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Occurrence of Chrysanthemum virus B in Taiwan and Preparation of Its Antibody Against Coat Protein Expressed in Bacteria

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ABSTRACT

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Chrysanthemum virus B (CVB) is well known for its global distribution and adverse effect on chrysanthemum production. From the year of 2000 to 2005, we conducted an extensive survey for the occurrence of CVB in different chrysanthemum plantations and cutting producing nurseries in Changhwa County. Among 504 chrysanthemum leaf samples collected from 9 different locations, 394 of them were detected with CVB infection in indirect ELISA. This is the first evidence showing the existence of CVB in chrysanthemum in Taiwan. We also found that the detection of CVB by ELISA was affected by the air temperature during seasonal field surveys. In seasons when temperature between 15-20℃, CVB infected plants were readily detected by ELISA and exhibited evident EIA readings. However, the EIA readings of the same samples would drop to healthy control levels when temperature rose above 25℃. This result implies winter is the appropriate time to perform CVB indexing in chrysanthemum in Taiwan. To characterize CVB isolates from Taiwan, their CP gene was amplified by reverse transcriptase polymerase chain reaction (RT-PCR) using a set of primer (CVB-up, CVB-dw) designed in this study according to the sequences available in GenBank. A DNA product of 1028 bp was amplified, cloned in pCRII-TOPO plasmid and subsequently sequenced. It was found to contain an open reading frame with 316 amino acid residues corresponding to the size of reported CVB CPs and it shared 78.4 to 88.0% and 83.1 to 93.7% identities in nucleotide and amino acid sequence, respectively, with 20 known CVB CP gene sequences documented in GenBank. In order to produce specific antiserum against CVB for its detection, we expressed the cloned CVB CP gene in bacteria culture and using bacteria expressed CP as immunogen for antiserum preparation. An antiserum (#107) was prepared against the expressed CVB CP and shown to be useful in ELISA to detect CVB in chrysanthemum plants. By comparing with a commercialized CVB antiserum (Agdia Inc. Elkhart, IN, USA), the reactivity in terms of EIA readings of antiserum #107 to the same dilution of infected chrysanthemum tissue was always higher than that of Agdia's CVB antiserum.

Key words : chrysanthemum, CVB, expression vector, RT-PCR, serology, bacteria-expressed virus coat protein

INTRODUCTION

Chrysanthemum is an important cut flower crop in Taiwan and many other countries worldwide. Except certain varieties selected for potted chrysanthemum are produced through seeds, most chrysanthemum cultivars for cut flower purpose are propagated by cuttings. Therefore, virus and viroid diseases may become a limiting factor for chrysanthemum production. The adverse effect will be worse for cultivars propagated vegetatively for many years. There are five viruses and two viroids reported to infect chrysanthemum[10,11,15,18,20]. Among them, *Chrysanthemum virus B*[1,2,3,14,16], *Tomato aspermy virus* (TAV)[1,2,3,13,17,21] and Chrysanthemum stunt viroid (CSVd) [3,12,16] are considered to be the most widespread and economically important ones. In a previous report, we have found TAV distributed widely in many commercial chrysanthemum varieties in Taiwan. The occurrence of CVB, however, is not yet scientifically confirmed in chrysanthemum plantations in Taiwan. CVB belongs to the Genus, *Carlavirus*. It was first described by Noordam (1952)[22] to infect *Chrysanthemum morifolium* from the Netherlands. The virus may induce a slight loss of flower quality, mild leaf mottling, vein clearing, mosaic or even severe necrosis symptoms on some sensitive cultivars. However, most commercial cultivars are usually infected by CVB without showing visible symptoms. In this study, we conducted an extensive field survey since 2000 to 2005 and confirmed that CVB was indeed widely spread in many commercial chrysanthemum varieties and different plantations as well as in cutting nurseries. The local isolates were molecularly characterized and compared with those known CVB strains in the literature. In this study, we prepared antiserum against our Taiwanese CVB strain using bacteria-expressed coat protein as immunogen. The reactivity of the antiserum was compared in ELISA with a commercialized antibody purchased from Agdia. Result showed that the antiserum to bacteria-expressed CVB CP was applicable in the routine indexing of CVB in chrysanthemum.

MATERIALS AND METHODS

Field survey for the occurrence of CVB in chrysanthemum plantations

From 2000 to 2005, we conducted nine field surveys for the occurrence of CVB in chrysanthemum plantations including some private cutting nurseries in Changhua County, the major chrysanthemum cut flower production area in Taiwan. Leaves and, if possible, a shoot fragment about 5-10 cm in length were taken from chrysanthemum plants and brought back to laboratory for indexing of CVB infection. Indirect ELISA as described previously[4,7,8] and an antiserum against CVB purchased from Agdia Inc. (Elkhart, IN, USA) were routinely applied to determine the incidence of CVB in chrysanthemum plantations. However, since 2003 an antiserum prepared against bacteria-expressed CP of our local CVB strain was used to substitute the Agdia's CVB antiserum in the ELISA indexing of field surveys.

