamiana* plants. The samples loaded on the gel in panel A (I) are the total DNA extracted from *N. benthamiana* plant inoculated with ChLCV-OM (lane 3-5). A total DNA extracted from healthy plant is loaded in lane 1 whereas plant inoculated with TYLCV-OM clone was loaded in lane 2 as a hybridization control. Panel A (II) includes the plants inoculated with TYLCV-OM (lane 3-5). Healthy genomic DNA was loaded in lane 1 whereas; ChLCV-OM inoculated plant was loaded in lane 2. The combination of ChLCV-OM/TYLCV-OM inoculated plants was loaded in lanes 6-8 in both panels AI and AII). Approximately equal amounts of the total DNA (10µg) were loaded in each case. The positions of the viral circular (oc), linear (lin), single stranded (SS), and supercoiled (SC) DNA forms are indicated. Panel B represents the real-time quantitative PCR analyses of the *N. benthamiana* plants inoculated with the ChLCV-OM (I), the TYLCV-OM (II), and the combination of ChLCV-OM/TYLCV-OM (III). Dark blue bars represent plants inoculated with TYLCV-OM, whereas light bars represent plants inoculated with ChLCV-OM

suppressor of gene silencing (Zhang *et al.*, 2012). Thus, there is a possibility that the same protein of the TYLCV-OM may be a suppressor of gene silencing and help the ChLCV-OM to accumulate at a higher rate. However, this hypothesis needs to be confirmed by further detailed studies.

Our results confirmed that leaf curl disease of radish in Oman is caused by mixed infection of TYLCV-OM and ChLCV-OM. Both viruses have a synergistic interaction, which results in the increase of symptoms and virus accumulation. We have demonstrated by qPCR that the copy number of both viruses increased by two orders of magnitude upon the mixed infection in *N. benthamiana*. TYLCV-OM is a major threat to the Omani agricultural crops, and the results presented here show that this virus can infect radish and to our knowledge this is first report of TYLCV-OM infecting radish. Moreover, our results have shown a synergistic interaction between TYLCV-OM and

ChiLCV-OM in a model host *N. benthamiana*. It is thus necessary to monitor the spread of these viruses regularly. With the diversity of viruses reported earlier in Oman, and the present evidence of synergism, further recombination and increased host range is a possibility.

## Acknowledgments

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