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Nucleotide sequence analysis of the coat protein genes of two isolates of *Sweet potato feathery mottle virus* from central Taiwan

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ABSTRACT

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Two potyvirus-like isolates, CY1 and CY2, were collected from sweet potato displaying leaf symptoms of mosaic or vein mottling at Chia-Yi area, Taiwan, by single-lesion isolation on *Chenopodium quinoa plants*. Using the degenerate primers for potyviruses, a 1.2 kb and a 1.3 kb DNA fragments were amplified from CY1- and CY2-infected tissues of *C. quinoa*, respectively, by reverse-transcription polymerase chain reaction (RT-PCR). After cloning and sequencing, the two cDNA fragments were found to be of 1205 and 1351 bp, and corresponding to a part of the 3' end of nuclear inclusion (NIb) region and the 5' end of the coat protein (CP) region of potyviruses. For amplification of the region corresponding to the 3' end of CP region, the 3' untranslated region (3'-UTR), and the poly A tail, RT-PCR was conducted with the oligo(dT) primer and the specific primers designed from the known sequence. The assembled cDNA sequences of 1249 and 1383 bp, respectively, from CY1 and CY2 were elucidated to reflect the 3'-terminal region of nuclear inclusion b (NIb) protein gene [85 nt (28 aa) / 213 nt (71 aa)], the complete CP gene [939 nts (313 aa) /945 nts (315 aa)], and 3'-UTR (both 225 nt) and a poly (A) tail. Sequence analysis indicated that the two viruses were isolates of *Sweet potato feathery mottle virus* (SPFMV). The two isolates showed 80.6% nucleotide identity and 86.3% amino acid identity in their CP genes. A putative proteolytic cleavage site Q/S was predicted between NIb protein and CP. A DAG triplet for aphid transmissibility of potyviruses was found at the 9-11 residues from the N-terminus of both CP genes. Phylogenetic analysis of CP sequences revealed that SPFMV-CY1 belonged to the group C and was closely related to the isolate 6, Ita1 and MD1/1, while SPFMV-CY2 belonged to the group O and was closely related to the isolates 115/1S, 5, Arua 10a, O and TZ4. The sequence relationships between the two isolates and potyviruses revealed that SPFMV-CY1, SPFMV-CY2 were closely related to the sweet potato infecting potyviruses, SPVY, SPVG and SPLV, reflecting a more recent evolutionary relationship.

Key words: *Sweet potato feathery mottle virus*, nucleic acid cloning, sequence analysis

INTRODUCTION

Sweet potato feathery mottle virus (SPFMV), a member of potyvirus, occurs wherever sweet potato is grown. It is the most important and widespread virus among the 20 viruses detected in sweet potato [20, 26]. SPFMV particles are elongated, flexuous rods of 810-865 nm with a single-stranded, positive sense RNA genome of 10.6 kb, 10-15% larger than that of typical potyviruses [18, 19]. Mixed infection of SPFMV with other viruses usually generated synergistic effects and caused more serious loss in crop yield [6, 9].

SPFMV was first described in the United States about 60 years ago [30]. The virus are transmitted by aphids in a nonpersistent manner [4, 14]. The host range of SPFMV is restricted in the genus *Chenopodium* of the family *Chenopodiaceae*, and the genus *Ipomoea* of the family *Convolvulaceae*. Some isolates can infect *Nicotiana benthamiana* Domin [6, 22]. In addition, several isolates have been identified according to symptomatology, host range, aphid transmissibility and serological relationship [6, 21]. Based on the host range and symptomatology, four major strains have been described, including the common (C) strain that infects *Convolvulaceae* and induces foliar symptoms on sweet potato, but does not infect *Chenopodium amaranticola* Coste & Reyn. [16, 19], the russet crack (RC) strain that induces lesions on roots of some sweet potato cultivars and produces local lesions on *C. amaranticola* [5, 6], the yellow vein (YV) strain that induces severe symptoms on *Ipomoea nil* (L.) Roth, and the 835 strain from Guatemala that infects *N. benthamiana*. Strains RC, YV, and 835 are serologically close related, whereas strain C is distantly related to the others [1]. The occurrence of SPFMV was first noticed in Taiwan in 1972 [8, 16]. Based on host reactions, aphid transmissibility and stability in saps, SPFMV was originally designated as SPV-A in Taiwan [16].

Based on the coat protein (CP) and the complete genome sequences of members of the family *Potyviridae*, the taxonomy of these viruses become available and can be used to distinguish sequences of strains of the same virus species [2, 28]. Based on comparisons of the amino acid sequences of potyvirus coat protein core regions, there were four levels of sequence identities: the lowest level of 20-30% corresponding to different genera, the second level of 55-75% reflects the differences between distinct species, the third level of 74-88% reflects the existence of subspecies and the fourth level of 90-99% corresponds to strains of one species [29]. The study of comparisons of all the 1220 complete CP genes of potyviruses suggest that 76-77% nucleotide identity is the optimal species demarcation criterion for the CP [2]. Many isolates of SPFMV have been characterized from different regions of the world where sweet potato is cultivated. The sequences data deposited in GenBank revealed the genetic grouping [15, 21] of SPFMV isolates. Four genetic groups have been classified according to the size of CP and the nucleotide and amino acid identities of the CP among strains [30]. The amino acid data from the computer-assisted translation indicated that the amino acid sequences of group C were shorter than those of the other groups [23, 30]. The major difference is resulted from the absence of CP amino acid residues 62 and 63. All the isolates of SPFMV contain the DAG triple peptide, the motif which is essential for aphid transmission of potyviruses. The phylogenetic analysis indicated that all the isolates from Tanzania and Uganda belong to group EA, while the strains from Australia, Korea, America, China and Egypt belong to group RC [30]. The CPs of the isolates belonging to group C, originated from many different countries, differ in their lengths [30]. The available sequence data indicate that SPFMV isolates from East Africa form a distinguishable group EA that is not found so far outside that region [30].