Virus source and storage

A chrysanthemum sample (CH3) bearing mild mottling symptoms on the leaves was collected from a chrysanthemum cutting nursery in Changhua County. The material reacted positively with Agdia's CVB antibody. Cutting of this sample was rooted in a pot with peat moss medium for later experiment. Leaves of the sample were cut into 1 cm square pieces, immersed in 50% glycerol and preserved in -20℃ freezer for long term storage of the virus isolate.

Electron microscopy

Field collected chrysanthemum samples detected serologically with CVB infection were further checked in a Hitachi electron microscopy for the existence of typical CVB virus particles. Leaf tissue was prepared by dipping method as described by Christie *et al* (1987)[10] followed by negatively stained with 5% uranyl acetate for observation.

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNAs was routinely extracted from 0.1 g of virus-infected leaf tissue by the QIAamp total plant RNA mini kit (Qiagen, Hilden, Germary). The first strand of cDNA was synthesized by a cDNA synthesis kit (Strategene, La Jolla, CA) from a 3 μ*l* of total RNA as template with addition of a downstream CVB CP gene specific primer (CVB-dw: 5'-ATCTTCACAATGACATCCAT-3') designed based on CVB's CP gene sequence published by Levay and Zavirev (1991)[18]. Subsequently, another upstream primer (CVB-up: 5'-TAGGTTGTGGAGTGGTTACA-3') was added for PCR amplification of CP gene of CVB. PCR was performed in 50 μ*l* volume containing 2 μ*l* cDNA, 1 pmole of upstream and downstream primers and 0.5 U of ExTaq polymerase (Takara Shuzo Co., Shiga, Japan) with buffer system recommended by manufacturer [13]. Cycling conditions of PCR were optimized to an initial denaturation of 94℃ for 2 min followed by 30 cycles of 94 ℃ for 1 min, 52℃ for 1 min, and 72℃ for 2 min. An elongation step at 72℃ for 6 min was conducted during the last cycle. Amplified PCR product was analyzed by electrophoresis in a 1.2% agarose gel[23].

Cloning and sequence analysis

PCR amplified DNA products were cloned into pCRII-TOPO vector (Invitrogen, Carlsbad, CA) according to manufacturer's instruction. Plasmid clones with expected size DNA inserts were screened and used for sequencing analyses[23]. Sequencing of the target DNA insert was done by an automatic DNA sequencer (ABI PRISM 377, Perkin-Elmer, CA, USA) with the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems, CA, USA). Sequence data was analyzed by ScanDNASIS program (Hitachi Software Engineering America, Ltd., California, USA).

Construction of bacteria plasmid expressing CVB CP

Based on the sequence data obtained from the previous experiment, a primer pair was designed for site-specific amplification of the complete CP gene of CVB[3]. The restriction enzymes *Nco*I and *Xho*I digestion sites (underlined) were created at the 5'-end of the upstream (CVB-up1: 5'-AGTCACCATGGCTCCCAAA-3') and downstream (CVB-dw1: 5'- TCAGTCACTATATCTCGAGTGT -3') primer, respectively, to facilitate subsequent directional cloning of the CP gene into expression vector plasmid pET28b(+) (Novogen, Inc., Madison, WI, USA). Using pCRII-TOPO plasmid containing CVB CP gene insert as template, CVB CP gene with upstream and downstream cloning sites was amplified by the aforementioned primers (CVB-up1 and CVB-dw1). The amplified DNA fragment was digested with *Nco*I and *Xho*I, and ligated with a *Nco*I-*Xho*I cleaved pET28b(+). The recombinant pET28b(+) vector was subsequently transformed into E. *coli* strain Rosetta(DE3) for protein expression. Bacterial clones containing recombinant pET DNA were identified by PCR using primer pair (T7 promoter/T7 terminator).

