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Identification of *Squash leaf curl Philippines virus* on *Benincasa hispida* in Taiwan

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ABSTRACT

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During a disease survey of wax gourd (*Benincasa hispida* Cogn.) in Taiwan, virus-like symptoms of yellowing, vein enation, rugose mosaic and leaf curling were observed and further characterized. A naturally infected wax gourd sample (Wg1) was collected from experimental field at Beidou, Changhua County, Central Taiwan. The plant was tested negative with indirect ELISA using the antisera against eight RNA viruses commonly found in cucurbits, including *Cucurbit aphid-borne* yellows virus, *Cucumber green mottle mosaic virus*, *Cucumber mosaic virus*, *Melon vein-banding mosaic virus*, *Papaya ringspot virus*-watermelon type, *Watermelon silver mottle virus* and *Zucchini yellow mosaic virus*. However, it showed positive to a geminivirus, tentatively named isolate of *Squash leaf curl virus* Wg1 (SqLCV-Wg1), with polymerase chain reaction (PCR) using primer pair that is specific to begomovirus genomes. After cloning and sequencing, the PCR product was identified to be 1116 nucleotides (nt) in length, encompassing 126, 771, and 219 nt of the AV2, CP, and AC3 gene coding regions, respectively. Sequence alignment with the corresponding regions of other begomoviruses revealed that SqLCV-Wg1 is most closely related to *Squash leaf curl Philippines virus*, with percent nucleotide identities of 97.6, 97.7, and 93.8 for AV1, CP, and AC3 genes, respectively. The CP gene of SqLV-Wg1 was further cloned into the vector pET21d(+), which was then used to express proteins in *Escherichia coli* BL21(DE3) and to produce specific antiserum for future diagnosis purposes. This is the first record of cucurbitaceous plants infected with *Squash leaf curl virus* in Taiwan.

Key words: cucurbit, *Benincasa hispida*, *Squash leaf curl virus*, identification

INTRODUCTION

Benincasa hispida Cogn., commonly known as wax gourd or white gourd, is a fast-growing, long-season, warm-climate vegetable. The plant produces edible fruits with green peel and white pulp containing numerous small seeds, which can grow up to 50 pounds. The unopened fruits can be stored in a cool place for months and used later in winter. Thus, this gourd is called Tong Qwa, meaning "Winter Melon" in Chinese. Wax gourd is not only a flavor vegetable at the low price, but could also reduce weight for people of obesity and prevent development of ulcers [(9)]. According to Taiwan Agriculture Annual Report in 2005, the growing area of wax gourd has

been increased to about 1500 hectares, among which 600 hectares concentrating in Changhua County. In recent years, wax gourd cultivation has been increasing in the southern and central Taiwan.

During a disease survey of wax gourd in Taiwan, several diseased plants with virus-like symptoms such as yellowing, vein enation, rugose mosaic and leaf curling were discovered in fields at Beidou, Changhua County, Central Taiwan. The diseased plants showed growth decline. It has been documented in Taiwan that wax gourd is infected by several RNA viruses, including *Cucurbit aphid-borne yellows virus* (CABYV), *Cucumber green mottle mosaic virus* (CGMMV), *Cucumber mosaic virus* (CMV), *Melon vein-banding mosaic virus* (MVbMV), *Papaya ringspot virus* W type (PRSV-W), *Tomato spotted wilt virus* (TSWV), *Watermelon silver mottle virus* (WSMoV), and *Zucchini yellow mosaic virus* (ZYMV)[7, 11, 22]. However, none of these RNA viruses could induce the symptoms similar to those on the diseased wax gourd plants described as above..

The first case of DNA viruse infecting cucurbits was discovered in squash leaf curl disease[1] in Philippine in 1977. The causal agent, *Squash leaf curl virus*, was further identified through nucleotide sequence analysis in 1991[14]. Several begomoviruses have been reported in the Southeast Asia regions, such as "*Squash leaf curl virus-Vietnam*" and "Loofa yellow mosaic virus-Vietnam" infecting the cucurbits in Vietnam[18], as well as "*Tomato leaf curl virus*" causing a yellow leaf disease of cantaloupe and wax gourds from Thailand[19]. As for DNA viruses on cucurbits[4, 5, 10], the geminivirus infecting wax gourd has not yet been reported in Taiwan.

In this study, we described the identification, molecular characterization, and phylogenetic analysis of a SqLCV associated with the new disease in wax gourds in Taiwan. This pathogen was tentatively named *Squash leaf curl virus* Wg1 isolate (SqLCV-Wg1). The specific antiserum against SqLCV-Wg1 was also prepared for rapid diagnosis of SqLCV-Wg1. This is the first report associated with the detection and molecular characterization of a SqLCV in Taiwan.