In this paper, we present and analyze sequence data of the complete CP genes of the two isolates of SPFMV collected from Chia-Yi area of central Taiwan. Their sequences were compared with those of seventy-three isolates of SPFMV and twenty-four other potyviruses deposited in GenBank, to analyze their relationships with other SPFMV strains and *Potyvirus*.

MATERIALS AND METHODS

Plant materials and isolation of viruses

The sweet potato [*Ipomoea* batatas (L.) Lam. cv. Tainung 66] showing mosaic or mottle symptoms were collected from Chia-Yi area, Taiwan. Mechanical transmissions was performed by grinding infected tissues with a mortar and pestle in 0.1 M phosphate buffer, pH 7.5, and the mixture was mechanically introduced, with cotton-tipped swabs onto carborundum-dusted leaves of *Chenopodium quinoa* Willd. plants. After three single-lesion transfers, the *C. quinoa* plants showing local lesions uniformly were maintained in the greenhouse as the virus source. Crude extracts from dipping of infected leaves of *C. quinoa* were negatively stained with 2% dodeca phosphotungstic acid and examined by a JEOL-100 electron microscope (JEOL, Tokyo, Japan).

Viral RNA extraction and cDNA amplification

ULTRASPEC™ RNA isolation system (Biotex Laboratories, Houston, TX) was used to prepare total RNA from the *C. quinoa* plants with coalescent chlorotic spots

10 days post-inoculation (dpi). The potyviral degenerate primer PotI [11] was used for reverse transcription (RT) to synthesize the first strand cDNA from 2 μg of total RNA using the cDNA synthesis kit (Strategene, La Jolla, CA). The polymerase chain reaction (PCR) was performed for amplification of the coat protein (CP) gene from the synthesized cDNA, using Ex-Taq polymerase (Takara, Shiga, Japan) according to the manufacturer's protocol and the degenerate primers PotI and PotII derived from conserved regions of the genomes of potyviruses [10, 11, 12]. The thermal cycling scheme used for 35 cycles was as follows: template denaturation at 94℃ for 1 min, primer annealing at 55℃ for 2 min and DNA synthesis at 72℃ for 3 min. The amplification products were analyzed by electrophoresis on 1% agarose gel. For amplification of the 3' end of the CP region, untranslated region (3'-UTR) and the poly A tail, RT-PCR was conducted using the oligo (dT) primer and the specific primers (5'-CCTGAGCAACATAACATTGGG-3' for CY1 isolate and 5'-TTCCAAGCATGGTATGAGGG-3' for CY2 isolate) designed from the known sequence of cDNA fragments amplified.

Cloning and sequencing

The PCR amplified products were cloned into the PCRII-TOPO vector (TOPO TA cloning kit, Invitrogen, Carlsbad, CA), according to the instructions of the manufacturer. Plasmid clones with expected inserts were identified and used for sequence analysis. Sequencing of the cDNA clones from at least two independent clones was performed by dideoxynucleotide chain termination method [25], carried out using the T7 Sequenase version 2.0 DNA Sequenceing Kit (United States Biochemical Corp., Cleveland, OH) according to manufacturer's instructions. Nucleotide sequences were assembled and amino acid sequences were predicted using PC/GENE 6.85 software (IntelliGenetics, Inc., University of Geneva, Geneva, Switzerland). Multiple sequences were aligned by the PILEUP program in the BESTFIT program of the GCG package (Wisconsin Package version 10.0, Genetic computer Group, WI, USA). Phylogenetic analysis of the CP genes of CY1 and CY2 with those of other SPFMV isolates (Table 1) and potyvirues from the GenBank was conducted using the NEIGHBOR-JOINING routines of PHYLIP software package version 3.63 (by Joseph Felsenstein and the University of Washington). The GenBank accession numbers of the potyvirus isolates used in this study: BCMV (*Bean common mosaic virus*, NC 003397), BYMV (*Bean yellow mosaic virus*, D83749), CVbMV (*Chili vein-banding mottle virus*, U72193), DsMV (*Dasheen mosaic virus*, AF048981), LMV (*Lettuce mosaic virus*, X97705), OYMV (*Onion yellow dwarf virus*, NC005029), PepMoV (*Pepper mottle virus*, AB126033), PeSMV (*Peper severe mosaic virus*, AM1811350), PLDMV (*Papaya leaf-distortion mosaic virus*, NC 005028), PPV (*Plum pox virus*, X81083), PRSV (*Papaya ringspot virus*, X97251), PSbMV (*Pea seedborne mosaic virus*, D10930), PVA (*Potato virus* A, Z21670), PVY

Table 1. Seventy-three isolates of *Sweet potato feathery mottle virus* (SPFMV) used in this study