Analysis of the bacteria-expressed CVB CP

Bacteria clones identified to have CVB CP inserted pET28b(+) were grown overnight at 37℃ in M9 medium containing 50 μg/ml kanamicin and 34 μg/ml chloramphenicol. When optical density (OD_{600}) of bacteria culture filtrate reached 0.6-0.7, 1 mM of isopropyl β-D-thiogalactopyranoside (IPTG) was added to the medium to induce protein expression. Four hours after IPTG induction, bacteria culture was subjected to centrifugation at 8000 rpm for 10 min. Bacteria cells were resuspended in TE buffer (10 mM Tris-HCl, pH8.0, 0.5mM EDTA) and frozen at -20℃ overnight. After thawing, the bacteria cells were further disrupted by a VCX 600 sonicator (Sonics & Materials Inc., CT, USA) followed by centrifugation at 3000 rpm for 10 min to remove cell debris. Supernatants were treated with an equal volume of protein denaturing solution (0.25 M Tris-HCl, pH 6.8, containing 2% (w/v) of SDS, 4% (v/v) of 2-mercaptoethanol and 10 % (w/v) of sucrose) and heated in boiling water bath for 3 min. The sample was then analyzed in SDS-polyacrylamide gel electrophoresis (SDS-PAGE)[21]. Size and expression level of viral CP was identified by western blotting analysis using Agdia's CVB antiserum.

Purification of bacteria-expressed viral CP

A bacteria clone with satisfactory CP expression ability was selected and grown in a 1000 ml flask of M9 medium containing 50 μg/ml kanamicin and 34 μg/ml chloramphenicol. Protein expression was induced and processed similarly as aforementioned procedure. Bacteria expressed CP was further purified by a preparative SDS-PAGE protocol as described previously[8, 9, 21]. Purified protein was adjusted to a concentration of 1.0 OD_{280} per ml for immunological studies[8].

Antiserum preparation and serological test

Antiserum against bacteria-expressed CVB CP was prepared by immunizing New Zealand white rabbit intramuscularly. The immunization protocol was the same as those described previously[4, 8]. Indirect ELISA as described in previous paper[4, 7, 8] was carried out to determine the reactivity of the prepared antiserum and compared with Agdia's commercialized CVB antibodies.

RESULTS

Survey for the occurrence of CVB in Taiwan

From the year of 2000 to 2005, we totally surveyed 9 different locations of chrysanthemum plantations including some private cutting nurseries in Changhua County (Table 1). A total of 504 chrysanthemum plants were collected and indexed by indirect ELISA. The results showed 394 of the collected samples were positively reacted with CVB antiserum. Among the infected chrysanthemum plants, most of them were major commercial cut flower varieties such as Huang-Xiu-Fang; some of them were from various varieties in germplasm collection of private nurseries and some were from the mother plants for producing commercial cuttings.

Symptomatology and electron microscopy

Of the 394 plants reacted with CVB antiserum, most of them were symptomless (Fig. 1A) while only a small

Table 1. Survey for the occurrence of *Chrysanthemum virus B* (CVB) on nine locations of chrysanthemum cut flower plantations and cutting nurseries at Changhwa County during 2000 to 2005.

Location	Date (Yr/M)	Number of samples collected	No. of sample detected [1]	Average EIA readings [2]
A	2000/Jan	90	90	1.207
B	2001/Dec	88	87	1.184
C	2002/Jan	51	28	0.836
D	2002/Jan	45	42	1.517
E	2002/Jun	20	19	0.355
F	2003/Jul	34	21	0.430
G	2003/May	77	9	1.731
H	2003/Nov	81	78	0.789
I	2005/Jan	18	17	2.189
Total		504	394	

[1] Field collected samples were indexed in indirect ELISA by the use of CVB antiserum purchased from Agdia Inc. (Elkhart, IN, USA). Antigen coating type of indirect ELISA as previously described was conducted. Routinely 0.1 g of leaf tissue randomly taken from each field collected chrysanthemum plant was grinded in 3 ml of coating buffer (30x of dilution) for indexing in indirect ELISA. A virus free control chrysanthemum plant kindly provided by Taiwan flower Co. was used as healthy control. Absorbance readings of test samples higher than two times of healthy control chrysanthemum readings are considered to be positive reaction.

[2] Figure represent the average value of EIA readings obtained from of the infected samples. All EIA readings (A_{405nm}) are taken 40 min after the addition of enzyme substrate solution.

percentage of plants exhibiting mild mottling symptoms on their leaves (Fig. 1B). Based on our observation, the percentage of plants bearing visible symptoms was higher during winter season than that in mid summer time, and EIA readings obtained from the symptomatic plants were always higher than those from symptomless ones. To confirm the infection of CVB in plants detected by Agdia's antiserum, we chose some plants and examined in electron microscopy. Consistently, flexuous rod shaped virus particles about 680 nm in length, typical of carlaviruses, were observed in negatively stained chrysanthemum samples (data not shown).