MATERIALS AND METHODS

Field observation and virus source

In the summer of 2005, a disease survey of wax gourd was conducted in the experimental field at Beidou, Changhua County, Central Taiwan. The naturally infected plants showing virus-like symptoms were collected and tested by indirect ELISA using specific antisera aginst CABYV, CGMMV, CMV, MVbMV, PRSV-W, TSWV, WSMoV or ZYMV. Virus cultures were maintained in wax gourd plants. Inoculations were done with whitefly vector [*Bemisia tabaci* (Gennadius)].

Polymerase chain reaction (PCR)

The forward and reverse specific primers gen-793-f (5' gtgttcatggtctacggtg 3') and gen-793-r (5' aaccacaacctgcggaaag 3')[3, 12] for amplification of geminivirus CP gene were used in PCR. Total DNAs were extracted from infected wax gourds using commercial kit (Viogene, Plant DNA Extraction Miniprep System,TW) according to the manufacturer's instructions. Amplification of the DNA was performed in a volume of 50 μl of reaction mixture containing: 3 μl DNA template, 5 μl 10x PCR buffer, 4 μl of 2.5 mM dNTP, 0.1 μl Ex-Taq Polymerase (Takara Shuzo Co., Shiga, Japan), 2.5 μl of 20 mM gen-793-f and 2.5 μl of 20 mM gen-793-r primers. Thirty-five cycles of PCR were performed using thermal cycler (Biometra Uno-thermoblock, Biometra biomedizinische Analytik GmbH) with denaturation at 94 ℃ for 2 min, primer annealing at 58 ℃ for 1 min, and DNA synthesis at 72 ℃ for 1-min 20-sec followed by a final elongation step at 72 ℃ for 8 min[13]. Eight μl of amplified products were analyzed by electrophoresis in 1 % agarose gel in TAE buffer. Bands were visualized by ethidium bromide staining.

Cloning and sequence analyses

The amplified DNA products were cloned into dT&A cloning vector and then transformed to *E. coli*. ECOS competent cell (Yeastern Biotech Co., Ltd,TW) according to the instructions provided by the manufacturer. DNA sequences of the correct clones were confirmed by an automatic DNA sequencing system (ABI 3730 XL DNA Analyzer, Applied Biosystems, CA). The deduced amino acid sequences were analyzed using the Translate program of the GCG software (GCG Wisconsin Package, Accelrys Inc., San Diego, CA). MegAlign program (DNASTAR, Inc) was used for pairwise and multiple sequence alignments of DNA or protein, and to create phylogenetic trees as well as reports and tables showing the numerical data underlying the comparisons. Sequences of other related cucurbit-infecting begomoviruses for comparisons were obtained from the EMBL Database under the following accession numbers: *Squash leaf curl Philippines virus*, AB085793.1; *Squash leaf curl China virus*, AF509741.1; *Chinese squash leaf curl virus*, S77090.1; *Tomato leaf curl New Delhi virus*, U15015.2, as described by Kon et al[12].

Expression of coat protein (CP) gene

The specific primers, gen-exp-f (5'-accatggcgaagcgaccagccgatatt 3') and gen-exp-r (5' cccggatccgaatcataaaaatagatc 3'), corresponding to the 5'- and 3'-termini of CP gene of SqLCV-Wg1 isolate was used to amplify the CP gene. Primers also contained *Nco*I and *Bam*HI restriction sites as non-template overhangs for cloning purposes. The reaction was carried out in 35 cycles of 45sec denaturation at 94℃, 45 sec annealing at 55℃ and 1 min 20 sec elongation at 72℃. The PCR products were directly cloned to dT&A cloning vector . The plasmid pET21d (+) was used for subcloning the PCR product above-mentioned, and expression of the CP gene of SqLCV-Wg1 (Wg1-CP) in *E. coli* BL21 (DE3) in Luria-Bertani (LB) medium [21]. A 5-ml overnight culture of BL21(DE3) cells containing plasmids was added to 50 ml of LB and the culture was grown to an optical density at 600 nm (OD_{600}) of 0.6, and then induced with isopropyl-1-thio-beta-D-galactoside (IPTG, Serva, Heidelberg, Germany) in a final concentration of 0.2 mM. The culture was than incubated for 3 h at 37℃ [15, 17]. Bacterial cells were harvested by centrifugation and stored at - 80 ℃ until used.

Preparation of CP-enriched fractions

Wg1-CP gene was partially purified from 125 ml of the bacterial culture according to the procedure described. The pellet and supernatant fractions obtained from centrifugations of bacterial cells were sonicated and partially resuspended in a small volume of phosphate-buffered saline (PBS) or sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoretic buffer, and subjected to further testing. The major portion of fusion proteins was in the pellet fractions. The pellets were re-suspended and further purified using His Excellulose Spin Kit (Yeastern Biotech Co., Ltd) as instructed by the manufacturer.