Virus species/isolate	Genetic group	Geographical origin (region, country)	Accession no.	Reference
115/1S	O	Kenya	AY523540	Unpublished
25/4A	C	Kenya	AY523543	Unpublished
45/3S	C	Kenya	AY523544	Unpublished
46b	EA	Kenya	AY523548	Unpublished
5	O	Cordoba, Argentina	U96624	(15)
51/9S	C	Kakamega, Kenya	AY459591	Unpublished
54/9S	EA	Kakamega, Kenya	AY459592	Unpublished
6	C	Cordoba, Argentina	U96625	(15)
85/7S	EA	Kisii, Kenya	AY459593	Unpublished
97/5S	C	Kisumu, Kenya	AY459594	Unpublished
Apa	EA	Apach, Uganda	AJ781787	(30)
Arua10a	O	Arua, Uganda	AY459595	Unpublished
Aus120-7	RC	Kimberley, E Australia	AM050889	Unpublished
Aus142-AC	C	Kimberley, E Australia	AM050891	Unpublished
Aus142-ARC	RC	Kimberley, E Australia	AM050890	Unpublished
Aus2	RC	Perth, W Australia	AJ781775	(30)
Aus4c	C	Perth, W Australia	AJ781778	(30)
Aus5	RC	Perth, W Australia	AJ781776	(30)
Aus55-4C	C	Broom, Australia	AM050892	Unpublished
Aus5C	C	Perth, W Australia	AJ781779	(30)

Table 1. Seventy-three isolates of *Sweet potato feathery mottle virus* (SPFMV) used in this study (con.)

Virus species/isolate	Genetic group	Geographical origin (region, country)	Accession no.	Reference
Aus6	RC	Perth, W Australia	AJ781777	(30)
Bag	EA	Bagamoyo, Tanzania	AJ781780	(30)
BAU	O	Bauchi, Nigeria	AJ010699	(15)
Bkb1	EA	Bukoba, Tanzania	AJ781781	(30)
Bkb2	EA	Bukoba, Tanzania	AJ781782	(30)
Bny	EA	Bushenyi, Uganda	AJ539130	(21)
C	C	North Carolina,USA	S43451	(1)
Canar3	EA	Canary Islands, Spain	AY459600	Unpublished
CH	O	China	Z98942	(12)
CH2	C	China	AJ001440	(12)
Jinan	RC	Shandong, China	AJ310201	(7)
CY1	C	Taiwan	EF492048	This study
CY2	O	Taiwan	EF492049	This study
Egypt1	RC	Cairo, Egypt	AJ515378	(13)
Egypt9	RC	Cairo, Egypt	AJ515379	(13)
Ita1	C	Latium, Italy	AM076411	(23)
KAB1	EA	Kabarole, Uganda	AJ010698	(15)
kby1	EA	Mipigi, Uganda	AJ781791	(30)
kby2	EA	Mipigi, Uganda	AJ781792	(30)
K1	RC	Korea	AF015540	(24)
K2	O	Korea	AF015541	(24)
MAD	EA	Antisirabe, Madagascar	AJ010700	(15)
MBL	EA	Mbale, Uganda	AJ010701	(15)
Mbl2	EA	Mbale, Uganda	AJ781788	(30)
MD1/1	C	South Africa	AY459601	Unpublished
Mis1	EA	Misungwi, Tanzania	AJ781783	(30)
Mpg2	EA	Mpigi, Uganda	AJ781789A	(30)
Nak	EA	Nakasongola, Uganda	J781790	(30)
NAM 3	EA	Namulonge, Uganda	AJ010702	(15)
NAM 1	EA	Namulonge, Uganda	AJ010704	(15)
NAM12	C	Namulonge, Uganda	AJ010703	Unpublished
NAM6	EA	Namulonge, Uganda	AJ010705	(15)
NIG 3	O	Niger	AJ010705	(15)
O	O	Japan	D16664	(17)
Pink-2C	C	Kimberley, E Australia	AM050893	Unpublished
Portugal	EA	Portugal	AY459599	Unpublished
Putisrabe	EA	Madagascar	AY459597	Unpublished
RAK	EA	Rakai, Uganda	AJ010706	(15)
Rakai6e	EA	Uganda	AY523537	Unpublished
RC	RC	North Carolina, USA	S43450	Unpublished
RUK	EA	Rukungigi, Uganda	AJ010707	(15)
Ruk55-2	EA	Uganda	AY523550	Unpublished
S	RC	Japan	D86371	Unpublished
Sor	C	Soroti, Uganda	AJ539129	(21)
Spain1C	C	Spain	AY518937	Unpublished
Spain1RC	EA	Spain	AY518938	Unpublished
Tar1	EA	Tarime, Tanzania	AJ781784	(30)
Tar2	EA	Tarime, Tanzania	AJ781785	(30)
TZ1	EA	Bagamoyo, Tanzania	AJ539131	(21)
TZ2	EA	Bagamoyo, Tanzania	AJ539132	(21)
TZ4	O	Tanzania	AY459598	Unpublished
Unj1	EA	Unguja, Tanzania	AJ781786	(30)
XN3	RC	China	AY459602	Unpublished

(*Poato virus* Y, PVU09509), SMV (*Sugarcane mosaic virus*, NC 002634), SPFMV (*Sweet potato feathery mottle virus*, EF492048), SPLV (*Sweet potato latent virus*, EF492050), SPMSV (*Sweet potato mild specking virus*, SPU61228), SPVG (*Sweet potato virus* G, Z83314), SPVY (*Sweet potato virus* Y, AY4559611), TEV (*Tobacco etch virus*, M15239), TuMV (*Turnip mosaic virus*, AF530055), WMV2 (*Watermelon mosaic virus* 2, AB218280), YMV (*Yam mosaic virus*, YMU42596), and ZYMV (*Zucchini yellow mosaic virus*, NC 003224). The repeatability of the branching orders obtained was estimated using the SEQBOOT program of (PHYLIP software) for bootstrap resampling (100 bootstrap reiterations) the multiple sequence alignment. Bootstrap consensus trees were then built using the CONSENSE program and the NEIGHBOR unrooted tree was drawn using the DRAWTREE program of the PHYLIP software.