Seasonal variation on the ELISA indexing of CVB

During the field surveys, we found some CVB-infected chrysanthemum plants showed very high EIA readings when they were ELISA indexed in winter. However, EIA readings of the same plants dropped to levels near those of healthy control plants in summer times. To study in detail about the seasonal effect on the

Fig. 1. Photographs showing (A) symptomless chrysanthemum plants determined by ELISA to be infected by *Chrysanthemum virus* B (CVB); (B) CVB-induced mild mottling symptoms on chrysanthemum leaves observed during winter months.

indexing result of CVB, we conducted a series of indirect ELISA indexing by the use of Agdia's CVB antiserum on three selected CVB infected chrysanthemum plants. The result is shown in Fig. 2. From January of 2000 to December of 2001, a total of eight indexing experiments were carried out. Monthly average air temperature of each indexing date was recorded for evaluation. The results confirmed that EIA readings of all three test plants collected at temperatures between 15-20℃ were always higher than those collected at temperatures above 25℃. All three plants responded consistently to temperature change from summer to winter by resulting higher EIA readings. Similarly, when temperature rose again in next summer EIA readings of all three plants dropped again.

Cloning and sequencing of the CP gene of CVB

A 1028 bp DNA product was consistently amplified from total RNAs extracted from chrysanthemum samples positively reacted with CVB antiserum by the use of CVB-specific primer set (CVB-up/CVB-dw) (Fig. 3). After cloning and sequencing, this DNA fragment was found to have an open reading frame with 316 amino acid residues corresponding to the size of reported CVB CP (Fig. 4). Comparing with 20 reported CVB CP gene sequences, it shared 78.4 to 88.0% and 83.1 to 93.7% in the identities of nucleotide and deduced amino acid sequence, respectively (Table 2). This result indicates the 1028 bp sequence amplified from chrysanthemum is indeed originated from a

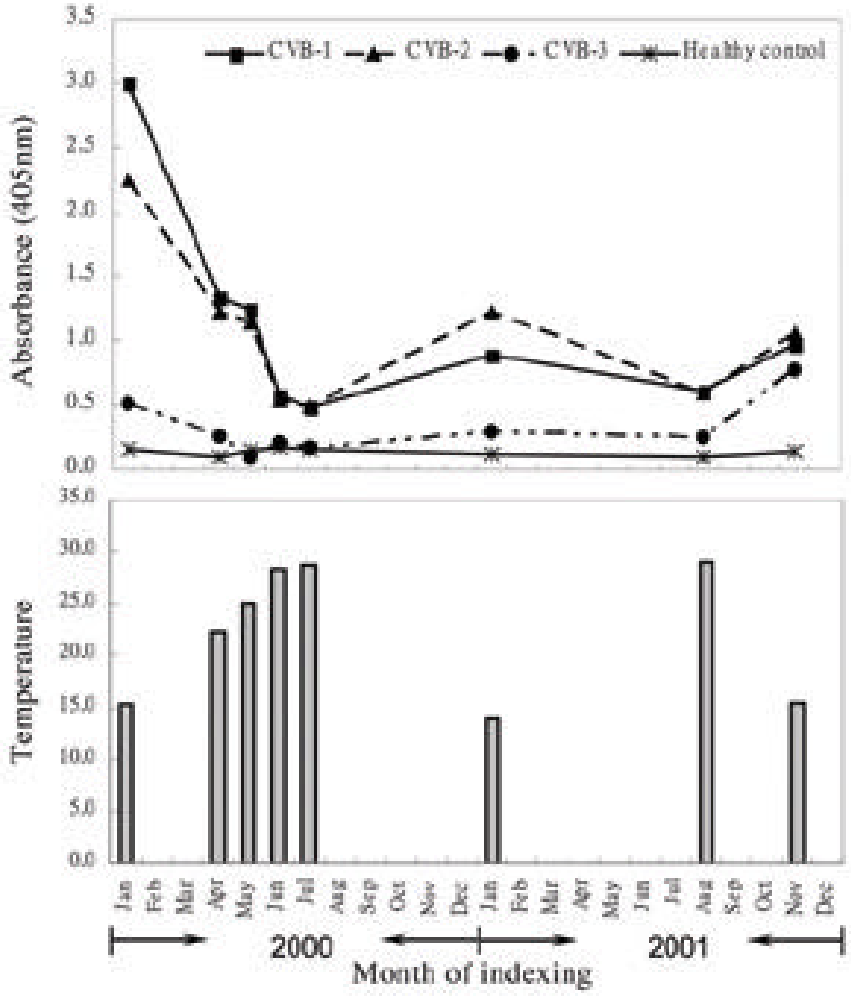

Fig. 2. Fluctuation of EIA readings of three *Chrysanthemum virus B* (CVB) infected-chrysanthemum plants as indexed by indirect ELISA in 8 different months from the year of 2000 to 2001 (A) in relation to the corresponding average air temperature (B). EIA readings obtained from three different CVB-infected chrysanthemum plants (CVB-1, -2 and -3) and healthy control plant are shown by different lines. Antigen coating type of indirect ELISA as previously described was conducted by the use of an antiserum against CVB purchased from Agdia Inc. (Elkhart, IN, USA). Routinely 0.1 g of leaf tissue randomly taken from each chrysanthemum plant was grinded in 3 ml of coating buffer (30x of dilution) for indexing in indirect ELISA. All EIA readings (A_{405nm}) were taken 40 min after the addition of enzyme substrate solution.

