Eur J Plant Pathol (2010) 128:1–5
DOI 10.1007/s10658-010-9630-0

SHORT COMMUNICATIONS

Odontoglossum ringspot virus causing flower crinkle in *Phalaenopsis* hybrids

You-Xiu Zheng · Bing-Nan Shen · Ching-Chung Chen · Fuh-Jyh Jan

Accepted: 26 April 2010 / Published online: 23 May 2010

Abstract A new disorder exhibiting flower crinkle on *Phalaenopsis* orchids bearing white flowers has been observed in Taiwan, China and Japan for several years. This disorder decreased the flower longevity and was considered as a physiological syndrome. The objective of this study was to identify and characterize the real causal agent of this new *Phalaenopsis* disorder. Five plants of *Phalaenopsis* hybrids "V3" (Phal. Yukimai × Phal. Taisuco Kochdian) with flower crinkle symptoms were collected and tested by enzyme-linked immunosorbent assay with antisera against 18 viruses. The extract of leaves and flowers from one diseased plant (96-Ph-16) reacted positively only to antiserum against *Odontoglossum ringspot virus* (ORSV), while those from the other four plants (96-Ph-7, 96-Ph-17, 96-Ph-18 and 96-Ph-19) reacted positively to the antisera against ORSV and *Cymbidium mosaic virus* (CymMV). Five ORSV isolates, one each from flowers of those five diseased *Phalaenopsis* orchids, were established in *Chenopodium quinoa*. A CymMV culture was isolated from the flowers of one of the ORSV/CymMV mix-infected *Phalaenopsis* orchids (96-Ph-19). To determine the causal agent of the flower crinkle disease, healthy *Phalaenopsis* seedlings were singly or doubly inoculated with the isolated ORSV and/or CymMV. Results of back inoculation indicated that ORSV is the sole causal agent of the crinkle symptom on petals of *Phalaenopsis* orchid. The CP gene of the ORSV isolates from this study shared 97.3–100% nucleotide identity and 96.2–100% amino acid identity with those of 41 ORSV isolates available in GenBank. This is the first report demonstrating ORSV as the sole virus causing flower crinkle disease on *Phalaenopsis* orchids.

Keywords *Odontoglossum ringspot virus* · Flower crinkle · *Phalaenopsis* · Orchid virus

Y.-X. Zheng · B.-N. Shen · C.-C. Chen · F.-J. Jan (✉)
Department of Plant Pathology,
National Chung Hsing University,
Taichung 402, Taiwan
e-mail: fjjan@nchu.edu.tw

Phalaenopsis orchid is one of the most popular ornamental plants in the *Orchidaceae*, because of its long-lasting flowers and beautiful appearance. Viruses represent the major constraints to *Phalaenopsis* production because the diseases result in reductions of flower quality and substantial yield losses. More than thirty orchid-infecting viruses have been identified (Lawson and Hsu 1995; Zettler et al. 1990; Zheng 2007). Among these viruses, *Cymbidium mosaic virus* (CymMV), *Odontoglossum ringspot virus* (ORSV), *Cucumber mosaic virus* (CMV) (Zettler et al. 1990), *Orchid fleck virus* (OFV) (Lawson and Brannigan 1986), Dendrobium vein necrosis virus (DVNV) (Lesemann 1977), *Tomato spotted wilt virus* (TSWV) (Baker et al. 2007) and *Impatiens necrotic spot virus* (INSV) (Baker et al. 2007) have been reported to infect *Phalaenopsis* orchids. Most orchid viruses cause symptoms on

leaves, however in 2006, a new disorder exhibiting symptoms of flower crinkle on white flower-bearing *Phalaenopsis* hybrids, especially on Phal. Sogo Yukidian "V3", in Taiwan, China and Japan was found. The *Phalaenopsis* flowers with crinkle generally displayed shorter flower longevity. In Japan, flower crinkle was called "HANAFUKE" meaning flower senescence (personal communication with Kazuo Yoneda, Nihon University). Since no obvious symptoms were expressed on *Phalaenopsis* leaves the cause of flower crinkle was unclear and the disorder was considered a result of physiological dysfunction. The objective of the present work was to determine the causal agent of the flower crinkle disease on *Phalaenopsis* orchids in Taiwan.

