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Identification and characterization of a mechanical transmissible begomovirus causing leaf curl on oriental melon

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Abstract Oriental melon plants, *Cucumis melo* var. *makuwa* cv. Silver Light, showing virus-induced symptoms of mosaic, leaf curl and puckering were observed in the fields of eastern Taiwan in 2007. A virus culture, designated as SL-1, isolated from the diseased melon was established in systemic host plants, *Nicotiana benthamiana* and oriental melon, by mechanical inoculation. SL-1 did not react to the antisera against common cucurbit-infecting RNA viruses. Viral DNAs extracted from the diseased plant were amplified with the degenerate primers for begomoviruses. The full-length genomic DNA-A and DNA-B of SL-1 were sequenced and found to be closest, with 97.7% and 90.6% nucleotide identity, respectively, to *Tomato leaf curl New Delhi begomovirus* (ToLCNDV) cucumber isolate from a group of cucurbit-infecting begomoviruses. The virus SL-1 was designated as ToLCNDV oriental melon isolate (ToLCNDV-OM). The pathogenicity of ToLCNDV-OM was confirmed by agroinfection. Progeny virus from the agroinfected *N. benthamiana* plants was able to infect oriental melon by mechanical inoculation and caused symptoms similar to the original diseased melon in the field. The ToLCNDV-OM also infected five other species of cucurbitaceous plants by mechanical inoculation. This is the first report of a new ToLCNDV isolate causing severe disease on oriental melon in Taiwan.

Keywords Tomato leaf curl New Delhi virus · Cucurbit-infecting geminivirus · Mechanical transmission

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Introduction

The *Geminiviridae* is characterized by viruses with monopartite or bipartite circular, single-stranded DNA genomes encapsidated in geminate particles. Geminiviruses infect a broad range of monocotyledonous and dicotyledonous plants and have caused adverse effects on crops worldwide. Based on genome organization, host range and insect vector, geminiviruses have been classified into four genera: *Mastrevirus*, *Curtovirus*, *Topocuvirus* and *Begomovirus*. Begomoviruses are the largest group of geminiviruses with more than 180 species identified (Fauquet et al. 2008).

Begomoviruses are transmitted by whiteflies and infect economically important crops causing stunting of plants, curling and yellowing of leaves, and low yield of fruits. Several begomovirus species infecting cucurbitaceous plants have been characterized. *Squash leaf curl virus* (SLCV) found in the south-western desert of the USA (Cohen et al. 1983) was the first recorded cucurbit-infecting begomovirus. Subsequently, cucurbit-infecting begomoviruses have become a serious problem in tropical and subtropical areas, including *Watermelon chlorotic stunt virus* (WmCSV) in Yemen (Jones et al. 1987), *Cucurbit leaf curl virus* (CuLCuV) in western USA and northern Mexico (Brown et al. 2000), *Melon chlorotic leaf curl virus*-Guatemala (MCLCuV-GT) in Central America (Brown et al. 2001), *Squash leaf curl Philippines virus* (SLCPHV) and *Luffa yellow mosaic virus* (LYMV) in Philippines and Vietnam (Revill et al. 2003), *Squash leaf curl China virus* (SLCCNV) and *Squash leaf curl Yunnan virus* (SLCYNV) in China (Hong et al. 1995; Xie and Zhou 2003), SLCPHV in Taiwan (Tsai et al. 2007), and *Tomato leaf curl New Delhi virus* (ToLCNDV) in Thailand and Pakistan (Ito et al. 2008; Tahir and Haider 2005).

ToLCNDV is a bipartite begomovirus with two approximately 2.7 kbp DNA genomic components, designated as DNA-A and DNA-B. Although ToLCNDV was first discovered on tomato, it is also reported to cause extensive damage in cucurbitaceous plants. Several new disease reports revealed that ToLCNDV caused severe symptoms on bitter gourd (Tahir and Haider 2005), bottle gourd (Ito et al. 2008), cantaloupe (Samretwanich et al. 2000a), cucumber (Samretwanich et al. 2000b), muskmelon (Samretwanich et al. 2000d), watermelon (Mansoor et al. 2000), and wax gourd (Samretwanich et al. 2000c), in Thailand or Pakistan. Most ToLCNDV isolates were only transmitted by whiteflies naturally while only the potato isolate has been shown to be mechanically transmitted to its original host (Usharani et al. 2004).

In this study, a mechanically transmissible ToLCNDV isolate causing leaf curl symptoms on oriental melon was isolated and characterized and its pathogenicity and mechanical transmissibility were analyzed by agroinfection and mechanical inoculation. This is the first report of a ToLCNDV isolate causing severe disease on oriental melon in Taiwan.