Antiserum production

The antiserum against the bacterially expressed Wg1-CP was prepared in a New Zealand White rabbit given three subcutaneous injections of 1 mg recombinant protein at three weekly intervals. The purified proteins were emulsified with an equal volume of Freund's complete adjuvant for the first injection and with Freund's incomplete adjuvant for the following two subsequent injections. The recombinant protein was soluble in both types of adjuvant. The rabbit were bled 5 weeks after the last injection [16]. The serum fractions were collected and stored at -20℃ until required.

Indirect enzyme-linked immunosorbent assay (I-ELISA)

Clear Polystyrene 96-well plates were coated with crude extracts diluted 1,10 and 100 fold in coating buffer (0.05 M sodium carbonate, pH 9.6, containing 0.01 % sodium azide). The above antiserum specific to Wg1-CP at 1:250, 1:500, 1:1000 and 1:2000 dilution in conjugate buffer (phosphate-buffered saline containing 0.05 % Tween 20, 2% polyvinylpyrrolidone-40, and 0.2% ovalbumin) were used [6]. Goat anti-rabbit immunoglobulin G (IgG) alkaline phosphatase (AP) conjugate (Kirkegaard & Ferry Laboratories, Gaithersburg, MD) was diluted to 1:5000 in conjugate buffer. Substrate solution was prepared by dissolving ρ-nitrophenyl phosphate tablets (Sigma Chemical, St. Louis, MO) in substrate buffer (97 ml of diethanolamine and 0.2 g of sodium azide per liter, pH 9.8) to a final concentration of 1 mg/ml [8]. Absorbance at 405 nm (A_{405nm}) was recorded using a Thermo Labsystems Multiskan reader (Thermo electron corr. U.S.A.) 30 min after the addition of substrate. The naturally infected wax gourd plants showing systemic mild mottling and leaf curl symptoms were collected as samples.

RESULTS

Field observation and virus isolation

From July to September 2005, eleven wax gourd plants including 2 resistant varieties (TVI4204) which are resistant to CABYV, CGMMV, CMV, MVbMV, PRSV-W, TSWV, WSMoV and ZYMV and 9 susceptible varieties (TV7296, TVI10688, TVI10689 and TVI11577) with a novel type of virus-like symptoms including mottling, yellowing, vein enation, rugose mosaic, or leaf curling were observed and collected. Out of them a wax gourd plant (Wg1) with systemic mild mottling and leaf curl (Fig. 1) but negatively reacted with antisera against CABYV, CGMMV, CMV, MVbMV, PRSV-W, TSWV, WSMoV and ZYMV was collected for further analyses.

Polymerase chain reaction (PCR)

After examination of several RNA viruses frequently found in cucurbits, it was inferred that DNA viruses, such as geminiviruses, might be the causal agents in Wg1 plant. Therefore, total DNAs extracted from Wg1 was assayed by PCR with the specific primers devised from CP gene of bipartite geminiviruses. Specific fragments (1.1 kb) (Fig. 2) were amplified, which demonstrated that Wg1 plant was infected by geminiviruses. To further characterize the virus associated with the diseased wax gourd plants, the specific

PCR products were cloned. Three clones were selected to sequence and analyze. It revealed that the PCR product consisted of 1116 nucleotides spanning 5' ,3' - terminal 126 nucleotides of the AV2 gene, the entire 771 nucleotides of the CP coding region (257 aa), and the 3' - terminal 219 nucleotides of AC3 gene (73 aa).

Sequence analysis

BLAST analysis revealed that the new virus is closely related to *Squash leaf curl virus* (Fig. 3), and it was tentatively named SqLCV-Wg1. To further analyze phylogenetic relationships between SqLCV-Wg1 and other

Fig. 1. Symptoms of wax gourd (Wg1) infected by *Squash leaf curl virus*. The leaves and shoot tip of a wax gourd plant (Wg1) were shown with systemic mild mottling, vein clearing, and leaf curling(as arrowed).

