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Identification and characterization of a potyvirus causing chlorotic spots on *Phalaenopsis* orchids

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Abstract A putative virus-induced disease showing chlorotic spots on leaves of *Phalaenopsis* orchids was observed in central Taiwan. A virus culture, phalaenopsis isolate 7-2, was isolated from a diseased *Phalaenopsis* orchid and established in *Chenopodium quinoa* and *Nicotiana benthamiana*. The virus reacted with the monoclonal antibody (POTY) against the potyvirus group. Potyvirus-like long flexuous filament particles around 12–15×750–800 nm were observed in the crude sap and purified virus preparations, and pinwheel inclusion bodies were observed in the infected cells. The conserved region of the viral RNA was amplified using the degenerate primers for the potyviruses and sequence analysis of the virus isolate 7-2 showed 56.6–63.1% nucleotide and 44.8–65.1% amino acid identities with those of *Bean yellow mosaic virus* (BYMV), *Beet mosaic virus* (BtMV), *Turnip mosaic virus* (TuMV) and *Bean common mosaic virus* (BCMV). The coat protein (CP) gene of isolate 7-2 was amplified, sequenced and found to have 280 amino acids. A homology search in GenBank indicated that the virus is a potyvirus but no highly homologous sequence was found. The virus was designated as Phalaenopsis chlorotic spot virus (PhCSV) in early 2006. Subsequently, a potyvirus, named Basella rugose mosaic virus isolated from malabar spinach was reported in December 2006. It was found to share 96.8% amino acid identity with the CP of PhCSV. Back-inoculation with the isolated virus was conducted to confirm that PhCSV is the causal agent of chlorotic spot disease of *Phalaenopsis* orchids in Taiwan. This is the first report of a potyvirus causing a disease on *Phalaenopsis* orchids.

Keywords New disease · New host record · Orchid virus · Phylogenetic analysis · Potyvirus

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Introduction

Phalaenopsis orchids are popular ornamental plants with beautiful flowers, a long florescence period and enormous diversity. Due to the improvement in breeding and tissue culture technologies, *Phalaenopsis* orchids have become one of the most important exported potted flowers in Taiwan. However, virus diseases often cause great loss in *Phalaenopsis* cultivation.

More than 29 orchid-infecting viruses have been identified. Among them, *Cymbidium mosaic potexvirus* (CymMV; Kado and Jensen 1964), *Odontoglossum ringspot tobamovirus* (ORSV; Inouye and Gara 1996),

Cucumber mosaic cucumovirus (CMV; Zettler et al. 1990), *Orchid fleck rhabdovirus* (OFV; Lesemann and Begtrup 1971) and Dendrobium vein necrosis closterovirus (DVNV; Lesemann 1977) were reported to be able to infect *Phalaenopsis* orchids. In addition, we recently reported a tospovirus, Capsicum chlorosis virus (CaCV-Ph), causing chlorotic ringspots on *Phalaenopsis* orchids (Zheng et al. 2008). CymMV, ORSV (Wey 1988), CMV (Ko 1988) and CaCV-Ph (Zheng et al. 2008) are the only four viruses that have been reported to infect *Phalaenopsis* orchids in Taiwan.

Genus *Potyvirus* covers about 200 definitive and tentative species of plant viruses but none of them has been reported to infect *Phalaenopsis* orchids. In October 2004, *Phalaenopsis* plants bearing virus-like symptoms of chlorotic spots on the leaves were observed in central Taiwan and a potyvirus-like culture was isolated from the diseased orchids (Chen et al. 2006). We report here the isolation, serological and molecular characterization, and back-inoculation of this potyvirus. It was named Phalaenopsis chlorotic spot virus (PhCSV) when a brief account of this work was reported (Chen et al. 2006).

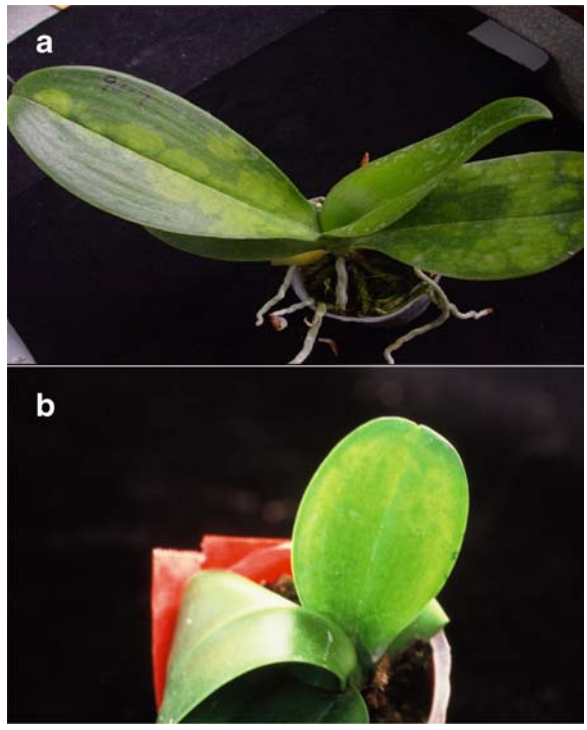

Fig. 1 *Phalaenopsis* orchid infected with Phalaenopsis chlorotic spot virus (PhCSV). **a** *Phalaenopsis* leaf showing chlorotic spots and **b** *Phalaenopsis* seedling inoculated with PhCSV displaying chlorotic spots

Materials and methods

Virus sources and maintenance

In 2004, a virus culture (isolate 7-2) was collected from a diseased *Phalaenopsis* orchid showing chlorotic spot symptoms on leaves (Fig. 1a) in central Taiwan. The virus was maintained in the systemic hosts, *Nicotiana benthamiana*, or *Chenopodium quinoa*, by mechanical inoculation using the inoculum prepared by grinding infected leaf tissues in 30 vol of 10 mM phosphate buffer (pH 7.0; Jan et al. 2000).