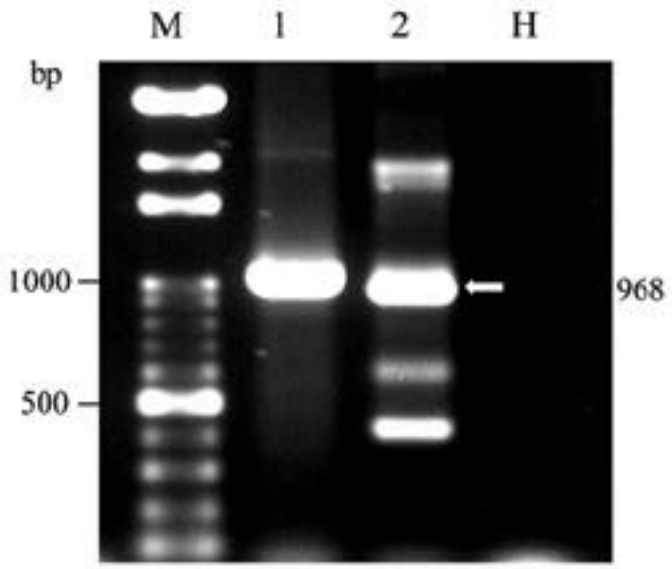

Fig. 3. Amplification of *Chrysanthemum virus B* (CVB) coat protein (CP) gene by reverse transcriptase-polymerase chain reaction (RT-PCR). A 1028 bp DNA product was amplified from field collected chrysanthemum specimen using primer set (CVB-up/CVB-dw) by RT-PCR (lane 1). The product was cloned into pCR II-TOPO plasmid which was subsequently used as template for PCR amplification of CVB CP gene by the use of primer set (CVB-up1/CVB-dw1). A 968 bp product was thus obtained (lane 2). Lane H is the RT-PCR amplification from healthy chrysanthemum using CVB-up/CVB-dw primers. Lane M is 100 bp DNA ladder markers.

Table 2. Percent identities of the nucleotide and amino acid sequence of the coat protein (CP) gene of *Chrysanthemum virus B* (CVB) to those of reported CVB CP gene sequences in GenBank.

Accession numbers of reported CVB CP	Percent identity[1]	
	nt	aa
S60150	83.1	92.1
AJ812569	85.3	93.0
AJ748853	81.2	91.5
AJ748852	81.5	91.0
AJ629843	81.4	88.3
AJ621815	79.6	90.2
AJ621814	82.9	92.1
AJ619744	81.1	91.1
AJ619743	81.3	90.7
AJ619742	81.5	90.5
AJ585514	87.4	93.4
AJ585240	82.4	91.5
AJ585051	83.1	88.2
CVI581993	88.0	93.7
CVI580956	81.3	92.4
CVI580955	78.4	80.2
CVI580954	80.7	91.1
CVI580931	81.9	91.8
CVI580930	80.9	90.2
CVI564858	82.0	92.1
AJ812733	79.4	83.1

[1] Percent identities of the respective nucleotide and amino acid sequences were analyzed by the ScanDNASIS programs (Hitachi Software Engineering America, Ltd., California, USA).

Fig. 4. Nucleotide and deduced amino acid sequences amplified from 3'-terminal region of the RNA genome of a CH3 isolate of *Chrysanthemum virus B* (CVB) derived from Changhua County in Taiwan. The sequence was obtained by reverse transcriptase polymerase chain reaction (RT-PCR) amplification using CVB-up and CVB-dw primers (underlined). Symbol of * indicates codon for translation termination.

local CVB strain.

Expression of coat protein gene of CVB in E. coli

Based on the CP gene sequenced (Fig. 4), a primer pair (CVB-up1/CVB-dw1) incorporated with two restriction enzyme sites was designed and used to amplify the cloned 1028-bp sequence in pCRII plasmid by PCR. A 968-bp product was thus amplified and subsequently constructed in expression vector pET28b(+) by directional cloning strategy as shown in Fig. 5. Recombinant pET28b(+) was then transformed into *E. coli* Strain Rosetta (DE3) for protein expression. After IPTG induction, bacteria lysates were analyzed in SDS-PAGE (Fig. 6A) and western blotting by the use of Agdia's CVB antiserum (Fig. 6B). A 36-kDa protein was identified in SDS-PAGE and shown to react strongly with Agdia's CVB antiserum in bacterial lysates with recombinant pET28b(+) but not in control bacteria clone containing only non-inserted pET28b(+) (Fig. 6). This result indicates the 36-kDa protein is antigenically related to CVB CP and is the product derived from the expression of cloned CVB CP sequence in pET28b(+). In order to mass-produce the expressed CVB CP for immunization, a high expressing clone was selected for large-scaled culture and the expressed protein was purified by preparative SDS-PAGE (Fig. 6). About 8.7 mg of bacteria expressed CP was purified from 1000 ml of *E coli*. strain Rosetta (DE3) culture.