In 2007, five plants (96-Ph-7, 96-Ph-16, 96-Ph-17, 96-Ph-18 and 96-Ph-19) of Phal. Sogo Yukidian "V3" (Phal. Yukimai × Phal. Taisuco Kochdian) bearing abnormal flowers with crinkle symptom (Fig. 1a, b) were collected in central Taiwan and tested by ELISA (Zheng et al. 2008) with 18 anitsera of plant viruses. The extracts of leaves and flowers from one diseased plant (96-Ph-16) (Fig. 1b) reacted positively only to the antiserum against ORSV, while those from the other four plants (96-Ph-7, 96-Ph-17, 96-Ph-18 and 96-Ph-19) reacted positively to antisera against ORSV and CymMV (Fig. 1a). Total RNA was extracted from flowers of those five *Phalaenopsis* orchids with symptoms of flower crinkle using the method described by Napoli et al. (1990). The specific primer pairs, FJJ2003-65 (5′-AGGGTTAAGTTACCACAATAA)/FJJ2003-66 (5′-AAACCACACGCCTTATT) and FJJ2003-67 (5′-GTTAGTGATATTCGTATTGAT)/FJJ2003-68 (5′-AACGTTATTTTCCTAAATAT), for the amplification of coat protein (CP) genes of CymMV and ORSV, respectively, were used for reverse transcription-polymerase chain reaction (RT-PCR) (Zheng et al. 2008). The result of RT-PCR confirmed those of ELISA tests and consequently we hypothesized that flower crinkle disease is probably caused by ORSV.

To determine if ORSV indeed caused the crinkle symptom on *Phalaenopsis* flowers, ORSV (96-Ph-O7, 96-Ph-O16, 96-Ph-O17, 96-Ph-O18, 96-Ph-O19)