Electron microscopy

Ultra-thin sections of symptomatic leaves of *N. benthamiana* plants infected with isolate 7-2 were prepared by the protocol described previously (Jan and Yeh 1995; Zheng et al. 2008). Ultra-thin sections were cut with a diamond knife and collected on formvar-coated, carbon-stabilized copper grids (75 mesh). Sections on grids were stained with 2% uranyl acetate for 15 min followed by 2% lead acetate for 1.5 min, and examined with a JOEL 200 CX electron microscope (JOEL Ltd., Tokyo, Japan). For crude sap, formvar-coated, carbon-stabilized copper grids (300 mesh) were floated for 3 min on one drop of crude sap extracted from isolate 7-2 infected leaf tissues. Samples were stained with 2% uranyl acetate for 10 s and examined with the electron microscope (Chen et al. 2005).

Host range and back-inoculation

To characterize the biological properties of the isolate 7-2, plants from 29 species representing nine families were inoculated mechanically as described above. *Phalaenopsis* seedlings were also inoculated with the isolate 7-2 to confirm that the virus is indeed the causal agent for the disease. Inocula were prepared by propagating the virus in *C. quinoa* and grinding the infected leaves in 10 mM phosphate buffer. Inoculated plants were kept in a greenhouse with insect screens for observing symptom development at least 30 days after inoculation. Both symptomatic and asymptomatic inoculated-plants were analyzed with indirect ELISA

using the antiserum against the isolate 7-2 generated in this study to confirm the virus infection.

Virus purification and production of polyclonal antibody to the isolate 7-2 (PhCSV)

Virions of the isolate 7-2 were purified by the procedures described by Gonsalves and Ishii (1980) with some modifications (Jan and Yeh 1995). Purified virus was resuspended in PE buffer (0.1 M potassium phosphate containing 0.01 M EDTA, pH 7.0) and used to immunize a New Zealand white rabbit for generating polyclonal antibody (PAb) against the isolate 7-2 by the procedures described previously (Jan and Yeh 1995).

ELISA

The procedure of indirect enzyme-linked immunosorbent assay (indirect ELISA) followed the method described by Clark and Adams (1977) with some modifications (Zheng et al. 2008). Rabbit antisera for ORSV, CymMV, CMV, *Impatiens necrotic spot virus* (INSV), *Tomato spotted wilt virus* (TSWV), *Groundnut ringspot virus* (GRSV), *Watermelon silver mottle virus* (WSMoV), Peanut chlorotic fan-spot virus (PCFV), *Turnip mosaic virus* (TuMV), *Zantedeschia mild mosaic virus* (ZaMMV), *Zantedeschia mosaic virus* (ZaMV), *Dasheen mosaic virus* (DsMV), *Carnation mottle virus* (CarMV), *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), CaCV-Ph or isolate 7-2 were individually prepared. Wells of polystyrene microtitration plates (Greiner Bio-One, Frickenhausen, Germany) were coated with crude extracts diluted to 50-fold in coating buffer (0.05 M sodium carbonate, pH 9.6, 0.02% sodium azide). The antisera to each virus diluted to 4,000-fold in conjugate buffer (phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20 and 0.2% ovalbumin) were used and followed by addition of alkaline phosphatase (AP) conjugated goat anti-rabbit IgG at 1:5,000 dilution in conjugate buffer. The AP substrate tablets (Sigma-Aldrich, St. Louis, MO, USA) were prepared in the substrate buffer (9.7% diethanolamine, and 0.02% sodium azide, pH 9.8) to a final concentration of 1 mg ml^{-1}. The level of reaction was recorded at 405 nm using a Labsystems Multiskan EX microplate reader (Labsystems, Vantaa, Finland) at 10–30 min after the addition of the substrate. Commercial ELISA kits for *Cymbidium ringspot virus* (CymRSV) (Agdia, Inc., Indiana, USA) and the POTY monoclonal antibody for detecting potyviruses (V087-R2, ADI, LLC., Arkansas, USA) were also used to react with crude extract from isolate 7-2-infected plants according to the manufacturer's protocol.

Immunoblotting

Immunoblotting was done as previously described (Jan and Yeh 1995; Zheng et al. 2008). Leaf extracts of mock-inoculated and virus-infected *C. quinoa* were separated by SDS-PAGE and then transferred to a PVDF transfer membrane (Bio-Rad, California, USA). The membrane was blocked and incubated with antiserum against 7-2 virions, followed by addition of AP conjugated IgG and colour development by chromogenic substrates (NBT/BCIP). Extracts from leaves of uninfected plants and normal serum were used as controls.