RESULTS

Isolation of the virus and RNA extraction

The sweet potato plants with mosaic or mottle symptoms collected from Chia-Yi area, Taiwan were used as the source for virus isolation. Two different types of chlorotic spots developed on inoculated leaves of *C. quinoa* after mechanical inoculation (Fig. 1A). The faint chlorotic spots developed on plants of *C. quinoa* 14-16 dpi (Fig. 1B left) and the distinct chlorotic spots 7-10 dpi (Fig. 1B right). The host reactions on sweet potato and *C. quinoa* were similar to those reported by Liao *et al.*[16]. When the crude extracts from leaf-dipping of *C. quinoa* were examined under the electron microscope, flexuous rods of 800-850 nm in length were observed (data was not shown). Two typical isolates, CY1 originated from a faint chlorotic spot and CY2 from a distinct chlorotic spot, were selected for further studies. The *C. quinoa* plants infected with each isolate were maintained in the greenhouse as the source for total RNA extraction.

Reverse transcription-polymerase chain reaction (RT-PCR) and cloning

The RT-PCR products amplified from total RNA of *C. quinoa* plant tissues with different chlorotic spots by the potyvirus specific primer pair PotI/PotII were cloned into PCRII-TOPO plasmid and subsequently sequenced. A cDNA fragment of 1205 bp was amplified from the total RNA extracted from the CY1-infected *C. quinoa* plants with chlorotic spots (Fig. 1B left), and a cDNA fragment of 1351 bp was amplified from the CY2-infected *C. quinoa* plants with distinct chlorotic spots (Fig. 1B right). Both cDNA fragments reflected a part of the 3' end of NIb region and the 5' end of the (CP) region, of a potyvirus.

For amplification of the 3' end of the CP region, the 3'-UTR, and the poly A tail, the specific forward primers, 5'-CCTGAGCAACATAACATTGGG-3' and 5'-TTCCAAGCATGGTATGAGGG -3' were designed for CY1 and CY2 isolates, respectively. Then the DNA

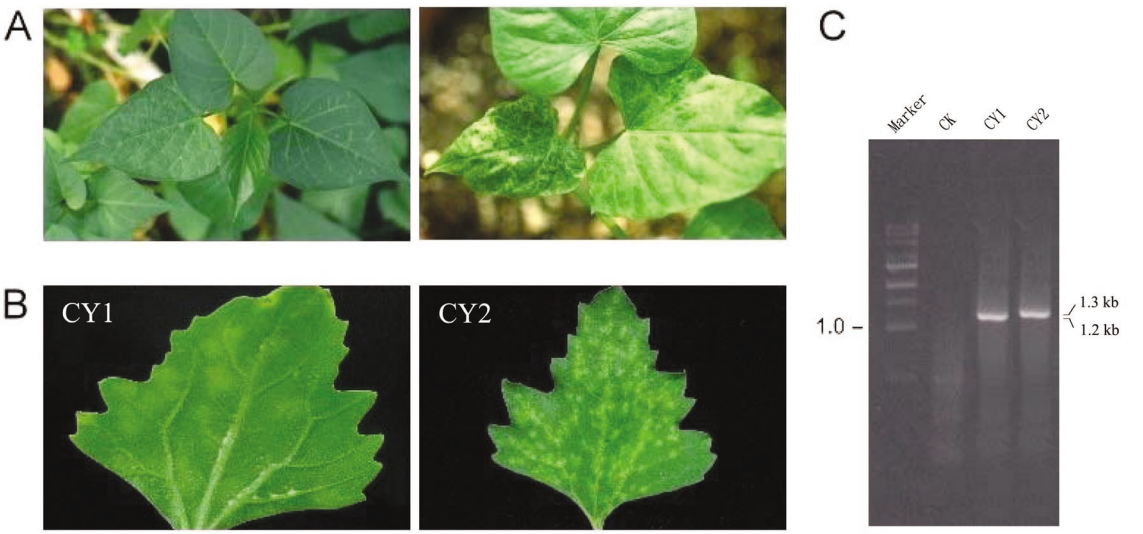

Fig. 1. Symptoms on sweet potato cultivar TN66 and *C. quinoa*, and agarose gel electrophoresis of two cDNA fragment corresponding to the 3'-terminal region of CY1 and CY2 virus collected. (A) Vein mottle and mosaic on samples of sweet potato cultivar TN66 collected from field. (B) Faint chlorotic spots on *C. quinoa* 14 dpi (isolate CY1, left) and distinct chlorotic spots an *C. quinoa* 10 dpi (isolate CY2, right). (C) The DNA fragment amplified from total RNA isolated from CY1- or CY2-infected tissue of *C. quinoa* with spot symptoms with primers PotI and PotII by RT-PCR. Lane CK, a uninfected *C. quinoa* control. Lane CY1, the CY1-infected *C. quinoa*. Lane CY2, the CY2-infected *C. quinoa*.