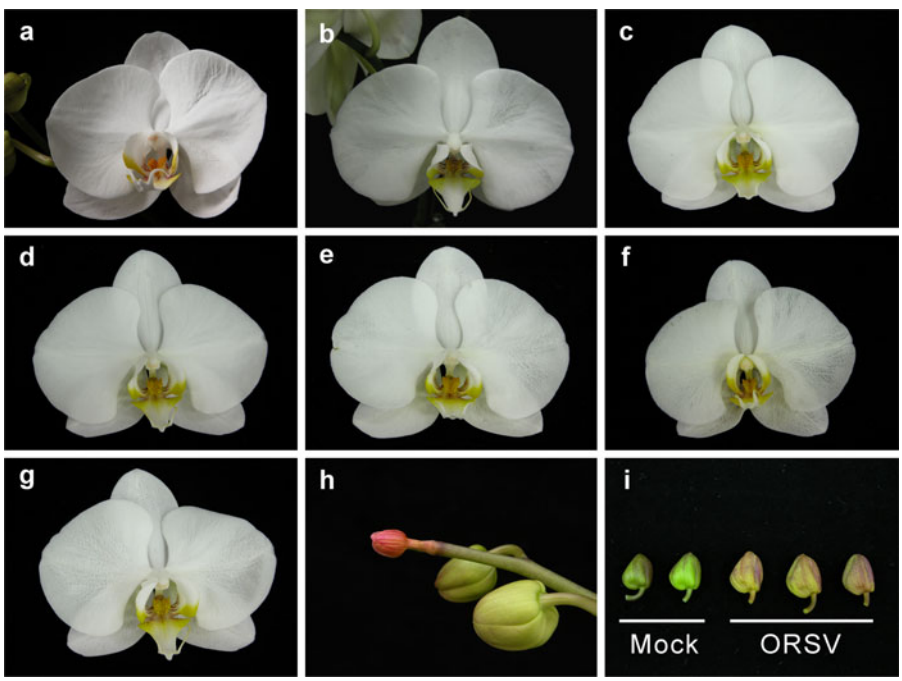

Fig. 1 Flower crinkle symptoms of *Phalaenopsis* orchids bearing white flowers. *Phalaenopsis* flower naturally infected with *Cymbidium mosaic virus* (CymMV) and *Odontoglossum ringspot virus* (ORSV) (**a**), and with ORSV alone (**b**). Back inoculation of ORSV resulted in crinkle symptom on petals, no matter whether CymMV was not inoculated (**e**), or inoculated first (**f**) or last (**g**). The crinkled regions fused progressively and the petals became transparent (**a**, **b**, **e**, **f**, **g**). Some flower buds of ORSV-infecting plants turned red (**h**, **i**) and shriveled eventually. The mock (**c**) and CymMV alone-infected (**d**) plants bore normal flowers

and CymMV (96-Ph-C19) were isolated by mechanical inoculation from crinkled flowers of *Phalaenopsis* plants and maintained in the local lesion host, *Chenopodium quinoa* Willd. (Jan et al. 2000). To confirm that virus isolates 96-Ph-O7, 96-Ph-O16, 96-Ph-O17, 96-Ph-O18 and 96-Ph-O19 were indeed ORSV, the CP genes were amplified by RT-PCR from total RNAs extracted from virus-infected *C. quinoa* using ORSV specific primers FJJ2003-67/68 (Zheng et al. 2008). The expected 0.56 kbp RT-PCR products were cloned and sequenced to reveal identical sequences for all five ORSV isolates. ORSV CP genes from this study shared high nucleotide (97.3–100%) and amino acid (96.2–100%) identities with others available in NCBI GenBank from various orchids around the world including *Phalaenopsis* (AF455274, AJ606105–107, AM398154, DQ915440 and EU653020), *Cymbidium* (AF406768, AF406770–73, AF406775, AJ564563, AY360407 and U89894), *Oncidium* (AF455273 and AY376394), *Cattleya* (AF406769), *Aranda* (AF406776 and AF406778), *Epidendrum* (AF405727) and *Rhyncovanda* (AF406777). There was no considerable difference between identities reached with *Phalaenopsis*-infecting ORSV and viruses from other orchids. Thus it cannot be assumed that a particular ORSV strain or isolate is causing flower crinkle disease.

Upon back inoculation, plants of two white flower-bearing *Phalaenopsis* hybrids, Phal. Sogo Yukidian "V3" (Phal. Yukimai×Phal. Taisuco Kochdian) [Table 1, Expt 1 and 2] and *Doritaenopsis* Join Angel "274-1" (Dtps. Casablanca Joy×Phal. Taida inlong "KC1932") [Table 1, Expt 3] were used for mechanical inoculation with one CymMV isolate (96-Ph-C19) and two ORSV isolates (96-Ph-O7 and 96-Ph-O16), to confirm their roles in the flower crinkle disorder. Each virus isolate was propagated in *C. quinoa* and inoculated onto leaves (Table 1, Expts 1 and 3) or flower stalks (Table 1, Expt 2) of *Phalaenopsis* hybrids V3 and 274-1. Following virus inoculation, ELISA and RT-PCR were conducted for virus detection. Additionally, V3 *Phalaenopsis* plants that were naturally infected with CymMV were inoculated with ORSV 96-Ph-O7 or ORSV 96-Ph-O16. Inoculated plants were kept in a greenhouse with appropriate temperature settings for flower induction with symptom development monitored daily for at least 3 months. Infection rates (presence of virus in inoculated tissues) of *Phalaenopsis* V3 plants inoculated with ORSV 96-Ph-O7 or ORSV 96-Ph-O16 reached 100% (Table 1, Expt 1). Virus inoculations on flower stalks were less effective and varied from 62.5% to 100% (Table 1, Expt 2). Regardless of leaves or flower stalks as inoculation sites, a 100% disease incidence (crinkling of flowers) was reached in V3 plants inoculated with ORSV 96-Ph-O7 or ORSV 96-Ph-O16 while none of the CymMV 96-Ph-C19 infected V3 plants showed crinkling of flowers. When leaves of *Doritaenopsis* Join Angel "274-1" plants were inoculated with ORSV 96-Ph-O7 or ORSV 96-Ph-O16, infection rates were 89–100% and disease incidences were 83.3–100% (Table 1, Expt 3). CymMV 96-Ph-19 infected "274-1" plants did not show crinkling of flowers. When V3 plants naturally infected with CymMV were inoculated with ORSV 96-Ph-O7 or ORSV 96-Ph-O16, flower symptoms