Serology

An antiserum (#107) was prepared against the expressed CVB CP and shown to be useful in ELISA for the detection of CVB in chrysanthemum plants. By comparing with Agdia's CVB antiserum, the reactivity in terms of EIA readings of #107 to the same dilution of infected chrysanthemum tissue was always higher than that of Agdia's CVB antiserum (Table 2). In fact, antiserum #107 has been applied in field survey for the occurrence of CVB in chrysanthemum plantations since 2003 (Table 3) and the results showed the antiserum was comparable to the traditionally prepared antiserum purchased from Agdia (data not shown). Antiserum #107 could also react with IPTG induced bacteria lysate containing CVB CP gene inserted pET28b(+) and CVB-infected chrysanthemum tissues in SDS-immunodiffusion test (data not shown). It did not react with healthy control chrysanthemum antigens.

DISCUSSION

Since 1970s chrysanthemum cut flower production has been the leading exporting ornamental crop in Taiwan[5]. Currently, there are still about 1600 ha of land producing chrysanthemum cut flowers[5,17]. Changhua County represents the major chrysanthemum production area for many decades[17]. However, in recent years growers in Yunlin County successfully developed greenhouse chrysanthemum production system and

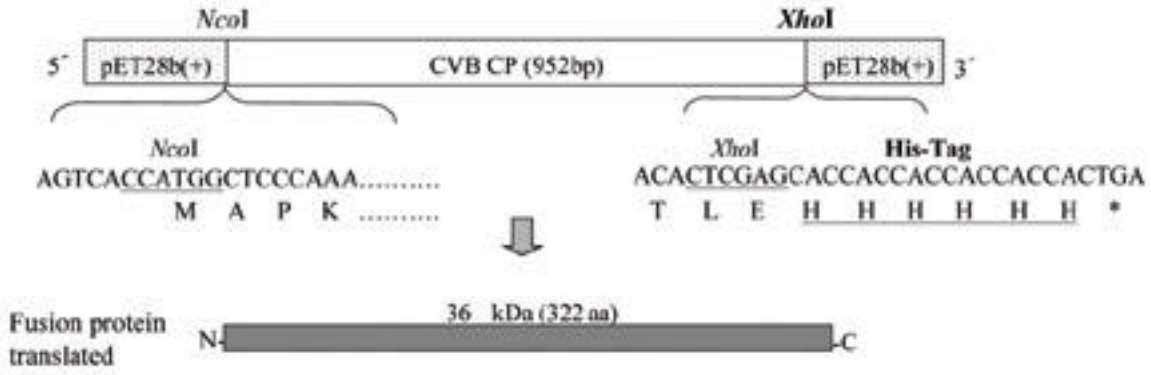

Fig. 5. Schematic representation of the construction of full-length coat protein gene of *Chrysanthemum virus B* (CVB) in the expression vector pET-28b(+). The upper chart shows the detail sequence linkage between CVB CP (□) and pET-28b(+)( ). Protein translation starts from the ATG codon of the *Nco*I site and terminates at the TGA codon provided by the expression vector. The expressed protein contains CVB CP and 6 repeated histidine residues encoded in pET-28b(+) sequence. The lower chart indicates the relative size of the translated protein with a molecular weight of 36 kDa estimated by ScanDNASIS programs (Hitachi Software Engineering America, Ltd., California, USA).