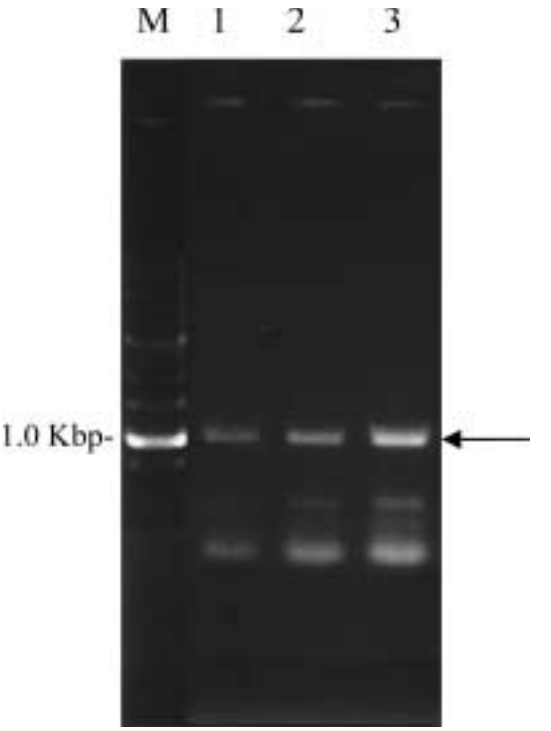

Fig. 2. Electrophoreses of polymerase chain reaction (PCR) products corresponding to the coat protein (CP) gene of SqLCV-Wg1 amplified using specific primer (gen-793-f , gen-793-r). Total DNAs extracted from infected wax gourds were used as templates for PCR with CP gene-specific primer pair as described previously. The amplified DNA products were analyzed by electrophoresis through a 1 % agarose gel, and observed on a UV-light box by staining with ethidium bromide. Lanes 1-3, products of PCR amplification of three independent samples. The expected size of SqLCV-Wg1 CP gene, about 1.1 kb in length, is indicated by the arrow on the right. Lane M, DNA standard ladder.

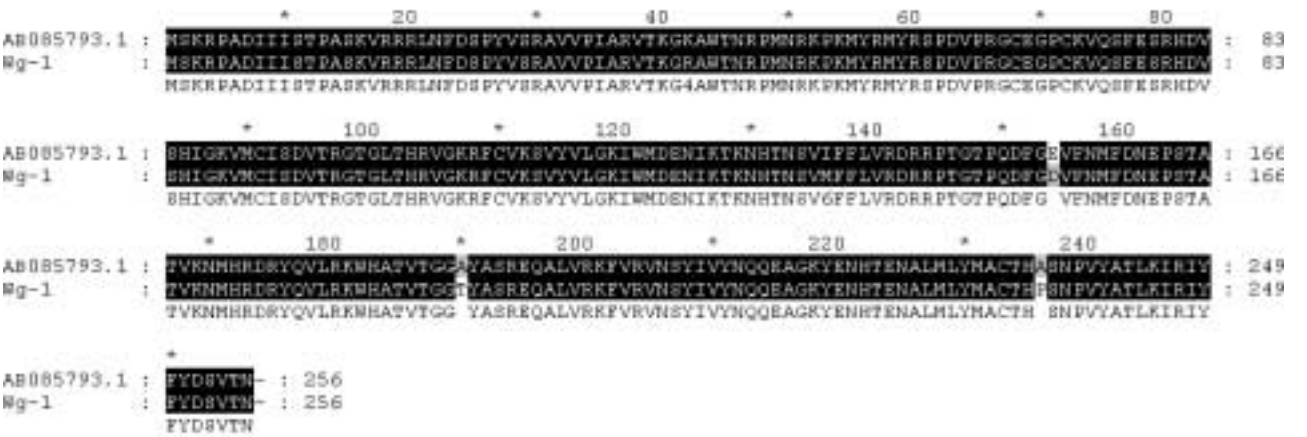

Fig. 3. The difference of amino acid sequences between SqLCV-Wg1 partial gene and *Squash leaf curl Philippines virus* (Accession No. AB085793). The region corresponding to the CP gene is indicated on the alignment. Amino acids are presented with the single-letter code. Identical amino acids were shown with white letters on black background. The consensus amino acid sequence between the two sequences is shown under the alignment.

Table 1. The percent identities between the CP gene regions of SqLCV-Wg1 virus DNA associated with the squash leaf curl disease and those of 10 most closely related bipartite geminiviruses, including *Squash leaf curl Philippines virus*; *Squash leaf curl China virus*; *Chinese squash leaf curl virus*; *Loofa yellow mosaic virus* DNA-A; and *Tomato leaf curl virus* deposited in GenBank (Benson, et al., 2006)[2]

Accession No.	Locus name	CP	
		nt	aa
AB085793	*Squash leaf curl Philippines virus*	97.7	98.0
AF509741	*Squash leaf curl China virus* [K] segment DNA-A	97.3	97.7
AF509743	*Squash leaf curl China viru* [B] segment DNA-A	96.5	96.9
AB027465	*Squash leaf curl China virus* DNA-A	96.1	96.5
AY184487	*Squash leaf curl China virus* - [Pumpkin :Coimbatore] segment	94.6	94.9
S77090	*Chinese squash leaf curl virus*	96.1	96.5
AF509739	*Loofa yellow mosaic virus* DNA-A	96.9	97.3
AY691900	*Tomato leaf curl virus*	94.9	95.3
AY691902	*Tomato leaf curl virus*	94.6	94.9
AY691899	*Tomato leaf curl virus*	94.6	94.9