fragments of 830 nts and the 780 nts for CY1 and CY2, respectively, were amplified by RT-PCR with oligo(dT) and the specific forward primers. Both large and small cDNA fragments of CY1 and CY2 were cloned and their sequences were determined and assembled for the complete CP sequences. A DNA sequence of 1249 nts, corresponding to the partial NIb (85 nt), the complete CP (939 nts) region, and the 3'-UTR (225 nts) followed by a poly-A tail of CY1 was elucidated (Fig. 2). Another DNA sequence of 1383 nts, corresponding to the partial NIb region (213 nts) the CP region (945 nts) and the 3'-UTR (225 nts) followed by a poly-A tail of CY2 was determined (Fig. 3). The deduced amino acid sequences of the complete CP gene contained 313 and 315 residues of CY1 and CY2, respectively, both showing a close relationship to SPFMV, indicating they were isolates of SPFMV. As with other potyviruses [27], the putative proteolytic cleavage site VYHQ/S between NIb and CP was predicted for the two isolates. For both CY1 and CY2, the first significant domain in the CP was the DAG box, which is involved in aphid transmission, was located at amino acid 9-11 residues from the proposed N-terminus of the CP. The MVWCIENGTSP conserved region of potyviruses was also conserved in both CPs of the two isolates (Fig. 2&3).

Sequence comparisons of strains of SPFMV and potyviruses

Sequence comparison of the CP gene of CY1 and CY2 isolates revealed 80.6% nucleotide identity and

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5'-CGATCTCAGCGAGTACACGCGTGTCCTTAACGAGATGTATGATGATAGCCTCCTTCAAGATAATGATTTAATGGTGTATCATCAATCTGA   90
   D  L  S  E  Y  T  R  V  L  N  E  M  Y  D  D  S  L  L  Q  D  N  D  L  M  V  Y  H  Q/ S  D

TAACCCCCCTGAATTTAAAGATGCAGGTGTGAACCCACCAGCCACCAAAGCCAAAAGGACCATACACACACCAGAAATCACAGAAATTAC   180
   N  P  P  E  F  K [D  A  G] V  N  P  P  A  T  K  A  K  R  T  I  H  T  P  E  I  T  E  I  T

TGAGCCCGAGGATCCCAAACAAGCTGCGCTTCGCGAAGCTAGACAGAAACAACCTGCTGTAACACCCGAATCATACGGTAGAGATACAGG   270
   E  P  E  D  P  K  Q  A  A  L  R  E  A  R  Q  K  Q  P  A  V  T  P  E  S  Y  G  R  D  T  G

TGAGAAGCCTATGCCCTCTGTTTCACCACAAAGGGTGAAAGACAAGGATGTCAATGTTGGTACGACAGGTACATTTTTAGTACCGCGAGT   360
   E  K  P  M  P  S  V  S  P  Q  R  V  K  D  K  D  V  N  V  G  T  T  G  T  F  L  V  P  R  V

TAAGCTTCATACTAGTAAAATGCGCCAACCCAGAGTCAATGGAATCTCTGTAGTAAACTTACAACACCTTGCAACCTATGAACCTGAGCA   450
   K  L  H  T  S  K  M  R  Q  P  R  V  N  G  I  S  V  V  N  L  Q  H  L  A  T  Y  E  P  E  Q

ACATAACATTGGGAATACACGCTCAACTCAGGAACAGTTTCAAGCATGGTATGAAGGTGTCAAGGGTGATTATGGTGTTGATGACGCTGG   540
   H  N  I  G  N  T  R  S  T  Q  E  Q  F  Q  A  W  Y  E  G  V  K  G  D  Y  G  V  D  D  A  G

AATGGCAATCTTGTTGAATGGATTGATGGTATGGTGCATAGAGAATGGAACATCTCCAAATATAAATGGCGTTTGGACGATGATGGATGG   630
   M  A  I  L  L  N  G  L  M  V  W  C  I  E  N  G  T  S  P  N  I  N  G  V  W  T  M  M  D  G

GGATGAACAAGTGACGTACCCCATAAAGCCATTACTGGATCATGCAGTGCCTACTTTTAGGCAAATCATGACACACTTCAGTGACGTTGC   720
   D  E  Q  V  T  Y  P  I  K  P  L  L  D  H  A  V  P  T  F  R  Q  I  M  T  H  F  S  D  V  A

TGAAGCGTATATAGAAATGAGGAATCGCACCAAGGCATATATGCCTAGGTATGGATTCCAACGTAATTTGACTGATATGAGTCTTGCGCG   810
   E  A  Y  I  E  M  R  N  R  T  K  A  Y  M  P  R  Y  G  F  Q  R  N  L  T  D  M  S  L  A  R

ATATGCATTTGATTTCTATGAGCTGCACTCAACAACACCTACACGTGCTAAAGATGCACACATGCAGATGAAAGCAGCTGCACTTAAGAA   900
   Y  A  F  D  F  Y  E  L  H  S  T  T  P  T  R  A  K  D  A  H  M  Q  M  K  A  A  A  L  K  N

TGCGCATAATCGGTTGTTTGGTTTGGATGGAAACGTCTCCACGCAAGAGGAGGATACGGAGAGGCACACTGCGACTGATGTTACTAGAAA   990
   A  H  N  R  L  F  G  L  D  G  N  V  S  T  Q  E  E  D  T  E  R  H  T  A  T  D  V  T  R  N

TATACATAACCTCTTAGGAATGAGGGGTGTGCACTAGGTGAAACTTTGCACTGTATTTATTACTTATGTGGTTTTTAGTATGCCTTTAAT  1080
   I  H  N  L  L  G  M  R  G  V  H  *