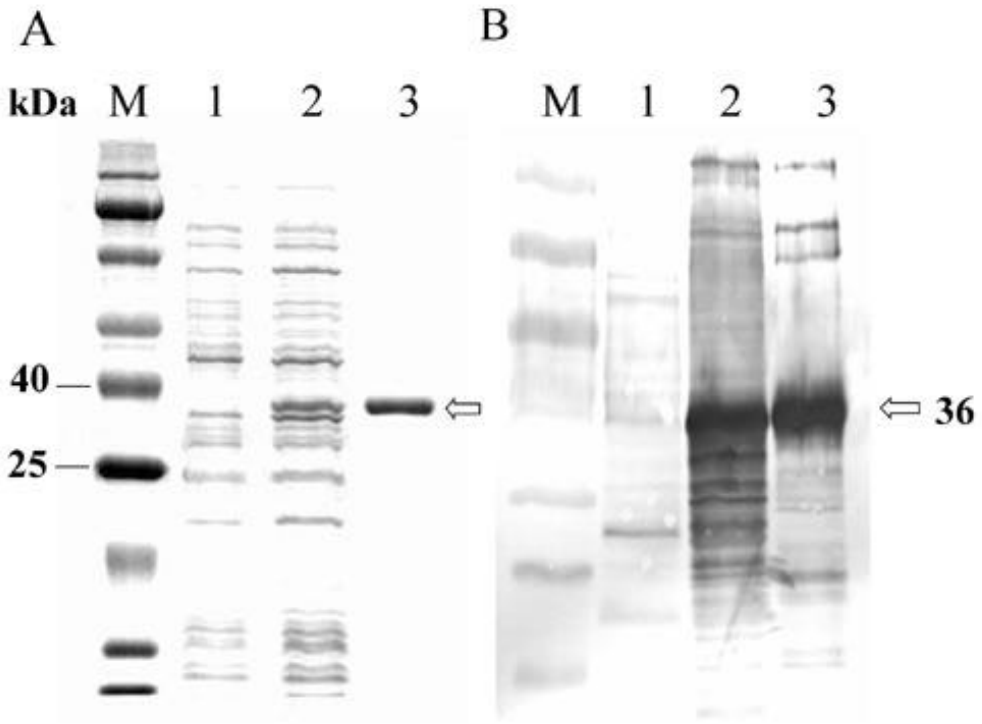

Fig. 6. Analyses of *Chrysanthemum virus B* (CVB) coat protein (CP) expressed by selected bacteria clones in Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (A) and western blotting (B) with antiserum against CVB (Agdia Inc., Elkhart, IN, USA). Lane M, protein standard markers; Lane 1, IPTG induced culture lysate of bacteria clone without CVB CP gene inserted pET-28b(+); Lane 2, IPTG induced culture lysate of bacteria clone containing CVB CP gene inserted expression vector pET-28b(+); Lane 3, purified CVB CP expressed in bacteria.

Table 3. Reactivity in indirect ELISA of the antiserum #107 against bacteria-expressed *Chrysanthemum virus B* (CVB) coat protein compared to a CVB antiserum (CVB-Agdia) purchased from Agdia Inc[1]

Sample number	Antiserum	
	#107	CVB-Agdia
1	2.232	1.460
2	1.284	0.940
3	0.426	0.661
4	2.469	1.611
5	0.834	0.624
6	0.824	0.732
7	2.494	1.801
8	2.128	1.475
CVB-infected chrysanthemum	3.158	1.635
Healthy chrysanthemum	0.100	0.101

[1] Antigen-coating type of indirect ELISA as described previously was conducted to compare the reactivity of antiserum #107 against bacteria-expressed CVB CP with that of CVB antiserum purchased from Agdia Inc.. Routinely 0.1 g of leaf tissue randomly taken from each chrysanthemum sample was grinded in 3 ml of coating buffer (30x of dilution) for indexing in indirect ELISA.

[2] Reactivities of the antisera are shown as the absorbance readings (A_{405nm}) taken 40 min after the addition of enzyme substrate solution. The figures shown are the average value of four replicate wells. Absorbance readings of test samples higher than two times of healthy control chrysanthemum readings are considered to be positive reaction.

gradually shared a significant percentage of exporting market. In a previous report, we documented the occurrence of TAV in chrysanthemum in Taiwan[3,19]. In this study, we further provide evidence showing that another internationally important chrysanthemum viral agent, CVB, also occurs in Taiwan. To our knowledge, this is the first report about the occurrence of CVB in our country. Our results indicated that commercial cut flower chrysanthemum variety such as Huang-Xiu-Fang and many others were commonly infected by CVB. Fortunately, CVB does not always induce visible symptoms on the infected plants, unless as shown in this study that in cold seasons when air temperature drops below 15℃. This is probably the reason why such extensive infection of CVB on chrysanthemum in Taiwan has never drawn the attention of our chrysanthemum growers. This situation also indicates most traditional old chrysanthemum varieties in Taiwan may have certain levels of resistance to CVB. However, during the recent field surveys we have found that some varieties newly introduced from Japan have suffered the infection of CVB by developing symptoms more severe than those old traditional ones (unpublished data). Therefore, we need to point out that there is still potential for CVB to jeopardize chrysanthemum production once new susceptible varieties become dominate in the industry. We strongly recommend that virus certification program, similar to those have been implemented many decades in most international chrysanthemum cutting production companies, should be introduced and established in the domestic chrysanthemum cutting production system to avoid CVB from jeopardizing our chrysanthemum industry.