Nt : nucleotide aa : amino acid.

begomoviruses, the program MegAlign was used for pairwise and multiple sequence comparisons. The percent sequence identities for nucleotide sequences of entire CP gene between SqLCV Wg1 and other begomoviruses ranged from 94.6 % (with *Tomato leaf curl virus*, AY691899; AY691902, *Squash leaf curl China virus*, AY184487) to 97.7 % (with *Squash leaf curl Philippines virus*, AB085793), and those for the amino acid ranged from 94.9 % (with *Tomato leaf curl virus*, AY691899) to 98.0 % (with *Squash leaf curl Philippines virus*, AB085793) (Table 1). The results suggested that SqLCV-Wg1 should be classified as an isolate of *Squash leaf curl Philippines virus*.

Expression and purification of Wg1-CP

To produce antiserum against the CP of SqLCV-Wg1 for diagnosis purposes, a clone containing the CP gene of SqLCV-Wg1 was used for further experiments. After digestion with restriction enzymes *Nco*I and *Bam*HI, the resulting DNA fragment carrying the CP gene was ligated into pET21d (+) plasmid (Fig. 4) and transformed *E. coli*. BL21 (DE3). After optimization of expression conditions, including time, temperature, and the concentration of IPTG inducer, the cells were harvested and the expected protein (31 kDa) was demonstrated by SDS-PAGE and Western blotting analysis (Fig. 5A, 5B). SDS-PAGE showed a strong band at a position corresponding to the expected molecular weight of about 31 kDa, consisting of the complete Wg1-CP with 6xHis tag. However, the major portion of fusion proteins was in the pellets. Therefore, the pellets were further suspended and the target proteins were purified using His Excellulose Spin Kit (Yeastern Biotech Co., Ltd). Polyclonal antiserum to the recombinant Wg1-CP was obtained from bleeds taken 3 weeks after the last

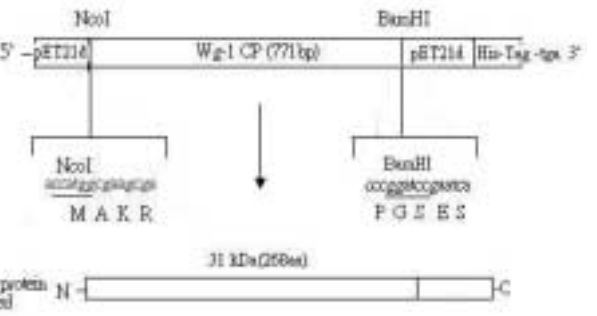

Fig. 4. Schematic representation of the construction of full-length coat protein gene of SqLCV - Wg1 in the expression vector pET-21d (+). The upper chart shows the organization of insertion relative to the vector, pET-21d (+), with the junction sequences between Wg-1 CP and the recognition sites of restriction enzymes *Nco*I and *Bam*HI indicated. Protein translation starts from the ATG codon of the *Nco*I site and terminates at the TGA codon provided by the expression vector. The lower chart indicates the relative size of the translated protein with a molecular weight of 31 kDa, as estimated by DNA Star programs.

injection. The anti-Wg1-CP sera had titres of 1:500 when tested in an I- ELISA with purified fused proteins in microtitre plates (data not shown).

Indirect enzyme-linked immunosorbent assay (I-ELISA)

Positive results were observed in indirect ELISA of crude extracts prepared from naturally infected wax gourd plants from fields at Beidou, Changhua County by using the antiserum against recombinant SqLCV-Wg1 CP. The healthy wax gourd plants tested negative in all the assays. The A_{405nm} reading of specific reaction was about five times