TAAATTCGTGTTCTTCAGTCCCGACAGAAATGGTTGGGTGTATCGACAGAGTGGGCTTCTAGCTTGGTCCATACACCTGAGAAGTTTCTG  1170

GTCTATTACGTATCATAAGGGACTCTTAAAAGTGAGGAGTACCTCGTAAGAAAAGCCTTTTTGGTTCGTGTTCGAGCCCAAAAAAAAAAA  1260

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3'                                                  1299
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Fig. 2. The nucleotide and deduced amino acid sequences of the cloned 3'-terminal region of CY1 genome (Accession No. EF492048). The cloned 1249 nucleotides of CY1 are shown starting from the second nt. The deduced amino acid sequence contains 313 residues encoding the complete sequence of coat protein (CP). The stop codon is indicated by an asterisk followed by 222 nts of a 3'-UTR. The putative protease cleavage site (Q/S) between nuclear inclusion protein b (NIb) and CP, the conserved region of the core of CP of potyviruses, and the DAG triplet aphid transmissibility, are underlined or boxed.

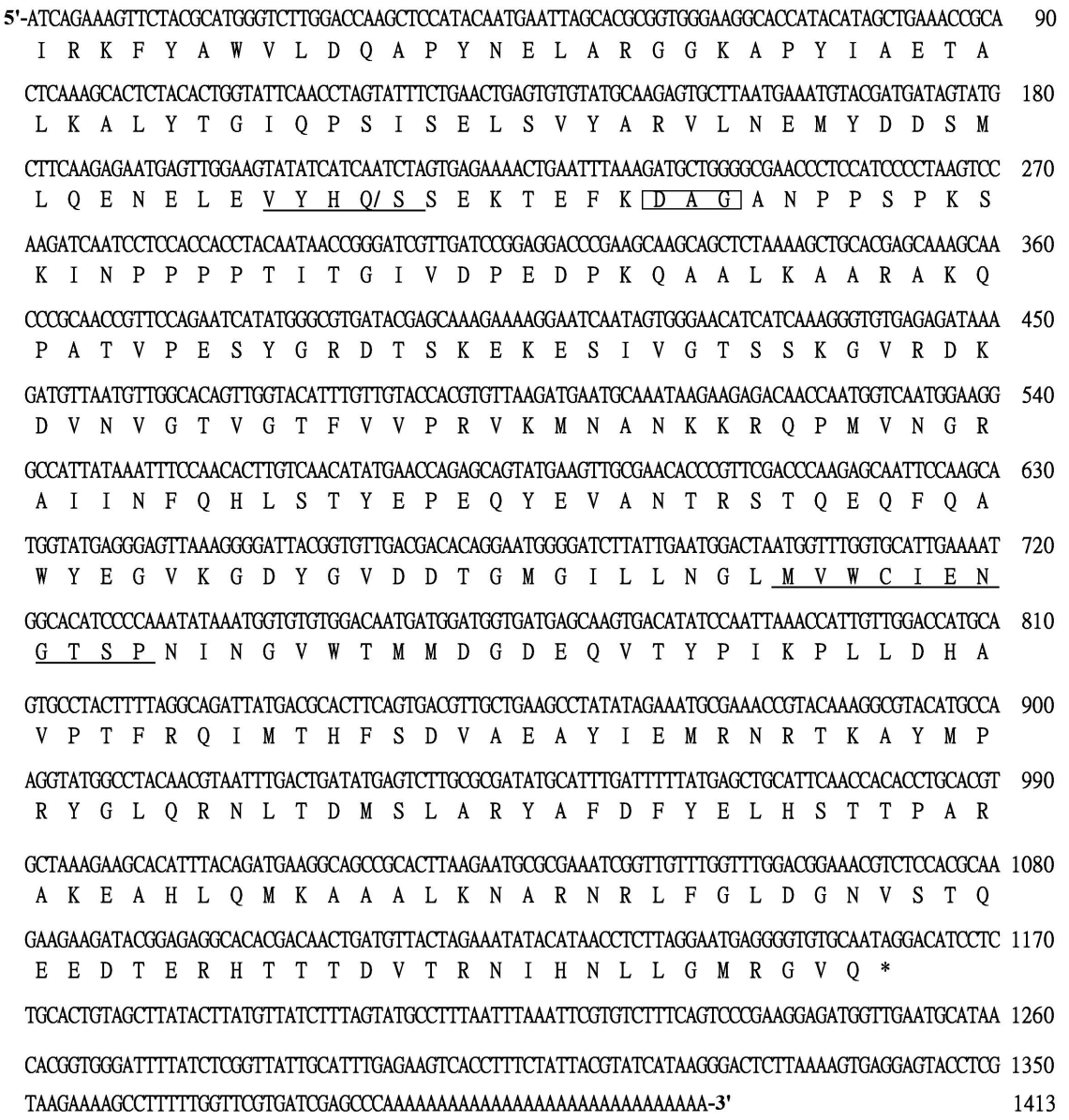

Fig. 3. The nucleotide and deduced amino acid sequences of the cloned 3'-terminal region of CY2 genome (Accession No. EF492049). The cloned 1383 nucleotides of CY2 are shown starting from the first nt. The deduced amino acid sequence contains 315 residues encoding the complete sequence of coat protein. The stop codon is indicated by an asterisk followed by 222 nts of a 3' untranslated region. The putative protease cleavage site (Q/S) between nuclear inclusion protein b (NIb) and CP, the conserved region of the core of the coat protein (CP) of potyviruses, and the DAG triplet aphid transmissibility, are underlined or boxed.