In order to implement virus certification system in chrysanthemum industry, the constant supply of high quality specific virus antisera for indexing purpose is crucial. Although antisera to chrysanthemum viruses can be purchased from some international companies, they are always expensive and their supplies are sometimes undependable. For this reason, we have attempted to prepare our own chrysanthemum virus antisera and succeeded to produce polyclonal antiserum against TAV using bacteria-expressed CP as immunogen[19]. In that study, we have shown that reactivity of the TAV antiserum is comparable to that prepared by traditional means. Therefore, the same strategy was followed in this study and again an antiserum (#107) to bacteria-expressed CVB CP was successfully prepared. Similarly, we were able to show again the bacteria-expressed CP antiserum to CVB is feasible to be applied in the routine virus indexing. During comparative studies, we were impressed by the EIA readings resulted by antiserum #107 being always higher than those reacted with Agdia's CVB antiserum. Furthermore, similar to other bacteria expressed viral CP antisera made in our laboratory[4,6,8,9,19], antiserum #107 reacts to healthy control antigens with very lower background readings (Table 3), which is considered as one of the key criteria for evaluation a high quality antiserum.

Our study shows that, despite the source of antisera used, EIA readings of CVB indexing obtained during cold season are always higher than those obtained in hot summer times. This result indicates that air temperature lower than 20℃ maybe in favor of the multiplication of CVB and therefore helps the accumulation of virus concentration in chrysanthemum tissue. Similar phenomenon was also observed in *Tuberose mild mosaic virus* (TMMV)[8] and many others[9,19]. This result implies that winter months are more appropriate than other seasons to perform indexing of CVB and many other viruses with similar properties. Correct conclusion is expectable if virus survey and indexing are carried out in conditions suitable for their multiplication.

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摘 要

林玫珠[1]、張清安[1,2]、陳金枝[1]、鄭櫻慧[1] 2005. 菊花病毒 B 於台灣菊花上之分佈及應用細菌表達鞘蛋白策略製備抗體之探討. 植病會刊 14:191-202. ([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]聯絡作者，電子郵箱：cachang@wufeng.tari.gov.tw)

菊花病毒 B(*Chrysanthemum virus B,* 簡稱 CVB) 乃一分佈全球，可造成菊花減產之重要病毒。自民國 89 年至 94 年，我們針對彰化縣內各菊花種苗繁殖圃及切花生產田區進行 CVB 發生情形之調查。在 9 個不同地點共收集 504 株菊花葉片樣品，以間接式酵素連結免疫分析法 (indirect ELISA) 進行病毒偵測，結果其中有 394 株被偵測出感染 CVB，這是台灣地區首次發現 CVB 感染菊花之證據。在田間偵測中我們同時發現 CVB 的偵測與樣品採集時間之氣溫變化具相關性；在 15-20℃ 的低溫季節可輕易地以 ELISA 檢測出感染 CVB 之菊花，且所得之 EIA 值均非常明確。然而，當溫度上升至 25℃ 以上，相同樣品之 EIA 值會下降，趨近於健康對照之 EIA 值；這些結果顯示台灣的冬季較適宜執行 CVB 之檢測。為了解台灣 CVB 分離株之特性，本試驗根據已發表之 CVB 鞘蛋白核酸序列設計出一組專一性引子對 (CVB-up 及CVB-dw)，可針對菊花樣品進行 RT-PCR 而穩定增幅出一個含 1028 個核啟酸之 DNA 產物。該DNA 序列可轉譯出一個含 316 胺基酸之蛋白質，且與 20 個登錄於 GenBank 之 CVB 鞘蛋白(CP) 基因序列比對後發現其核啟酸序列相同度介於 78.4 - 88.0% 之間，而胺基酸序列之相同度則介於 83.1-93.7%，證實此序列即為台灣菊花上所分離之 CVB 之鞘蛋白基因。為製作專一性抗體供未來 CVB 之偵測，本試驗遵循本研究室過去已經多次報告之策略，利用細菌表現病毒鞘蛋白之方式，將 CVB 之 CP 基因構築於細菌表達載體 pET28b(+) (Novogen, Inc., Madison, WI, USA) 中，再將其轉型於 *E. coli* strain Rosetta (DE3) 菌株，經由 IPTG 之誘導使細菌大量表達 CVB 之 CP。應用純化之表現蛋白為抗原，已經成功製備出一株抗血清 (#107)，此抗血清可應用 indirect ELISA 偵測感染 CVB 之菊花，與商用 CVB 抗血清 (Agdia Inc. Elkhart, IN, USA)比較，感病組織在相同稀釋倍數下本策略所製備之抗血清其 EIA 值經常大於購自 Agdia 之商用抗血清，且對健康無病菊花之背景值極低，證明 #107 確實為一優質之檢測抗體。

關鍵詞：菊花、菊花病毒B、表現載體、反轉錄聚合酵素連鎖反應、血清、細菌表現病毒鞘蛋白