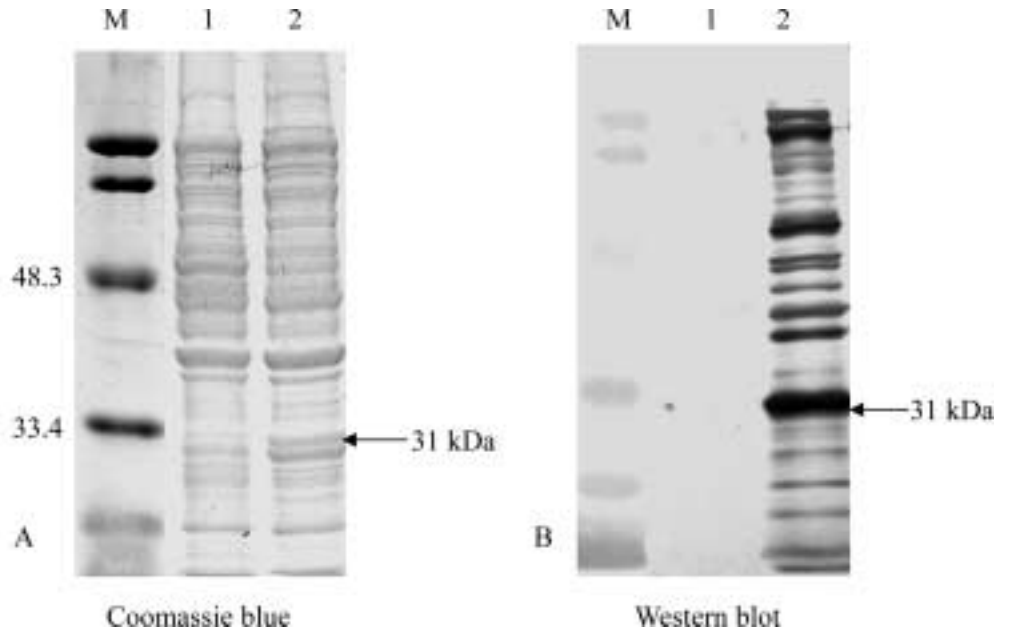

Fig. 5. Analyses of full-length coat proteins of SqLCV-Wg1 expressed by selected bacteria clones. Total bacteria lysate from selected bacteria clone containing pET21d(+) vector alone or pET21d(+) harboring SqLCV-Wg1 CP gene were separated on a 12.5 % polyacrylamide gel with 1% SDS, stained with coomassie blue (panel A), and transferred to PVDF membrane and reacted with antiserum against coat protein gene of SqLCV-Wg1 (panel B). Lane M, protein standard markers; lane 1, IPTG-induced culture lysate of bacteria clone containing pET-21d(+) vector alone; lane 2, IPTG-induced culture lysate of bacteria clone with Wg1 CP gene inserted into the expression vector pET-21d(+). The relative molecular weights were indicated on the left. The expected size and position of Wg1 CP is specified on the right.

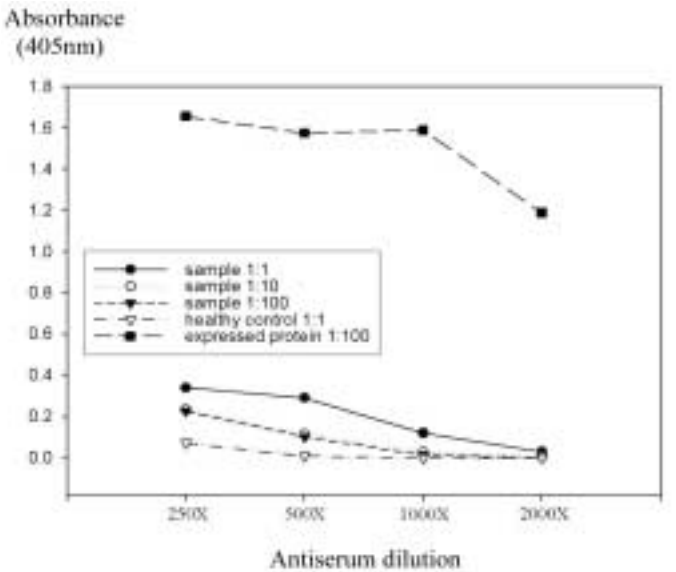

Fig. 6. Results of indirect enzyme-linked immunosorbent assay (I-ELISA) on naturally infected wax gourd plants using the antiserum against SqLCV-Wg1. Leaf samples collected from symptomatic wax gourd plants were ground in coating buffer in ratios of 1:1, 1:10, and 1:100 (weight/volume), and subjected to detection by I-ELISA using SqLCV-Wg1-specific antiserum at 250X, 500X, 1000X, and 2000X dilutions. The absorbance of each sample at 405 nm were recorded. Crude extracts of healthy wax gourd leaves and bacterial expressed SqLCV-Wg1 CP at 1:1 and 1:100 dilutions were used as the negative and positive control, respectively.

that of negative control (Fig. 6). The antiserum diluted 250 and 500 fold was proven useful in indirect-ELISA test.

DISCUSSION

This study demonstrated that a viral disease on wax gourds occurred in Taiwan, and its has not yet recorded previously. Eight RNA viruses frequently observed in cucurbits were not detected in these symptomatic wax gourd plants. Through cloning and nucleotide sequence analyses, the provisional identity of the causal pathogen was determined to be an isolate of *Squash leaf curl Philippines virus*[1], designated as SqLCV-Wg1.