86.3% amino acid identity with each other. The CP nucleotide sequences of CY1 and CY2 were compared with those of the 73 isolates selected from the GenBank (Table 1), including 33 isolates in group EA, 12 isolates in group RC, 10 isolates in group O and 18 isolates in group C (Table 2). The percentages of CP nucleotide and amino acid identities were agreed with the results of Tairo [30]. The phylogenetic tree analysis placed the CY1 to SPFMV group C and CY2 to SPFMV group O, respectively (Fig. 4). Our results indicate that SPFMV-CY1 is closely related to the isolates of 6 from Argentina, Ita1 from Italy, MD1/1 from South Africa; while SPFMV-CY2 is closely related to the isolates of 115/1S from Kenya, 5 from Argentina, Arua 10a from Uganda, O from Japan, and TZ4 from Tanzania. Sequence comparison based on the CP sequences of other potyviruses searched from the GenBank and EMBL databases revealed that CY1 and CY2 are closely related to other potyviruses infecting sweet potato, such as SPVY (*Sweet potato virus* Y), SPVG (*Sweet potato virus* G) and SPLV (*Sweet potato latent*

Table 2. The divergence within the four genetic groups of SPFMV

Subgroup	No. of isolates	Size of CP encoding region		% CP identity within group	
		Nucleotide	Amino acid	Nucleotide	Amino acid
EA	33	945	315	94.5-99.8	94.6-100
RC	12	945	315	97.6-99.3	97.1-99.4
O	10	945	315	91.7-97.6	94.6-96.8
C	18	936-939	312-313	94.0-99.0	93.6-99.4

Sequence comparison included the thirty-three isolates of genetic group EA, twelve isolates of genetic group RC, ten isolates of genetic group O and eighteen isolates of genetic group C.

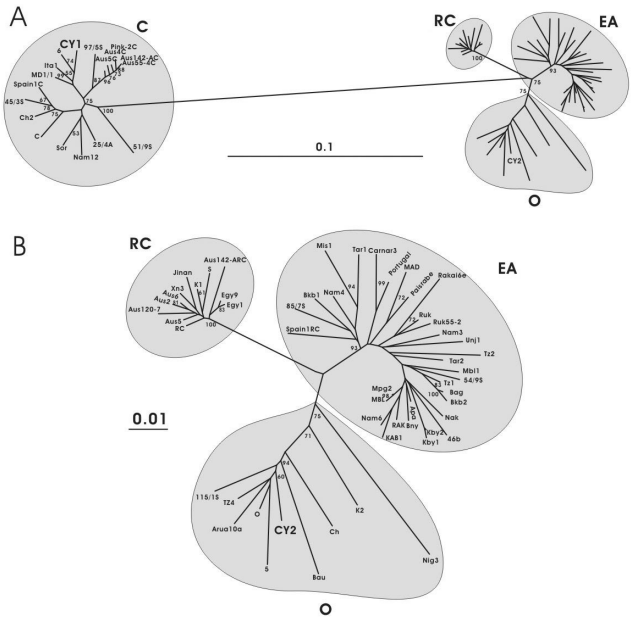

Fig. 4. Phylogenetic relationships of the nucleotide sequences of the CP genes of 73 isolates of *Sweet potato feathery mottle virus* (SPFMV). Thirty three isolates of group EA, 12 isolates of group RC, 10 isolates of group O and 18 isolates of group C are used for analysis. The scale to measure genetic distances is indicated at the lower left. (A) The four major phylogenetic groups of C, RC, EA and O. (B) Higher resolution of groups RC, O, and EA.

virus), reflecting a more recent evolutionary relationship; whereas they are distantly related to other potyvirues such as TEV, PVA, and TuMV (Fig. 5).

DISCUSSION

The virus isolates CY1 and CY2 were isolated from diseased sweet potato plants with mosaic or mottle, that collected in Chia-Yi area. Their symptomatological reactions on sweet potato and *C. quinoa* were similar to those induced by the virus described as SPV-A in Taiwan previously [8, 16]. Electron microscopy also indicated that both isolates are potential members of the genus *Potyvirus*. In this study, we presented molecular evidence of the two isolates, CY1 and CY2, by the sequence analysis of its 3' genomic region, including the complete CP gene and the entire 3'-UTR. Comparison of the CP nucleotide sequences CY1 and CY2 indicated that they belong to different genetic group C and O of SPFMV, respectively.

For detailed analysis of the phylogenetic relationships, a total of 73 SPFMV isolates from GenBank and Tairo [30], representing all SPFMV genetic groups, were used for comparison. Our results indicate that SPFMV-CY1 is closely related to the isolate 6 from Argentina, Ita1 from Italy, MD1/1 from South Africa, and SPFMV-CY2 is closely related to the isolate 115/1S from Kenya, 5 from Argentina, Arua 10a from Uganda, O from Japan, and TZ4 from Tanzania. This indicates that, unlike isolates in the EA group, SPFMV isolates in the other three genetic