Table 1 Flower reactions of white flower-bearing *Phalaenopsis* hybrids inoculated with *Odontoglossum ringspot virus* (ORSV) and *Cymbidium mosaic virus* (CymMV)

	Expt 1		Expt 2		Expt 3	
	V3[a]		V3		274-1[a]	
	Leaf[b]		Flower stalk[b]		Leaf	
Virus[c]	IR[d]	DI[e]	IR	DI	IR	DI
Mock	0/12	0/0	0/9	0/0	0/11	0/0
O7	7/7	7/7	7/7	7/7	8/9	8/8
O7+C19	6/6	6/6	10/10	10/10	9/9	9/9
O16	9/9	9/9	7/7	7/7	9/9	9/9
O16+C19	9/9	9/9	8/9	8/8	8/9	8/8
C19	9/9	0/9	6/8	0/6	7/7	0/7
C19+O7	7/7	7/7	5/8	5/5	6/6	5/6
C19+O16	4/4	4/4	5/5	5/5	6/6	5/6
C	8/8	0/8	–	–	–	–
C+O7	5/5	5/5	–	–	–	–
C+O16	5/5	5/5	–	–	–	–

[a] *Phalaenopsis* hybrids: V3, Phal. Sogo Yukidian "V3" (Phal. Yukimai×Phal. Taisuco Kochdian); 274-1, *Doritaenopsis* Join Angel "274-1" (Dtps. Casablanca Joy×Phal. Taida inlong "KC1932")

[b] Inoculation site

[c] O7: ORSV 96-Ph-O7; O16: ORSV 96-Ph-O16; C19: CymMV 96-Ph-C19; C: naturally CymMV-infected plants. The double inoculation was done in the order of the virus code

[d] IR: Infection rate based on the detection of the virus presence by indirect ELISA and RT-PCR

[e] DI: Disease incidence based on the development of the flower crinkle

were induced as a result of ORSV infection (Table 1, Expt 1).

V3 and "274-1" plants inoculated with ORSV 96-Ph-O7 or ORSV 96-Ph-O16 showed flower crinkle symptoms similar to the symptoms found on naturally infected plants (Fig. 1), regardless whether plants were inoculated with ORSV only (Fig. 1e) or occurred in mixed infections with CymMV (Fig. 1f, g). A difference in symptom expression between ORSV isolates could not be observed. All ORSV-infected V3 plants displayed crinkle symptoms on flowers indicating that this *Phalaenopsis* hybrid, V3, is very susceptible to ORSV infections with often severe symptoms while fewer ORSV-infected "274-1" plants expressed crinkle symptoms indicating that "274-1" plants are more tolerant to ORSV infections.

In the initial stage of symptom development, crinkled regions are particularly found on petals of *Phalaenopsis* orchids with crinkled regions subsequently becoming transparent. With symptoms progressing, crinkled regions gradually enlarged and fused. At that stage the petals became obviously transparent (Fig. 1e, f, g). In addition, the visual appearance of the flower was not as appealing and the blooming period of diseased orchids was shortened. New flowers showed systemic symptoms, some flower buds shriveled and dried up (Fig. 1h, i), hence decreasing flower numbers. CymMV-infected plants showed the phenotype of healthy, uninfected plants (Fig. 1c, d).