Molecular cloning and sequence analysis

Isolate 7-2 reacted positively with the POTY monoclonal antibody (MAb) for detecting potyviruses in indirect ELISA test, indicating that this orchid virus might be a potyvirus. For confirmation, total RNA was extracted from leaves of 7-2-infected *C. quinoa* using the method described by Napoli et al. (1990). The degenerate primer pair, Pot 1 (5′-GACTGGATC CATT(C/G/T)TC(A/G/T)AT(A/G)CACCA-3′) and Pot 2 (5′-GACGAATTCTG(C/T)GA(C/T)GC(C/G/T) GATGG(C/T)TC-3′) (Fig. 4; Colinet and Kummert 1993) was used for amplifying the conserved region including the 3′-end of NIb and 5′-end of CP genes of the potyvirus genome. The first-strand cDNA was synthesized with SuperScript™ III RNase H^- Reverse Transcriptase (Invirtogen, California, USA) according to the instructions of the manufacturer and polymerase chain reaction (PCR) amplification was performed using TAKARA LA *Taq* DNA polymerase (TAKARA BIO Inc., Shiga, Japan). The procedures of reverse transcription-PCR (RT-PCR), DNA cloning and sequencing were described previously (Zheng et al. 2008). Pot 2 and oligo d(T) primers were used to amplify the 3′ terminal region of the potyvirus genome. The Gap programme of the SeqWeb (version 3.1.2, Accelrys, California, USA) was used to determine the degree of the nucleic acid and amino acid

identities among potyviruses. The PROTDIST programme of PHYLIP version 3.63 (University of Washington, Washington, USA) was used to calculate the distance matrices of the coat protein sequences. The sequences and accession numbers of potyviruses on GenBank used for analysis are listed in Table 1.

Results

Symptoms and virus isolation

In October 2004, one *Phalaenopsis* plant bearing chlorotic spots (Fig. 1a) was tested by ELISA with antisera against CymMV, ORSV, CMV, INSV, TSWV, GRSV, WSMoV, PCFV, TuMV, ZaMMV, ZaMV, DsMV, CarMV, TMV, ToMV, CaCV-Ph, CymRSV or potyviruses. The diseased leaves reacted positively only to the POTY monoclonal antibody (MAb) which is for detecting potyviruses. A virus culture, isolate 7-2, was then established in *C. quinoa* from diseased *Phalaenopsis* orchids through three consecutive single-lesion isolations.

Host range and back-inoculation

From the 29 plant species mechanically inoculated, thirteen species were susceptible to the virus isolate 7-2. Among these susceptible species, *C. quinoa*, *N. benthamiana*, *Celosia argentea*, *Gomphrena globosa*, *Lycopersicum esculentum* and *Lisianthus russellianus* developed systemic symptoms with chlorotic spots or mosaic symptoms. Chlorotic local lesions were observed on inoculated leaves of *Amaranthus viridis*, *C. amaranticolor*, *C. ficifolium*, *C. murale*, *Vigna angularis*, *Cucurbita moschata* and *Capsicum anmuum* var. *grossum*. The virus isolate 7-2 induced chlorotic spots on both inoculated and systemic leaves of infected *C. quinoa* plants around 5 and 7 days, respectively, post-inoculation. Infections were also confirmed by positive reactions of ELISA tests with antiserum to isolate 7-2. *Amaranthus mangostanus*, *Carica papaya*, *Helianthus annuus*, *Brassica chinensis*, *V. unguiculata*, *Citrullus vulgaris*, *C. vulgaris*, *Cucumis melo*, *C. metuliferus* L35, *C. metuliferus* L37, *Cucurbita pepo* cv. field pumpkin, *Datura stramonium*, *N. occidentalis*, *N. rustica*, *N. tabacum* cv. Samsun

Table 1 Comparison of the nucleotide and deduced amino acid sequences of the coat protein (CP) gene and 3′ untranslated region (3′ UTR) of PhCSV with those of other nineteen reported potyviruses

Virus name	Acronym	Accession number	Sequence identity (%)		
			CP		3′UTR
			Nucleotide	Amino acid	
Basella rugose mosaic virus	BaRMV	DQ394891	92.7	96.8	88.5
Beet mosaic virus	BtMV	AY206394	67.3	70.3	46.4
Colombian datura virus	CDV	AB179622	60.1	65.1	36.4
Bean common mosaic virus	BCMV	AJ312438	63.0	62.6	40.9
Ceratobium mosaic virus	CerMV	AF022446	62.4	61.9	47.1
Dendrobium mosaic virus	DeMV	U23564	59.3	61.7	40.5
Watermelon mosaic virus	WMV	AY437609	61.6	61.3	42.6
Sarcochilus virus Y	SVY	AF185957	62.5	61.0	41.5
Dasheen mosaic virus	DsMV	NC_003537	61.0	60.1	40.3
Vanilla necrosis virus	VNV	L22907	61.2	59.9	42.9
Peanut stripe virus	PStV	AY968604	62.9	59.7	45.3
Calanthe mild mosaic virus	CalMMV	AB011404	61.5	59.3	43.4
Vanilla mosaic virus	VanMV	AJ616719	62.1	59.0	42.5
Potato virus Y	PVY	AY884984	61.3	58.4	42.4
Bean yellow mosaic virus	BYMV	NC_003492	61.0	57.8	45.6
Clover yellow vein virus	ClYVV	NC_003536	59.2	56.7	39.9
Turnip mosaic virus	TuMV	AB093610	57.9	55.2	39.2
Spiranthes mosaic virus 2	SpiMV 2	AY685219	58.8	54.9	41.0
Spiranthes mosaic virus 3	SpiMV 3	AY685218	58.3	53.5	43.4

and *N. tabacum* cv. Xanthi were not infected with isolate 7-2 as determined by symptom expression, ELISA and infectivity assay on *C. quinoa*.