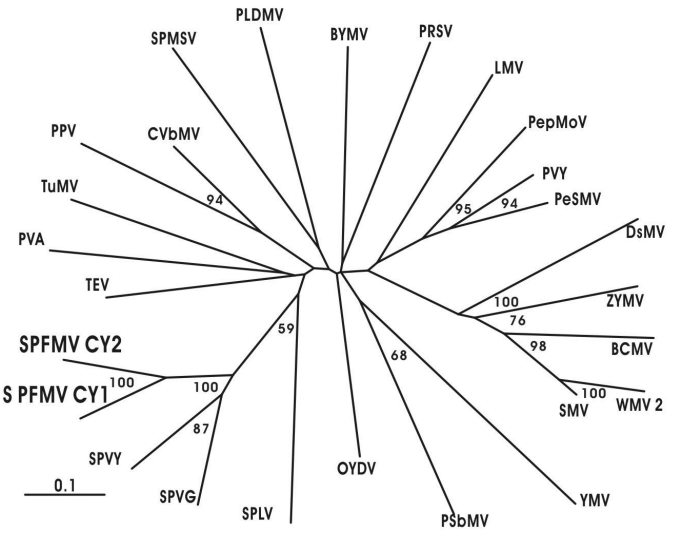

Fig 5. A phylogenetic tree calculated from the deduced amino acid sequences of the coat proteins of 24 potyviruses with highest percent identities selected from GenBank, using the NEIGHBOR-JOINING routines of PHYLIP software package version 3.63 (by Joseph Felsenstein and the University of Washington). The dendrograph was produced using the NEIGHBOR-JOINING algorithm with 100 bootstrap replicates shown at appropriate nodes. The scale to measure genetic distances is indicated at the lower left.

groups of C, O, and RC are not restricted in a particular geographical area [30].

The DAG box at the N-terminal region of CP is essential for aphid transmissibility of potyviruses [3]. From the presence of DAG motif in the CP sequences of CY1 and CY2, it is likely that the two SPFMV are transmitted by aphids in field. According to the complete coat protein sequence data in this study, specific primers could be designed as a diagnostic tool to survey the distribution of SPFMV in field in Taiwan by RT-PCR.

Because of the propagation of sweet potato by vine cutting or storage roots, mixed infection of SPFMV with other viruses frequently occur to cause severe loss of sweet potato production. It is important to build a system to examine the virus-free tissue culture stocks for preventing virus transmissions from vegetative propagation. Therefore, the development of a rapid and more sensitive method for indexing of SPFMV in tissue culture stocks is crucial. The cDNA fragment s of the CP regions of the SPFMV CY1 and CY2 also can be cloned into an expression system in bacteria for protein production. The expressed protein can be used as antigen for production of specific antiserum for large-scale survey of SPFMV.

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摘 要

王麗媛[1, 2]、陳冠君[2]、陳宗祺[2]、葉錫東[2, 3]. 2007. 甘藷羽狀斑駁病毒台灣分離株鞘蛋白基因序列分析. 植病會刊 16：203-213. ([1] 嘉義市農業試驗所嘉義分所植保系；[2] 台中市國立中興大學植物病理學系；[3] 聯絡作者，電子郵件：sdyeh@nchu.edu.tw；傳眞：+886-4-22852501)

於嘉義地區，自呈現斑駁及嵌紋的甘藷葉片接種於奎藜 (*Chenopodium quinoa*) 後，經單斑分離可得兩種不同大小型態之局部黃斑，分別命名爲 CY1 及 CY2 分離株。由感染 CY1 或 CY2 之奎藜萃取得總量核糖核酸 (total RNA) ，利用對應 Potyvirus 屬病毒基因體核糖核酸 3' 端之簡併式引子對 Pot1/PotII，以反轉錄聚合酶連鎖反應增幅出約 1.2 及 1.3 kb 兩個核酸片段。此核酸片段經選殖及核酸序列分析，分別爲長1205及1351個核苷酸 (nucleotide, nt) 序列。與登錄於美國國家生物科技資訊中心 (NCBI) 之馬鈴薯 Y 病毒屬 (*Potyvirus*) 核酸序列比對分析，得知爲甘藷羽狀斑駁病毒 (*Sweet potato feathery mottle virus*, SPFMV)。由此設計專一性引子，將鞘蛋白基因 3' 端非轉譯區 (3' untranslated region, 3'-UTR) 及 poly (A) 尾端解序完成；分別得全長含1249 (CY1) 及 1383 (CY2) 個核苷酸。由 5' 端起此序列分別爲 85 及 213 核苷酸之細胞核內含體b (nuclear inclusion b, NIb) 3' 端基因部分序列、SPFMV-CY1全長度鞘蛋白 (coat protein, CP) 基因爲 939 個核苷酸，SPFMV-CY2 爲 945 個核苷酸，而 3' 端非轉譯區 (3'-UTR) 均爲 225 個核苷酸及 poly A 尾端。其中所含之全長度鞘蛋白分別爲 313 及 315 個胺基酸所組成，兩者之間蛋白酶切位應落於符合 *potyviruses* 特質 Q/S 之間，且由鞘蛋白 N 端起第 9-11 個胺基酸位置具有代表 *potyvirus* 蚜蟲傳播能力之 DAG 序列。比較CY1、CY2 兩個分離株鞘蛋白基因核酸序列有80.3% 的相同度，86.3% 的相似度。與登錄於 NCBI 之 SPFMV 73 個系統鞘蛋白基因比對結果顯示，CY1與CY2 分別屬於基因群 C 及 O；於演化關係上，CY1 與 6、Ita、MD1/1 分離株親緣最近，CY2 與 5、O、Arua10a、 TZ4 及 115/1S 分離株親緣最近。又分析此兩個分離株與24個 *potyviruses* 的演化關係，結果顯示與其他能感染甘藷的病毒 SPVY、SPVG 及 SPLV 親緣關係最近，顯示 SPFMV 與違些病毒具相同演化歷程，其次爲TEV 、PVA 及 TuMV。

關鍵詞：甘藷羽狀斑駁病毒，核酸選殖，序列分析