It has been reported that the pathogen causing yellow leaf disease on cantaloupe and wax gourds in Thailand was ToLCV or closely related strains [18]. Although there were different symptoms between induced by ToLCV and SqLCV, it is difficult to distinguish *Squash leaf curl Philippines virus* from other Begomovirus members by serological assays [20]. The result of indirect ELISA indicated that the concentration of SqLCV virions in infected tissue is not high, so the optimal antigen dilution is suggested to be 1-10 fold. On the other hand, the optimal antiserum titer is 250-500 fold. In this condition, we could reliably detect the existence of *Squash leaf curl virus* in cucurbitaceous plants.

The nucleotide sequence of CP gene of SqLCV-Wg1 is closely related to *Squash leaf curl Philippines virus* (97.7.0%); *Squash leaf curl China virus* [K] segment DNA-A (97.3%); *Squash leaf curl China virus* [B] segment DNA-A (96.5%); *Squash leaf curl China virus* DNA-A (96.1%); *Chinese squash leaf curl virus* (96.1%); *Squash leaf curl China virus* [Pumpkin :Coimbatore] (94.6%); and *Tomato leaf curl virus* (94.6%) in partial nucleotide sequence comparisons (Table 1). A similar analysis was applied to the deduced amino acid sequences of the putative ORF products (Table 1). Through sequence analysis, it was found that SqLCV-Wg1 is most closely related to *Squash leaf curl Philippines virus* (accession number AB085793). The significant sequence identity of the partial genomic DNA of SqLCV-Wg1 is compared to other distinct geminivirus species in the genus *Begomovirus*.

This is the first record of cucurbits infected with *Squash leaf curl virus* in Taiwan. For future studies, it is necessary to investigate the natural host range of SqLCV-Wg1, the epidemiology, the development and spread of the disease by whitefly vectors [5], and methods to decrease the population density of the vectors and the incidence of the disease in the field. The sequence information and serological tools provided in this study would be helpful in the understanding and the management of the new disease.

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REFERENCES

1. Benigno, D. R. A. 1977. Leaf curl disease on squash. Philippine Agriculturalist 61: 304-305.
2. Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J. and Wheeler, D.L. 2006. GenBank. Nucleic Acids Res. 34: D16-20.
3. Briddon, R. W., and Markham, P. G. 1994. Universal primers for the PCR amplification of dicot-infecting geminiviruses. Molecular Biotechnology, 1: 202-205.
4. Brown, J. K., and Nelson, M. R. 1989. Characterization of watermelon curly mottle virus, a geminivirus distinct from squash leaf curl virus. Ann. Appl. Biol. 115: 243-252.
5. Brown, J. K., Idris, A. M., Olsen, M. W. and Miller, M. E. 2000. Cucurbit leaf curl virus, a new whitefly transmitted geminivirus in Arizona, Texas, and Mexico. Plant Dis. 84: 809.
6. Clark, M. F., and Adams, A. N. 1977. Characteristic of microplate method of enzyme-linked immunosorbent assay for the detection of plant virus. J. Gen. Virol. 34: 475-483
7. Deng, T. C., Tsai, C. H. and Liao, J. Y. 2005. Seed transmissibility of viruses in cucurbits. Seed and Nursery 7: 1-19.
8. Dijkstra, J., and de Jager, C. P. 1998. Practical Plant Virology: Protocols and Exercises. P277-284. Springer-Verlag Berlin; Heidelberg.
9. Grover, J. K., Adiga, G., Vats, V., and Rathi, S. S. 2001. Extracts of *Benincasa hispida* prevent development of experimental ulcers. J. Ethnopharmacol. 78:159-164.
10. Guzman, P., Sudarshana, M. R., Seo, Y. S., Rojas, M. R., Natwick, E., Turini, T., Mayberry, K., and Gilbertson, R. L. 2000. A new bipartite geminivirus (Begomovirus) causing leaf curl and crumpling in cucurbits in the Imperial Valley of California. Plant Dis. 84: 488.
11. Hseu, S. H., Huang, C. H., Chang, C. A., Yang, W. Z., Chang, Y. M. and Hsiao, C. H. 1987. The occurrence of five viruses in six cucurbits in Taiwan. Plant Prot. Bull. 29: 233-244.
12. Kon, T., Dolores, L. M., Murayama, A., Bajet, N. B., Hase, S., Takahashi, H., and Ikegami, M. 2002. Genome organization of an infectious clone of *Tomato leaf curl virus* (Philippines), a new monopartite *Begomovirus*. J. Phytopathology 150: 587-591.
13. Kon, T., Dolores, L. M., Bajet, N. B., Hase, S., Takahashi, H., and Ikegami, M. 2003. Molecular characterization of a strain of *Squash leaf curl China virus* from the Phillipines. J. Phytopathology 151: 535-539.
14. Lazarowitz, S. G., and Lazdins, I. B. 1991. Infectivity and complete nucleotide sequence of the cloned genomic components of a bipartite squash leaf curl geminivirus with a broad host range phenotype. Virology 180: 58-69.
15. Lee, N., Zhang, S. Q., Cozzitorto, J., Yang, J. S., and Testa, D. 1987. Modification of mRNA secondary