Materials and methods

Virus source and isolation

A diseased plant of *Cucumis melo* var. *makuwa* cv. Silver Light showing mosaic, leaf curling and puckering symptoms was collected from the field in April, 2007. *Chenopodium quinoa*, *C. amaranticolor*, *Nicotiana benthamiana* and oriental melon plants were mechanically inoculated using 10-fold dilution of the inoculum prepared by grinding leaf tissue of diseased melon in phosphate buffer (0.1 M potassium phosphate buffer, pH 7.0). Mosaic and leaf curling symptoms were observed on *N. benthamiana* and oriental melon plants but no symptoms were observed on *C. quinoa* and *C. amaranticolor*. Three series of end-point diluting inoculation of whole plant assay (Brakke 1970) were carried out on the systemic host oriental melon plants to isolate the virus. A virus culture SL-1 was established in oriental melon and *N. benthamiana* plants for further assays.

Indirect-enzyme-linked immunosorbent assay (indirect-ELISA)

Indirect-ELISA was used for detecting the presence of possible cucurbit-infecting RNA viruses as described by Yeh and Gonsalves (1984). Crude sap of diseased oriental melon leaf from the field and symptomatic leaf tissue of isolate SL-1 infected *N. benthamiana* and oriental melon plants were analyzed by indirect-ELISA using antisera against *Cucumber green mottle mosaic virus* (CGMMV), *Cucumber mosaic virus* (CMV), *Papaya ringspot virus* type-W (PRSV-W), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus* (WMV), *Watermelon silver mottle virus* (WSMoV) or *Zucchini yellow mosaic virus* (ZYMV).

Polymerase chain reaction (PCR) and sequence analysis

Total DNA was extracted from diseased tissue using a modified microprep method (Jan et al. 2000) and the isolated DNA was used in PCR amplification. Begomoviral DNA was amplified using degenerate primer pairs PAL1v1978-PAR1c715 for DNA-A (Gilbertson et al. 1991a) and BLV2-BLC1 for DNA-B (Green et al. 2001). PCR amplified products of partial begomoviral DNA-A and DNA-B were cloned into

pCRII-TOPO vector following the manufacturer's instructions (Invitrogen). The DNA clones were sequenced on an automatic DNA sequencer ABI PRISM 3730 (Applied Biosystems) at the Biotechnology Center, National Chung Hsing University, Taiwan. Using the sequence obtained, primer pairs FJJ2007-13 (AAGCTCTAGAACGTCTCCGTCTTTGTCG)-FJJ2007-14 (GACCTCTAGAATGGGGTGTTTTCCAGAT) for DNA-A and FJJ2007-15 (TGTGAATTCCGCTTGTTT)-FJJ2007-16 (GAATTCACAATTCCGATC) for DNA-B were designed for full-length begomoviral DNAs amplification. PCR amplified full-length DNA products were cloned and sequenced as above. Sequence searches were done with the BLAST database program (http://www.ncbi.nlm.nih.gov/BLAST) and sequence analyses were performed using the Clustal V algorithm of the DNASTAR MegAlign software (DNASTAR, Inc.). Phylogenetic analysis was done with Clustal W and the Neighbor-joining method using the MEGA4 software (Tamura et al. 2007). Begomovirus sequences used in these analyses are listed in Table 1.

Construction of infectious clones and agroinfection

Tandemly repeated infectious DNA clones of the SL-1 isolate were constructed by rolling circle amplification (RCA) as described by Wu et al. (2008) with some modification. Total DNA was isolated from tissue of SL-1 infected melon and the circular genomic DNAs were amplified by RCA using the TempliPhi Amplification kit (Amersham Pharmacia). The RCA products were subjected to limited digestion by restriction enzyme *Xba*I. The digested RCA products corresponding to dimeric begomovirus genomic DNAs approximately 5 to 6 kbp were eluted and cloned into a Ti-plasmid, pGANP (unpublished). The tandemly repeated constructs of DNA-A and DNA-B were differentiated by restriction enzyme digestion and individually designated as pGPhi-ToLCNDV-2A and pGPhi-ToLCNDV-2B. The accuracy of the DNA clones pGPhi-ToLCNDV-2A and pGPhi-ToLCNDV-2B were confirmed by sequencing and then transferred into *Agrobacterium tumefaciens* LBA4404 separately. Agroinfection was used to assay the infectivity of DNA clones of SL-1. Forty-eight hour cultures of agrobacteria containing pGPhi-ToLCNDV-2A or pGPhi-ToLCNDV-2B were mixed and co-inoculated by injecting into petioles of *N. benthamiana* and oriental melon plants using a 23-gauge needle. Progeny virus from agroinfected plants was subsequently mechanically inoculated to 48 *N. benthamiana* and 32 oriental melon plants for assaying the infectivity efficiency.