To determine whether the disease of chlorotic spots on *Phalaenopsis* orchids was caused by isolate 7-2, *Phalaenopsis* orchids were mechanically inoculated with inocula prepared from isolate 7-2-infected *N. benthamiana*. Inoculated *Phalaenopsis* seedlings produced chlorotic spots similar to those on naturally infected plants (Fig. 1b).

Electron microscopy

Electron microscopic examination of ultra-thin sections of isolate 7-2-infected *N. benthamiana* plants revealed the presence of pinwheel inclusion bodies (Fig. 2a). Long flexuous filament particles measuring about 12–15×750–800 nm were also observed in the crude sap of infected *C. quinoa* leaves (Fig. 2b).

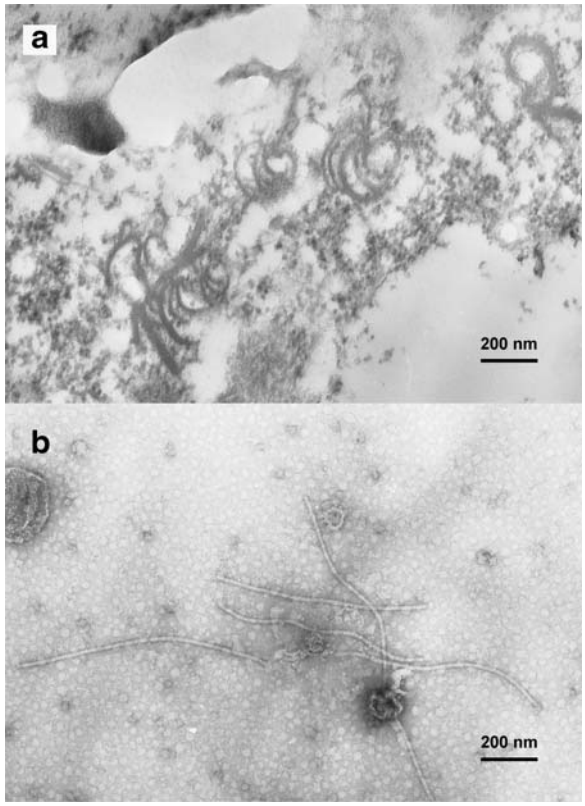

Fig. 2 Electron micrographs of PhCSV-infected tissues and viral particles. **a** Pinwheel inclusion bodies in ultra-thin sections from PhCSV-infected *Nicotiana benthamiana* plants and **b** long flexuous filament particles in the crude sap of infected *Chenopodium quinoa* leaves

Transmission electronic micrographs of viral particles and inclusion bodies showed the typical features of a potyvirus.

Serological analysis

In order to detect the virus specifically and rapidly, the virions were purified and the PAb against the isolate 7-2 was generated by immunizing a rabbit. One structural protein of 33–34 kDa was present in purified virus preparations in SDS-PAGE (Fig. 3a). The similar band of 34 kDa was observed in western blotting of the leaf extracts from virus-infected *C. quinoa* using 4,000-fold dilution of rabbit PAb (Fig. 3b).

Molecular cloning and sequence analysis

To classify and characterize the genomic sequence of 7-2, one cDNA fragment around 1,200 bp was amplified, cloned and sequenced from the total RNAs extracted from virus-infected *C. quinoa* by RT-PCR using the degenerate primers, Pot 1 and Pot 2, designed for amplifying the conserved region including the 3′-end of NIb and 5′-end of CP genes of potyvirus RNA (Fig. 4). When BLAST analysis against the GenBank databases was done, the 1,189 bp DNA fragment from isolate 7-2 shared 60.6%, 63.1%, 58.9% and 56.6% nucleotide identities and 55.6%, 65.1%, 52.5% and 44.8% amino acid identities with those of *Bean yellow mosaic virus* (BYMV), *Beet mosaic virus* (BtMV), *Bean common mosaic virus* (BCMV) and *Turnip mosaic virus* (TuMV), respectively, which are found to be closely homologous. The sequence comparison of the conserved region indicated that 7-2 should be a member of potyvirus.