structure and alteration of expression interaction alpha 1 in *Escherichia coli*. Gene 58: 77-86.
16. Liao, J. Y., Chang, C. A.,Yan, C. R., Chen, Y. C. and Deng, T. C., 2002. Detection and incidence of *Cactus virus X* on pitaya in Taiwan. Plant Pathol. Bull. 12: 225-234.
17. Lin, K., and Cheng, S. Y. 1991. An efficient method to purify active eukaryotic proteins from the inclusion bodys in *Escherichia coli*. Biotechniques 11: 748-753.
18. Revill, P. A., Ha, C. V., Porchun, S. C., Vu, M. T., and Dale, J. L. 2003. The complete nucleotide sequence of two distinct geminiviruses infecting cucurbits in Vietnam. Arch. Virol. 148: 1523-1541.
19. Samretwanich, K., Chiemsombat, P., and Ikegami, M. 2000. Yellow leaf disease of cantaloupe and wax gourd from Thailand caused by *Tomato leaf curl virus*. Plant Dis. 84: 200.
20. Sander, C., and Schneider, R. 1991. Database of homology-derived protein structures and the structural meaning of sequence alignment. Proteins 9: 56-68.
21. Tobias, J. W., Shrader, T. E., Rocap, G., and Vrashavsky, A. 1991. The N-end rule in bacteria. Science 254: 1374-1377.
22 Wu, F. Y., Hseu, S. H., and Huang, C. H. 1994. Virus of cucurit crops in Taiwan. Symposium on Techniques of Curcubits Protection. Plant Prot. Bull 36: 159-167.

摘 要

廖吉彥[1]、胡仲祺[2]、林子凱[3]、張清安[1]、鄧汀欽[1、4]. 2007. 感染冬瓜的南瓜捲葉病毒之鑑定. 植病會刊 16: 11-18. ([1] 台中縣霧峰鄉行政院農業委員會農業試驗所植物病理組；[2] 國立中興大學生物科技所；[3] 台中縣霧峰鄉行政院農業委員會農業試驗所園藝組；[4] 聯絡作者，電子郵件：tcde@wufeng.tari.gov.tw)

冬瓜 (*Benincasa hispida* Cogn.) 上發現一種新病害病徵，葉部褪綠、葉脈突起及褪色、葉面皺縮、甚至捲葉之現象，使得植株生長勢衰弱。採自彰化縣北斗鎮之冬瓜疑似病毒罹病株 (Wg1)，經瓜類作物的八種病毒血清檢測，包括瓜類蚜媒黃化病毒 (*Cucurbit aphid-borne yellows virus*, CABYV)，胡瓜綠斑嵌紋病毒 (*Cucumber green mottle mosaic virus*, CGMMV)，胡瓜嵌紋病毒 (*Cucumber mosaic virus*, CMV)，甜瓜脈綠嵌紋病毒 (*Melon vein-banding mosaic virus*, MVbMV)，木瓜輪點病毒-西瓜系統 (*Papaya ringspot virus* W type, PRSV-W)，番茄斑點萎凋病毒 (*Tomato spotted wilt virus*, TSWV)，西瓜銀斑病毒 (*Watermelon silver mottle virus*, WSMoV)，及矮南瓜黃化嵌紋病毒 (*Zucchini yellow mosaic virus*, ZYMV)，均呈陰性反應。針對begomovirus外鞘蛋白之高度保留區域核酸序列，經比對設計引子對，以PCR增幅之。經由勝任細胞的轉形作用與核酸解序，獲得1116個核苷酸，分爲AV2 (126個核苷酸)、CP (771個核苷酸)及AC3 (219個核苷酸)。經與其他 begomoviruses 比對，得知 Wg1 病毒分離株與 *Squash leaf curl Philippines virus* 最有親源關係，其核酸相同度在AV1, CP, and AC3 基因分別爲97.6, 97.7, and 93.8 %。利用載體 pET21d (+) 夾帶 SqLV-Wg1 的 CP 基因，在 *Escherichia coli* BL21 (DE3) 中表現病毒鞘蛋白，並用以注射紐西蘭白兔製備專一性的抗血清，供進一步診斷鑑定用。本文爲台灣瓜類感染 *Squash leaf curl virus* 的首次紀錄。