Infectivity and host reaction assay with mechanical inoculation

To characterize the biological properties and mechanical transmissibility of isolate SL-1, plants from 21 species representing 4 families were mechanically inoculated with the crude sap from agroinfected- or mechanically inoculated *N. benthamiana* plants. Inoculated plants were kept in a greenhouse with insect screens for observing symptom development for at least 35 days after inoculation. All inoculated-plants were indexed by PCR using primer pairs FJJ2007-38 (CCCAGCGTGACTGGCAAAGC)-FJJ2007-44 (CCAGCAGATATCATCATTTC) for DNA-A and FJJ2007-50 (CGTTCGAAAGTCGGATGTGA)-FJJ2007-56 (TCCGTCCTTCTGTGTTTCTG) for DNA-B.

Results

Virus source and isolation

Symptomatic oriental melon leaves exhibiting mosaic, leaf curl and puckering virus-like symptoms were observed in the field in 2007 (Fig. 1a). ELISA tests of the field-infected melon tissue gave negative reactions to the antisera against the seven common cucurbit-infecting RNA viruses used. However, DNA fragments of approximately 1.5 kbp and 2.5 kbp were amplified from total DNA isolated from the diseased melon by PCR with the degenerate primer pairs for begomoviral DNA-A and DNA-B, respectively (data not shown). These results indicated that the virus causing the melon leaf curl might be a begomovirus.

Symptoms of mosaic and leaf curling were observed on *N. benthamiana* (Fig. 1b) and oriental melon plants (Fig. 1c) at 12 days post-inoculation but no symptoms were observed on *C. quinoa* and *C. amaranticolor* plants at least 30 days post-inoculation. A virus culture SL-1 obtained by end-point diluting inoculation was established and maintained on *N. benthamiana* and oriental melon plants. The isolated SL-1 was also checked by PCR for the presence of begomovirus and

Table 1 List of begomoviruses used in sequence analyses

Viruses	Accession numbers		Acronym
	DNA-A	DNA-B	
Cucurbit leaf curl virus-[USA:Imperial Valley]	AF224760		CuLCuV-IV
Cucurbit leaf curl virus-[Arizona]	AF256200		CuLCuV-Az
Luffa yellow mosaic virus-[Vietnam]	AF509739		LYMV
Melon chlorotic leaf curl virus-[Guatemala]	AF325497		MCLCuV-GT
Squash leaf curl China virus-[China]	AB027465		SLCCNV-CN
Squash leaf curl China virus-[Vietnam:B]	AF509743		SLCCNV-B
Squash leaf curl China virus-[Vietnam:K]	AF509741		SLCCNV-K
Squash leaf curl China virus-[China:Hainan61]	AM260205		SLCCNV-Hn
Squash leaf curl China virus-[India:Coimbatore:Pumpkin]	AY184487		SLCCNV-Coi
Squash leaf curl China virus-[India:Lucknow:Pumpkin]	DQ026296		SLCCNV-Luc
Squash leaf curl Philippines virus-[Philippines:Munoz]	AB085793		SLCPHV-PH
Squash leaf curl Philippines virus-[Taiwan:Pumpkin]	DQ866135		SLCPHV-TW
Squash leaf curl virus-[USA:Imperial Valley]	M38183		SLCV-IV
Squash leaf curl virus-[Cairo]	DQ285019		SLCV-Cai
Squash leaf curl Yunnan virus-[China:Yunnan]	AJ420319		SLCYNV
Squash mild leaf curl virus-[USA:Imperial Valley]	AF421552		SMLCV
Squash yellow mild mottle virus-[Costa Rica]	AY064391		SYMMoV
Watermelon chlorotic stunt virus	AJ012081		WmCSV
Watermelon chlorotic stunt virus-[Iran]	AJ245652		WmCSV-IR
Tomato leaf curl New Delhi virus-[India:New Delhi:Mild]	U15016		ToLCNDV-Mld
Tomato leaf curl New Delhi virus-[India:New Delhi:Severe]	U15015	U15017	ToLCNDV-Svr
Tomato leaf curl New Delhi virus-[Pakistan:Solanum:1997]	AJ620187		ToLCNDV-Sn
Tomato leaf curl New Delhi virus-[Pakistan:Lahore]	AM258977		ToLCNDV-Lah
Tomato leaf curl New Delhi virus-[Chili pepper]	DQ116880		ToLCNDV-Chi
Tomato leaf curl New Delhi virus-[Potato]	AY286316	AY158080	ToLCNDV-Pot
Tomato leaf curl New Delhi virus-[India:Happur:Potato]	EF043230		ToLCNDV-Hap
Tomato leaf curl New Delhi virus-[India:Meerut:Potato]	EF043231		ToLCNDV-Mee
Tomato leaf curl New Delhi virus-[Pakistan:Multan:Luffa]	AM292302		ToLCNDV-Mul
Tomato leaf curl New Delhi virus-[Thailand:Luffa]	AF102276		ToLCNDV-Luf
Tomato leaf curl New Delhi virus-[Thailand:Bottle gourd]	AB368447		ToLCNDV-Bot
Tomato leaf curl New Delhi virus-[Thailand:Cucumber]	AB330079	AB330080	ToLCNDV-Cuc
Tomato leaf curl New Delhi virus-[Thailand:Muskmelon]	AB368448		ToLCNDV-Mus
Tomato leaf curl New Delhi virus-[Taiwan:oriental melon]	GU180095	GU180096	ToLCNDV-OM

for the absence of RNA viruses by ELISA. These results indicated that the cultured SL-1 virus could be a mechanically transmissible begomovirus.