For classifying the potyviruses, the nucleotide and amino acid sequences of the CP gene are required. The 3′ terminal region of viral genomic RNA was then amplified from 7-2 using primers Pot 2 and olig d(T) (Fig. 4). An expected ~2.0 kbp RT-PCR product was cloned and sequenced. The 2018 bp of 3′ terminal region encodes a predicted protein of 546 amino acids with a calculated molecular mass of 63.0 kDa. The polypeptide has a putative protease cleavage site at amino acid (aa) 266/267 (E*V*VY*Q*/*S*) (amino acids in italics indicate the similar recognition sites of NIa protease) and the CP of 280 amino acids

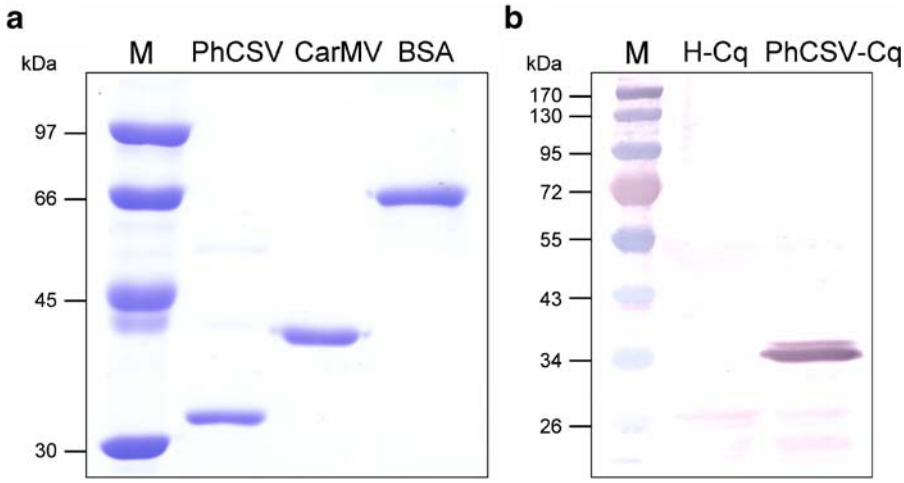

Fig. 3 SDS-PAGE and western blotting analysis of PhCSV CP. **a** One structural protein about 33–34 kDa is observed in purified viral particles of PhCSV and the Mr of *Carnation mottle virus* (CarMV) CP and bovine serum albumin (BSA) is about 40 and 67 kDa, respectively. **b** One ~34 kDa protein is shown in the leaf extracts of PhCSV-infected *C. quinoa* plant but not in the healthy *C. quinoa* plant reacting with PhCSV antiserum

encoded at C terminal region of this polypeptide has a calculated Mr of 31.5 kDa. When BLAST analysis against the GenBank databases was done, the CP gene of the 7-2 isolate shared low nucleotide and predicted amino acid identities of 57.9% to 67.3% and 53.5% to 70.3%, respectively, with those of the eighteen potyviruses having highest sequence identities (Table 1). The CP gene of the 7-2 isolate shared the highest nucleotide and amino acid identities of 67.3% and 70.3%, respectively, with those of BtMV (Table 1). The 3′ untranslated region (3′UTR) located at the downstream of the CP gene was 376 bp long without the poly A tail and showed 36.4% to 46.4% nucleotide identities with those of the other eighteen potyviruses (Table 1). During this manuscript preparation, the 3′-genomic sequence of a new potyvirus, Basella rugose mosaic virus (BaRMV), was published (Huang and Chang 2006). The CP gene of the 7-2 isolate shared nucleotide and amino acid sequence identities of 92.7% and 96.8%, respectively, with BaRMV (Table 1). The phylogenetic analysis revealed that the CP relationship of 7-2 and BaRMV was most closely related to that of BtMV (Fig. 5).

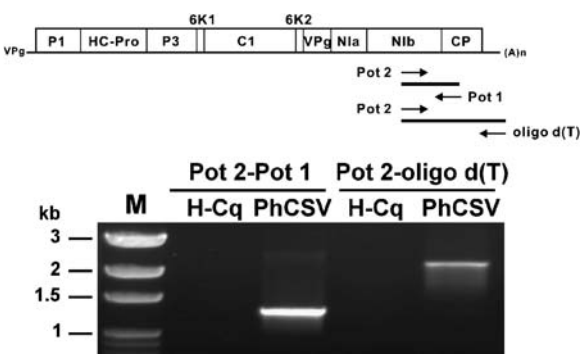

Fig. 4 RT-PCR of PhCSV genomic RNA. The conserved region and the 3′ terminal region of PhCSV genomic RNA were amplified using primer pairs, Pot 2/Pot 1 and Pot 2/oligo d (T), respectively

Discussion

Our results have demonstrated that the virus isolate 7-2 causing chlorotic spots on Phalaenopsis orchids is a potyvirus. Initial CP gene sequence analyses showed that none of the reported potyviruses shared more than 80% nucleotide or amino acid sequence identities with the isolate 7-2 indicating that the isolate 7-2 is a new potyvirus and was designated as Phalaenopsis chlorotic spot virus (PhCSV) in June 2006 when a brief account of this work was reported (Chen et al. 2006).