Our results provide evidence that ORSV is causing flower crinkle disease of the white flower-bearing *Phalaenopsis* hybrids, Phal. Sogo Yukidian "V3" (Phal. Yukimai × Phal. Taisuco Kochdian) and *Doritaenopsis* Join Angel "274-1" (Dtps. Casablanca Joy × Phal. Taida inlong "KC1932") orchids. In addition to flower crinkle of white flower-bearing *Phalaenopsis* orchids, *Phalaenopsis* hybrids with coloured flowers showed similar symptoms and ORSV infection was confirmed by ELISA in our studies (unpublished). Flower senescence disorder of red *Phalaenopsis* flowers in Japan were also found (Fukai 2008). It was therefore concluded that both V3—the white flower-bearing *Phalaenopsis* hybrid as well as other coloured flowering hybrids could exhibit flower crinkle when infected by ORSV.

In previous reports, only few viruses were detected in orchid flowers showing various symptoms (Corbett 1967; Inouye et al. 1988; Lawson and Hsu 1995; Lesemann 1977; Lesemann and Vetten 1985; Zettler et al. 1990). Flowers of *Phalaenopsis* orchid infected with CymMV and the orchid strain of *Tobacco mosaic virus* (TMV-O; synonym of ORSV) were deformed and reduced in size (Corbett 1967). This was the first report of virus symptoms on *Phalaenopsis* flowers, but evidence demonstrating TMV-O as the sole causal agent of the flower symptoms was not provided. Similarly, colour breaking and deformation of *Phalaenopsis* flowers was considered a result of ORSV infections (Inouye 1990) but ORSV was not unequivocally confirmed. Our results from virus transmission studies including back inoculation to orchids, ELISA and RT-PCR demonstrated that flower crinkle of white flower-bearing *Phalaenopsis* orchids is a symptom caused by infections with ORSV. Consequently, a new disease phenotype of ORSV on *Phalaenopsis* hybrids designated as "flower crinkle" is proposed to be added to the list of virus diseases of *Phalaenopsis*.

Acknowledgements We are grateful to Dr. Wen-Hsiung Ko, Professor Emeritus of the University of Hawaii at Manoa and Dr. Chung-Jan Chang, Professor of the University of Georgia for their critical review of the manuscript. This study was partially supported by a grant (97AS-14.3.1-BQ-B1) from the Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Executive Yuan, Taiwan.

References

Baker, C. A., Davison, D., & Jones, L. (2007). *Impatiens necrotic spot virus* and *Tomato spotted wilt virus* diagnosed in *Phalaenopsis* orchids from two Florida nurseries. *Plant Disease, 91*, 1515.

Corbett, M. K. (1967). Some distinguishing characteristics of the orchid strain of tobacco mosaic virus. *Phytopathology, 57*, 164–172.

Fukai, S. (2008). *Tissue observation of flower senescence*. Retrieved January 14, 2010, form the Japan Flower Growers Association, Division of Phalaenopsis Web site: http://phal.orchid-as.jp/hanafukehoukoku080811.pdf.

Inouye, N. (1990). Virus, fungal and bacterial diseases of orchids. *Plant Protection, 44*, 177–181.

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

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. *The Journal of General Virology, 81*, 2103–2109.

Lawson, R. H., & Brannigan, M. (1986). *Virus disease of orchids. Handbook of orchid pests and diseases* (pp. 2–49). Florida: American Orchid Society.

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. 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.

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. *The Plant Cell, 2*, 279–289.

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*, 621–625.

Zheng, Y.-X. (2007). *Identification and characterization of new Phalaenopsis orchid-infecting viruses and developing new transgenic approach for broad-spectrum resistance to viruses by expressing designed siRNA-generating synthetic nucleotides*. Dissertation: National Chung Hsing University.

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.