Viral DNA sequence analysis

BLAST analysis of the partial sequences of the 1.5-kbp DNA-A and 2.5-kbp DNA-B fragments showed 91–97% and 80–92% nucleotide sequence identities, respectively, with those of ToLCNDV isolates. Full length DNA-A and DNA-B amplification was achieved with the two primer pairs, FJJ2007-13-FJJ2007-14 and FJJ2007-15-FJJ2007-16, respectively. DNA-A of SL-1 virus was 2,739 nucleotides in length, containing six ORFs typical of other ToLCNDV, including two ORFs, AV1 (771 nt), and AV2 (339 nt), in viral sense and four ORFs, AC1 (1,086 nt), AC2 (405 nt), AC3 (387 nt), and AC4 (177 nt), in

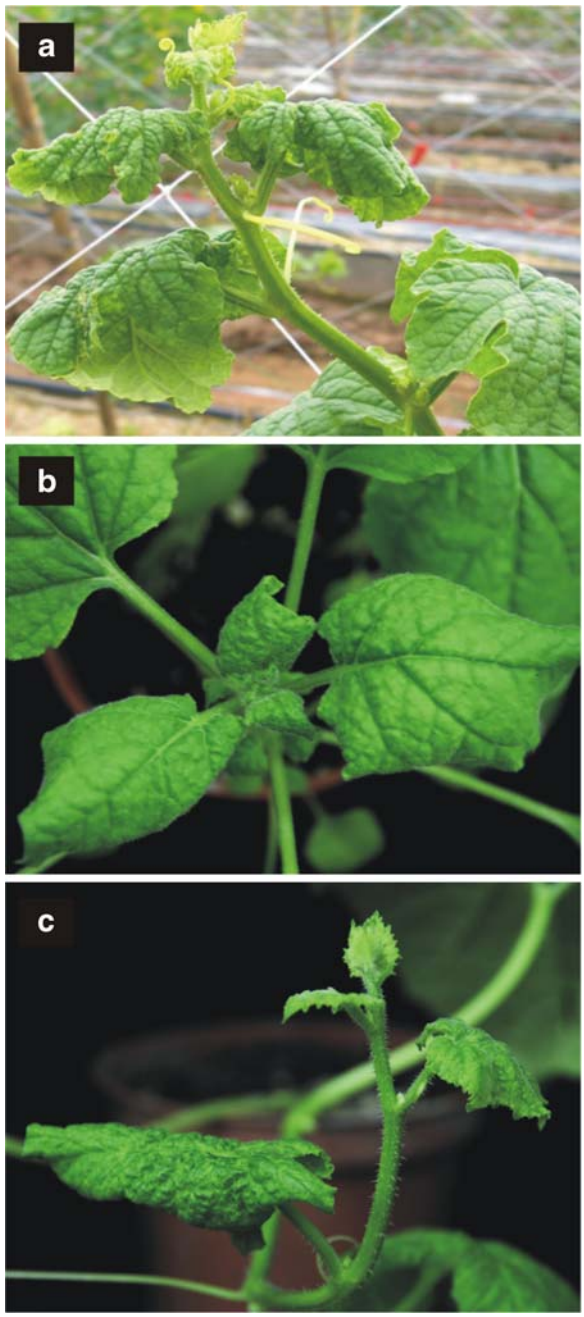

Fig. 1 Virus induced symptoms on oriental melon (*Cucumis melo* var. *makuwa* cv. Silver Light) and *Nicotiana benthamiana*. (**a**) Field-infected oriental melon, and (**b**) SL-1 virus mechanically inoculated *N. benthamiana* and (**c**) oriental melon, showing symptoms of mosaic, leaf curl and puckering

complementary sense. DNA-B of SL-1 isolate was 2,673 nucleotides in length, containing two ORFs, including BV1 (807 nt) in viral sense and BC1 (846 nt) in complementary sense. A common region (CR) of 158 nucleotides with 91% identity was also identified in the intergenic regions of DNA-A and DNA-B. An identical stem-loop region containing conserved nanonucleotides-TAATATTAC (Ikegami et al. 1988) was found in the CR. The iterative sequence (iteron)-GGCGTC, which is the putative binding site of the replication initiator protein (Rep), was also found in the CR.