Sixteen potyviruses, BYMV (Lesemann and Koenig 1985), *Calanthe mild mosaic virus* (CalMMV; Gara et al. 1998), *Colombian datura virus* (CDV; Fry et al. 2004), *Ceratobium mosaic virus* (CerMV; Mackenzie et al. 1998), *Clover yellow vein virus* (ClYVV; Inouye et al. 1988), Dendrobium mosaic virus (DeMV; Hu et al. 1995), *Dasheen mosaic virus* (DsMV; Jordan et al. 2002), Habenaria mosaic virus (HaMV; Inouye et al. 1998), Pecteilis mosaic virus (PcMV; Yora et al. 1983), Spiranthes mosaic virus 2 (SpiMV 2), SpiMV 3 (Guaragna et al. 2006), *Turnip mosaic virus* (TuMV; Lesemann and Vetten 1985; Lawson and Hsu 1995), Vanilla mosaic virus (VanMV; Wisler et al. 1987), Vanilla necrosis virus (VNV; Pearson and Pone 1988), Diurus

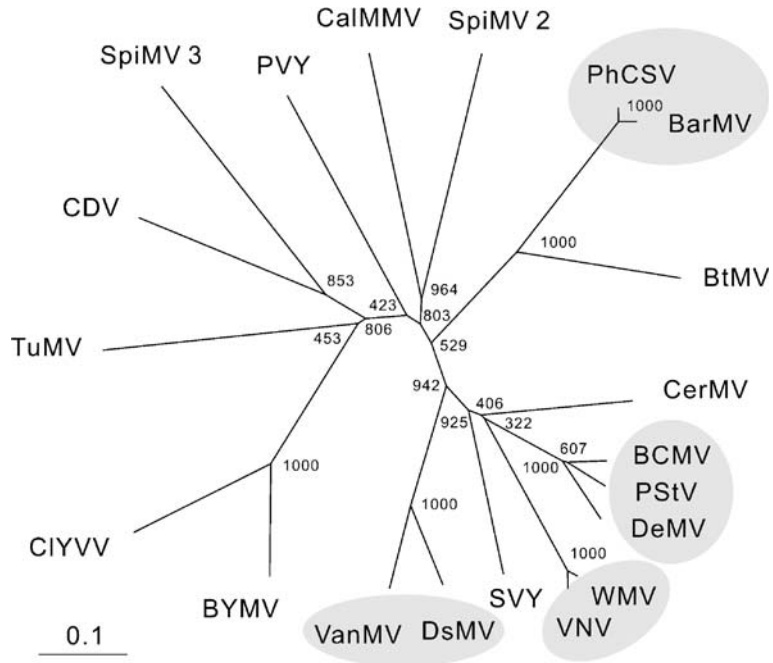

Fig. 5 Phylogenetic relationships of the CPs of PhCSV and other potyviruses. The scale for genetic distances is indicated at the bottom left and the viruses in shadows are highly homologous with each other. Sequence sources are same as in Table 1. Phylogenetic analysis was conducted using the Clustal-X and PHYLIP package version 3.63. The dendrogram was produced using the neighbour-joining algorithm with 1,000 bootstrap replicates

virus Y (DVY) and Sarcochilus virus Y (SVY; Gibbs et al. 2000) were reported to infect orchids. However, none of them has been reported to infect *Phalaenopsis* orchids.

Phylogenetic analysis of CP genes between PhCSV and other potyviruses indicated that three orchid-infecting potyviruses are the strains of other potyviruses (Fig. 5). VanMV shared high (>85%) CP amino acid identity with that of DsMV and was classified as a strain of DsMV (Farreyrol et al. 2006). VNV was considered to be a strain of *Watermelon mosaic virus* (WMV) based on serological, symptomatological and molecular characteristics (Wang et al. 1993). In addition, DeMV was shown to a member of the *Bean common mosaic virus* (Hu et al. 1995).

A potyvirus, named Basella rugose mosaic virus (BaRMV) from malabar spinach (*Basella rubra*) in Taiwan was reported in December 2006 (Huang and Chang 2006). Its CP gene shares 96.8% amino acid identity with that of PhCSV. PhCSV and BaRMV have different natural hosts but both infect *N. benthamiana* and *C. quinoa* systemically. PhCSV-infected *C. quinoa* plants developed chlorotic spots on inoculated and systemic leaves but the infected *N. benthamiana* plants showed mosaic symptoms. However, PhCSV cannot systemically infect *C. amaranticolor* which is the systemic host of BaRMV. Based on the CP gene sequence analyses (Table 1 and Fig. 5), these two viruses should be classified as the same potyvirus species, despite the difference in biological characteristics. Besides BaRMV, another potyvirus, Ullucus mosaic virus (UMV), has been reported to infect a member of the family *Basellaceae* (*Ullucus tuberosus*) (Brunt et al. 1982). However, this virus differs from BaRMV and PhCSV, since it only causes local lesions on *C. quinoa* (Brunt et al. 1982; Huang and Chang 2006). In addition, the host range of UMV is different from that of PhCSV. *Lycopersicum esculentum* is a local infection host for UMV, but is a systemic host for PhCSV. *Gomphrena globosa* is not infected by UMV which is a systemic host of PhCSV. Also, UMV caused local infection in *N. rustica* and *D. stramonium*, which are not infected by PhCSV (Brunt et al. 1982).

According to the sequence analyses of the 3′ terminal region of PhCSV RNA, two consensus motifs of potyviruses were found to be present in the NIb protein and CP, respectively. The GDD motif present at aa 101–103 of the PhCSV polyprotein is conserved in the C-terminus of potyviral NIb protein (Argos 1988;

Koonin and Dolja 1993) and the DAG motif at aa 290–292 of the polyprotein (aa 24–26 of CP) is related to the vector transmission of potyviruses (Harrison and Robinson 1988; Atreya et al. 1990; Shukla et al. 1994). Two highly conserved amino acids, R and D, at aa 436 and 480 of the polyprotein presenting at aa 170 and 214 of CP involve in virion assembly (Jacquet et al. 1998). The consensus motifs and amino acids are also present in BaRMV RNA.