The DNA-A sequence of SL-1 showed 41.8–97.7% nucleotide identity with those of 24 cucurbit-infecting begomoviruses in the GenBank database. It shared more than 92% nucleotide identity to those of ToLCNDV cucurbit isolates and had the highest identity (97.7%) with that of the ToLCNDV cucumber isolate (ToLCNDV-Cuc, AB330079). AV1 and AC1 ORFs of SL-1 shared 99% and 98% amino acid identity, respectively, with those of ToLCNDV-Cuc. Four other ORFs on the DNA-A of SL-1 also showed more than 95% amino acid identity with those of ToLCNDV-Cuc. The DNA-B of SL-1 isolate had the highest (90.6%) nucleotide identity with that of ToLCNDV-Cuc (AB330080). The BV1 and BC1 ORFs of SL-1 shared 90% and 97% amino acid identity, respectively, with those of ToLCNDV-Cuc. Among the eight ORFs of SL-1, BV1 showed the lowest amino acid identity to that of ToLCNDV-Cuc. When compared with biologically different isolates of ToLCNDV, the BV1 of SL-1 revealed a distinguishable difference in length (Fig. 2). The BV1 N terminal sequence of SL-1 was 28 and 13 amino acids shorter than the BV1 ORFs of two non-mechanically transmissible isolates, ToLCNDV-Cuc and ToLCNDV-Svr (Samretwanich et al. 2000b; Hussain et al. 2005) but equivalent in length to those of a mechanically transmissible isolate ToLCNDV-Pot (Usharani et al. 2004).

In the phylogenetic analysis of the DNA-A of 25 cucurbit-infecting begomoviruses, the SL-1 isolate clustered with cucurbit-infecting ToLCNDV isolates (Fig. 3). Based on begomovirus species demarcation criteria (Fauquet et al. 2008), the outcome of sequence alignments and phylogenetic analysis, SL-1 is an isolate of ToLCNDV named ToLCNDV-OM (ToLCNDV oriental melon isolate).

Pathogenicity and mechanical transmissibility assay with agroinfection

To illustrate the pathogenicity and mechanical transmissibility of ToLCNDV-OM, the tandemly-repeated genomic clones, pGPhi-ToLCNDV-2A and pGPhi-

Fig. 2 Comparison of the amino acid sequences of nuclear shuttle proteins between the mechanically and non-mechanically transmissible isolates of *Tomato leaf curl New Delhi virus* (ToLCNDV). Amino acid sequence of nuclear shuttle protein of ToLCNDV-OM was compared with those of mechanically transmissible ToLCNDV-Pot and non-mechanically transmissible ToLCNDV-Svr and ToLCNDV-Cuc isolates. The box indicates distinct difference between mechanically and non-mechanically transmissible ToLCNDV isolates. See Table 1 for virus accession numbers

ToLCNDV-2B for the DNA-A and DNA-B, respectively, were co-introduced into *N. benthamiana* and oriental melon by agroinfection. Agroinfection induced symptoms of mosaic, leaf curl and puckering on *N. benthamiana* at 12 days post-inoculation but not on oriental melon. However, the progeny virus from agroinfected *N. benthamiana* was successfully transmitted to oriental melons by mechanical sap-inoculation. In the assay of infection efficiency, all 48 tested *N. benthamiana* and 30 out of 32 oriental melon plants were infected by the progeny virus from agroinfected-*N. benthamiana* by mechanical inoculation. The sap-inoculated melons showed the same symptoms of mosaic, leaf curl and puckering as field-infected melon, at 12 days post-inoculation with additional yellowing symptom at 20 days post-inoculation (Fig. 4a). ToLCNDV-OM was also successfully mechanically transmitted to oriental melon from infected melon plants. These results indicate that ToLCNDV-OM is a mechanically transmissible isolate and is the causal agent of the mosaic, leaf curl and puckering symptoms on oriental melon in the field.