CymMV, ORSV (Wey 1988), CMV (Ko 1988) and CaCV-Ph (Zheng et al. 2008) are the only four viruses that have been reported to infect *Phalaenopsis* orchids in Taiwan. Symptoms of chlorotic spots caused by PhCSV on *Phalaenopsis* orchids were quite different from those of chlorotic ringspots caused by CaCV-Ph that we identified recently from *Phalaenopsis* orchids (Zheng et al. 2008).

In Taiwan, *Phalaenopsis* orchid which is an important agricultural commodity, often suffers heavy losses caused by viral diseases. Diverse virus-like symptoms were frequently observed in *Phalaenopsis* orchid nurseries. However, *Phalaenopsis* orchid-infecting viruses are hard to identify because of the difficulty in isolating the virus from diseased *Phalaenopsis* orchid plants and/or inoculating the isolated virus back to a healthy one mechanically. In our preliminary survey of around 150 diseased *Phalaenopsis* plants using ELISA around, 10% of plants were found to be infected with PhCSV. The PAb generated for detection of PhCSV in this study should be useful for detecting the virus in the nurseries and for obtaining virus-free plants for propagation.

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References

Argos, P. (1988). A sequence motif in many polymerases. *Nucleic Acids Research*, *16*, 9909–9916.

Atreya, C. D., Raccah, B., & Pirone, T. P. (1990). A point mutation in the coat protein abolishes aphid transmissibility of a potyvirus. *Virology*, *178*, 161–165.

Brunt, A. A., Phillips, S., Jones, R. A. C., & Kenten, R. H. (1982). Viruses detected in *Ullucus tuberosus* (Basellaceae) from Peru and Bolivia. *Annals of Applied Biology*, *101*, 65–71.

Chen, C. C., Chen, T. C., Lin, Y. H., Yeh, S. D., & Hsu, H. T. (2005). A chlorotic spot disease on calla lilies (*Zantedeschia* spp.) is caused by a tospovirus serologically but distantly related to *Watermelon silver mottle virus*. *Plant Disease*, *89*, 440–445.

Chen, C.-C., Zheng, Y.-X., Chen, Y.-K., & Jan, F.-J. (2006). Isolation and characterization of a new potyvirus causing chlorotic spots on moth orchids (*Phalaenopsis* spp.). *Phytopathology*, *96*, S22.

Clark, M. F., & Adams, A. N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology*, *34*, 475–483.

Colinet, D., & Kummert, J. (1993). Identification of a sweet-potato feathery mottle virus isolate from China (SPFMV-CH) by the polymerase chain reaction with degenerate primers. *Journal of Virological Methods*, *45*, 149–159.

Farreyrol, K., Pearson, M. N., Grisoni, M., Cohen, D., & Beck, D. (2006). Vanilla mosaic virus isolates from French Polynesia and the Cook Islands are *Dasheen mosaic virus* strains that exclusively infect vanilla. *Archives of Virology*, *151*, 905–919.

Fry, C. R., Zimmerman, M. T., & Scott, S. W. (2004). Occurrence of *Colombian datura virus* in the terrestrial orchid, *Spiranthes cernua*. *Journal of Phytopathology*, *152*, 200–203.

Gara, I. W., Kondo, H., Maeda, T., Inouye, N., & Tamada, T. (1998). *Calanthe mild mosaic virus*, a new potyvirus causing a mild mosaic disease of Calanthe orchid in Japan. *Journal of Phytopathology*, *146*, 357–363.

Gibbs, A., Mackenzie, A., Blanchfield, A., Cross, P., Wilson, C., Kitajima, E., et al. (2000). Viruses of orchids in Australia: Their identification, biology and control. *Australian Orchid Review*, *65*, 10–21.

Gonsalves, D., & Ishii, M. (1980). Purification and serology of *Papaya ringspot virus*. *Phytopathology*, *70*, 1028–1032.

Guaragna, M. A., Ndum, O., & Jordan, R. (2006). Detection and characterization of two previously undescribed potyviruses in the terrestrial orchid *Spiranthes cernua*. *Acta Horticulturae*, *722*, 209–218.

Harrison, B. D., & Robinson, D. J. (1988). Molecular variation in vector-borne plant viruses: Epidemiological significance. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *321*, 447–462.

Huang, C. H., & Chang, Y. C. (2006). Basella rugose mosaic virus, a new potyvirus infecting *Basella rubra*. *Plant Pathology*, *55*, 819.

Hu, J. S., Ferreria, S., Wang, M., Borth, W. B., Mink, G., & Jordan, R. L. (1995). Purification, host range, serology and partial sequencing of Dendrobium mosaic potyvirus, a new member of the *Bean common mosaic virus* subgroup. *Phytopathology*, *85*, 542–546.

Inouye, N., & Gara, I. W. (1996). Detection and identification of viruses of orchids in Indonesia. *Bulletin of the Research Institute for Bioresources Okayama University*, *4*, 109–118.

Inouye, N., Maeda, T., & Mistsuhata, K. (1988). A strain of *Clover yellow vein virus* isolated from *Calanthe* sp. *Acta Horticulturae*, *234*, 61–68.