Infectivity and host reaction assay with mechanical inoculation

Test plants inoculated with sap from viral progeny originated from agroinfection showed a similar reaction to those inoculated with sap from the field-isolated SL-1 virus culture. Five out of 21 plant species were susceptible to ToLCNDV-OM by mechanical inoculation. No symptoms were observed and no infection detected on the other 17 tested plant species, including *C. amaranticolor*, *C. quinoa*, *C. murale*, *Capsicum annuum* (red pepper), *C. annuum* var. *grossum* (sweet pepper), *Solanum lycopersicum*, *N. tabacum*, *N. occidentalis*, *N. edwardsonii*, *Datura stramonium*, *Phaseolus vulgaris*, *Vigna mungo*, *V. radiata*, *V. unguiculata*, *Citrullus lanatus*, *C. melo* var. *reticulatus* (muskmelon), *C. metuliferus* and *Cucurbita moschata*. Susceptible plants of the 5 cucurbit species showed symptoms at approximately 13–17 days post-inoculation. Severe mosaic, leaf curl and puckering symptoms were observed on oriental melon (*C. melo* var. *makuwa* cv. Silver Light), pickling melon (*C. melo* var. *conomon* cv. Silver Charm) (Fig. 4b), bottle gourd (*Lagenaria siceraria*) (Fig. 4e) and 5 cultivars of cucumber (*C. sativus*) (Fig. 4c). Symptoms of mosaic, leaf curl and mild puckering were observed on zucchini squash (*C. pepo* var. *zucchini*) (Fig. 4d) and loofah (*Luffa cylindrica*) (Fig. 4f). The presence of viral DNA in all symptomatic plants was confirmed by PCR.

Discussion

At least seven RNA viruses (Chen et al. 2008; Huang et al. 1993) including CGMMV, CMV, *Melon vein-banding mosaic virus*, *Melon yellow spot virus*, PRSV-W, WSMoV and ZYMV, and one DNA virus, SLCPHV (Tsai et al. 2007) have been reported to infect cucurbit plants in Taiwan. New virus-like symptoms of mosaic, leaf curl and puckering were observed on *C. melo* var. *makuwa* cv. Silver Light plants in the field during the spring season of 2007. Based on the high nucleotide sequence identity (97.7%

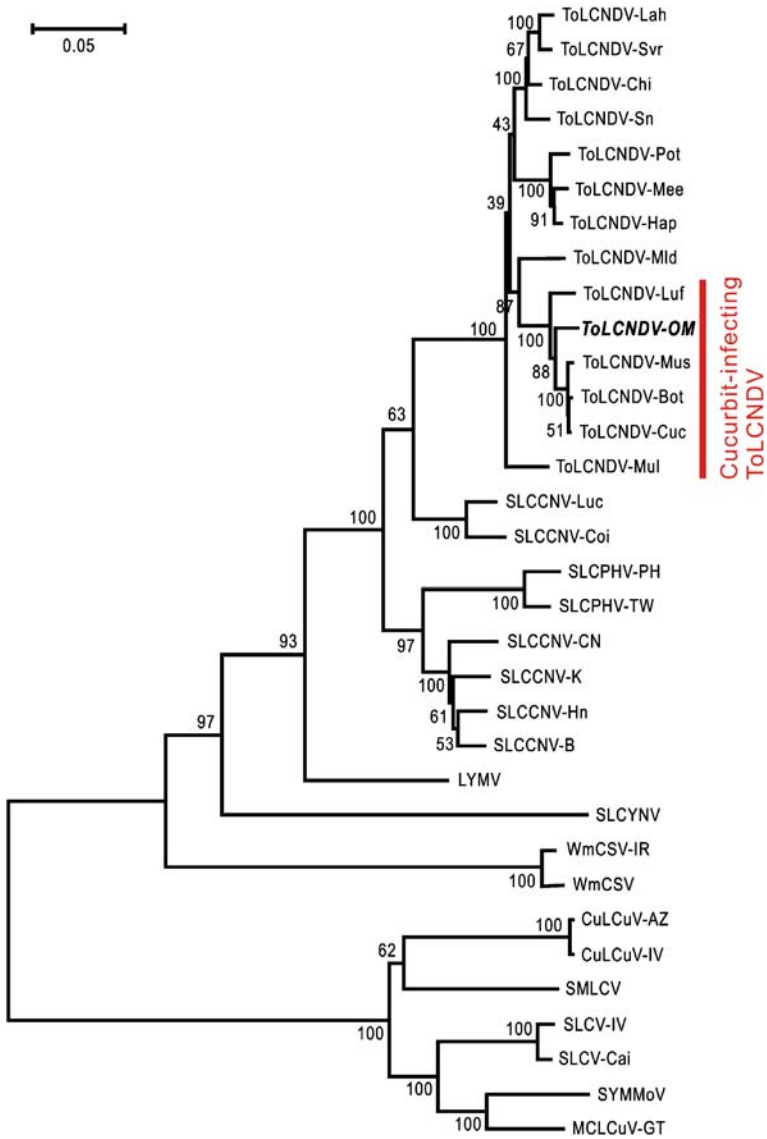

Fig. 3 Phylogenetic relationships of the DNA-A component of cucurbit-infecting begomoviruses and different isolates of *Tomato leaf curl New Delhi virus*. Phylogenetic trees were constructed by Clustal W and Neighbor-joining method with 1,000 boot-straps using the MEGA4 software. The scale bar representing the genetic distance is indicated at the upper left. See Table 1 for virus accession numbers

of DNA-A and 90.6% of DNA-B with ToLCNDV-Cuc) and the demarcation criteria in species identification (Fauquet et al. 2008), the SL-1 virus isolated from the diseased melon is considered an isolate of ToLCNDV and was accordingly named ToLCNDV-OM (ToLCNDV oriental melon isolate). The pathogenicity of ToLCNDV-OM was also confirmed by mechanical inoculation of the progeny virions from the agro-infected *N. benthamiana* onto oriental melon causing symptoms similar to those occurring in the fields. This indicated that the mechanically transmissible ToLCNDV-OM is responsible for mosaic, leaf curl and puckering symptoms on melon in the field.

The mechanical transmissibility of ToLCNDV-OM is convenient because it allows for host range assays without the necessity of whitefly breeding. ToLCNDV has been reported to infect many natural hosts, such as bitter gourd (Tahir and Haider 2005), bottle gourd (Ito et al. 2008), cantaloupe (Samretwanich et al. 2000a), chilli pepper (Hussain et al. 2004), cucumber (Samretwanich et al. 2000b), muskmelon (Samretwanich et al. 2000d), potato (Usharani et al. 2004), watermelon (Mansoor et al. 2000), wax gourd (Samretwanich et al. 2000c), and tomato (Padidam et al. 1995). In this study, several plant species which have been reported as natural hosts for other ToLCNDV isolates were infected by

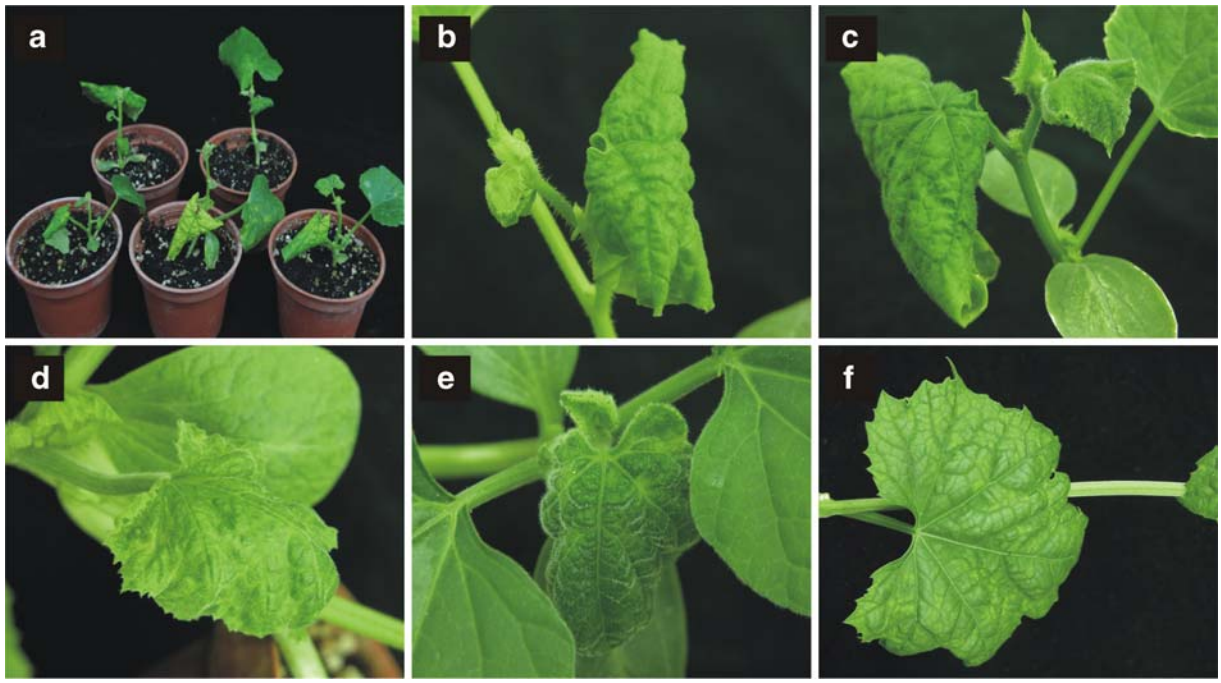

Fig. 4 Symptoms of cucurbitaceous plants mechanically inoculated with ToLCNDV-OM. Symptoms of mosaic, leaf curl and puckering were observed in ToLCNDV-OM-infected (**a**) oriental melon (*Cucumis melo* var. *makuwa* cv. Silver Light), (**b**) pickling melon (*C. melo* var. *conomon* cv. Silver Charm), (**c**) cucumber (*C. sativus*), (**d**) zucchini squash (*Cucurbita pepo* var. *zucchini*), (**e**) bottle gourd (*Lagenaria siceraria*), and (**f**) loofah (*Luffa cylindrica*)

ToLCNDV-OM with mechanical inoculation. Surprisingly, ToLCNDV-OM did not infect tomato plants, either by mechanical inoculation or agroinfection (data not shown). The muskmelon and pepper were also not infected by ToLCNDV-OM with mechanical inoculation. Although our results indicate that ToLCNDV-OM has a different host range from other reported ToLCNDV isolates, the same inoculation method should be applied to confirm the difference of host reaction. Interestingly, ToLCNDV-OM was obviously well adapted to cucurbit hosts according to the results of host reaction assays.