Inouye, N., Maeda, T., Mitsuhata, K., & Gara, I. W. (1998). Habenaria mosaic virus, a new member of potyvirus from

Habenaria radiata orchid. *Bulletin of the Research Institute for Bioresources Okayama University*, *5*, 155–168.

Jacquet, C., Delecolle, B., Raccah, B., Lecoq, H., Dunez, J., & Ravelonandro, M. (1998). Use of modified *Plum pox virus* coat protein genes developed to limit heteroencapsidation-associated risks in transgenic plants. *Journal of General Virology*, *79*, 1509–1517.

Jan, F.-J., Fagoaga, C., Pang, S. Z., & Gonsalves, D. (2000). A single chimeric transgene derived from two distinct viruses confers multi-virus resistance in transgenic plants through homology-dependent gene silencing. *Journal of General Virology*, *81*, 2103–2109.

Jan, F.-J., & Yeh, S.-D. (1995). Purification, in situ localization, and comparative serological properties of *Passionfruit woodiness virus*-encoded amorphous inclusion protein and two other virus proteins. *Phytopathology*, *85*, 64–71.

Jordan, R., Guaragna, M. A., Kinard, G., & Lynn, S. (2002). Detection and first report of *Dasheen mosaic virus* and a second potyvirus infecting the terrestrial orchid *Spiranthes cernua*. *Acta Horticulturae*, *568*, 253–260.

Kado, C. I., & Jensen, D. D. (1964). *Cymbidium mosaic virus* in *Phalaenopsis*. *Phytopathology*, *54*, 974–947.

Ko, N. J. (1988). Cytological identification of *Cucumber mosaic virus* infecting *Phalaenopsis*. *Proceedings of the National Science Council, Republic of China – Part B, Life Sciences*, *12*, 46–51.

Koonin, E. V., & Dolja, V. V. (1993). Evolution and taxonomy of positive-strand RNA viruses: Implications of comparative analysis of amino acid sequences. *Critical Reviews in Biochemistry and Molecular Biology*, *28*, 375–430.

Lawson, R. H., & Hsu, H. T. (1995). Orchid. In G. Loebenstein, R. H. Lawson, & A. A. Brunt (Eds.) *Virus and virus-like diseases of bulb and flower crops* (pp. 409–420). UK: Wiley.

Lesemann, D. E. (1977). Long, filamentous virus-like particles associated with vein necrosis of *Dendrobium phalaenopsis*. *Phytopathologische Zeitschrift*, *89*, 330–339.

Lesemann, D., & Begtrup, J. (1971). Electron microscopic demonstration of a bacilliform virus in *Phalaenopsis*. *Phytopathologische Zeitschrift*, *71*, 257–269.

Lesemann, D. E., & Koenig, R. (1985). Identification of *Bean yellow mosaic virus* in *Masdevallia*. *Acta Horticulturae*, *164*, 347–354.

Lesemann, D. E., & Vetten, H. J. (1985). The occurrence of *Tobacco rattle* and *Turnip mosaic viruses* in *Orchis* sp., and of an unidentified potyvirus in *Cypripedium calceolus*. *Acta Horticulturae*, *164*, 45–54.

Mackenzie, A. M., Nolan, M., Wei, K. J., Clements, M. A., Gowanlock, D., Wallace, B. J., et al. (1998). *Ceratobium mosaic potyvirus*: Another virus from orchids. *Archives of Virology*, *143*, 903–914.

Napoli, C., Lemieux, C., & Jorgensen, R. (1990). Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. *Plant Cell*, *2*, 279–289.

Pearson, M. N., & Pone, S. P. (1988). Viruses of Vanilla in the Kingdom of Tonga. *Australasian Plant Pathology*, *17*, 59–60.

Shukla, D. D., War, C., & Brunt, A. A. (1994). *The Potyviridae*. Wallingford: CAB International.

Wang, Y. Y., Beck, D., Gardner, R. C., & Pearson, M. N. (1993). Nucleotide sequence, serology and symptomatology suggest that Vanilla necrosis potyvirus is a strain of *Watermelon mosaic virus II*. *Archives of Virology*, *129*, 93–103.

Wey, G. C. (1988). Occurrence and investigation of important diseases on *Phalaenopsis* in Taiwan. *Report of the Taiwan Sugar Research Institute*, *122*, 31–41.

Wisler, G. C., Zettler, F. W., & Mu, L. (1987). Virus infections of *Vanilla* and other orchids in French Polynesia. *Plant Disease*, *71*, 1125–1129.

Yora, K., Saito, Y., Doi, Y., Inouye, T., & Tomaru, K. (1983). *Handbook of plant viruses*. Tokyo: Asakura Shoten.

Zettler, F. W., Ko, N. J., Wisler, G. C., Elliott, M. S., & Wong, S. M. (1990). Viruses of orchids and their control. *Plant Disease*, *74*, 621–626.

Zheng, Y.-X., Chen, C.-C., Yang, C.-J., Yeh, S.-D., & Jan, F.-J. (2008). Identification and characterization of a tospovirus causing chlorotic ringspots on *Phalaenopsis* orchids. *European Journal of Plant Pathology*, *120*, 199–209.