Many begomoviruses have been reported to be mechanically transmissible only to *N. benthamiana* plants. However, only a few begomoviruses, such as *Bean dwarf mosaic virus* (BDMV) (Morales et al. 1990), the Guatemala and Dominican Republic isolates of *Bean golden mosaic virus* (Gilbertson et al. 1991b), *Pepper golden mosaic virus* (PGMV) (syn. Texas pepper virus) (Stenger et al. 1990), and Watermelon curly mottle virus (WCMoV) (Brown and Nelson 1986) were shown to be mechanically transmissible to their original and/or other host plants. BDMV was sap-transmitted to bean plants with up to 100% efficiency (Morales et al. 1990), however, the mechanical transmission rate of PGMV to pepper was less than 20% (Stenger et al. 1990). WCMoV is a tentative species of cucurbit-infecting begomoviruses, which was reported to be mechanically transmissible to cantaloupe, cucumber, watermelon and zucchini squash with a 33–60% transmission rate (Brown and Nelson 1986). Although several ToLCNDV isolates have been identified, mechanical transmissibility to its original host has been proved only for the potato isolate (Usharani et al. 2004). In our study, the mechanical transmission rate of ToLCNDV-OM onto oriental melon is higher than 93%, indicating that ToLCNDV-OM is a cucurbit-infecting isolate with high mechanical transmission efficiency.

According to the high (97.7% and 90.6% to DNA-A and DNA-B, respectively) nucleotide identity and the illustration from the phylogenetic tree, ToLCNDV-OM is most closely related to the cucumber isolate, ToLCNDV-Cuc, from Thailand. Both isolates also shared more than 98% amino acid identity in AV1 (coat protein, CP) and AC1 (Rep) ORFs. However, a previous

study showed that ToLCNDV-Cuc was not mechanically transmissible to cucumber (Samretwanich et al. 2000b). In this study, we found that ToLCNDV-OM is mechanically transmissible and well adapted to 5 cucumber cultivars. It has been reported that ToLCNDV required both DNA-A and DNA-B for symptom development, but CP was not required for systemic infection and symptom development (Padidam et al. 1995). These findings suggest that CP is not the key element for the mechanical transmissibility of ToLCNDV-OM.

To investigate the possible determinants of the mechanical transmissibility of ToLCNDV-OM, its nucleotide and amino acid sequences of each ORF were compared with those in the non-mechanically transmissible isolates of ToLCNDV-Svr (Hussain et al. 2005) and ToLCNDV-Cuc, and the mechanically transmissible isolate ToLCNDV-Pot (Fig. 2). A difference, distinguishing the mechanically transmissible isolate from non-mechanically transmissible ones, in the length of the BV1 ORF (encodes for the nuclear shuttle protein, NSP) of these 4 ToLCNDV isolates was observed. The NSPs of the mechanically transmissible ToLCNDV-OM and ToLCNDV-Pot are 28 and 13 amino acids shorter in the N terminal region than ToLCNDV-Cuc and ToLCNDV-Svr, respectively (Fig. 2). Although we cannot exclude other possibilities, it is reasonable to consider that the loss of the NSP N terminus of ToLCNDV-OM may play a role in the mechanical transmissibility of this particular begomovirus. This could be confirmed by modifying the NSP region in the N terminus and assessing the possibility of mechanical transmission. In addition, the NSP, especially the first 60 amino acids of the N terminus, has previously been shown to play a key role in symptom development and as a virulence determinant (Hussain et al. 2005). The difference of the NSP N terminus may also account for other biological properties of these four ToLCNDV isolates originating from different hosts.

Begomoviruses extend their diversity through mutation, recombination and pseudorecombination, not only between the isolates or variants within the species but also between species (Varma and Malathi 2003). The adaptation to cucurbit plants and mechanical transmissibility of ToLCNDV-OM may allow this virus to cause a serious disease in the field. If mechanical transmissibility is transferred to other strains or begomoviruses via recombination, the disease-causing potential of these viruses will be more serious and complex.

ToLCNDV had not been characterized and recorded in Taiwan before our study. The environment and techniques for cucurbit cultivation in Thailand and Taiwan are similar. Combining this and results of sequence analysis in this study, it is possible that ToLCNDV-OM has been spread from Thailand either by insects, plant tissues or humans. The findings of the mechanical transmissibility and the host range difference of ToLCNDV-OM reported in this manuscript will help in understanding and managing the disease.

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