Orchid virus diseases in Taiwan and their control strategies

Characteristics of virus diseases

Causing systemic infection

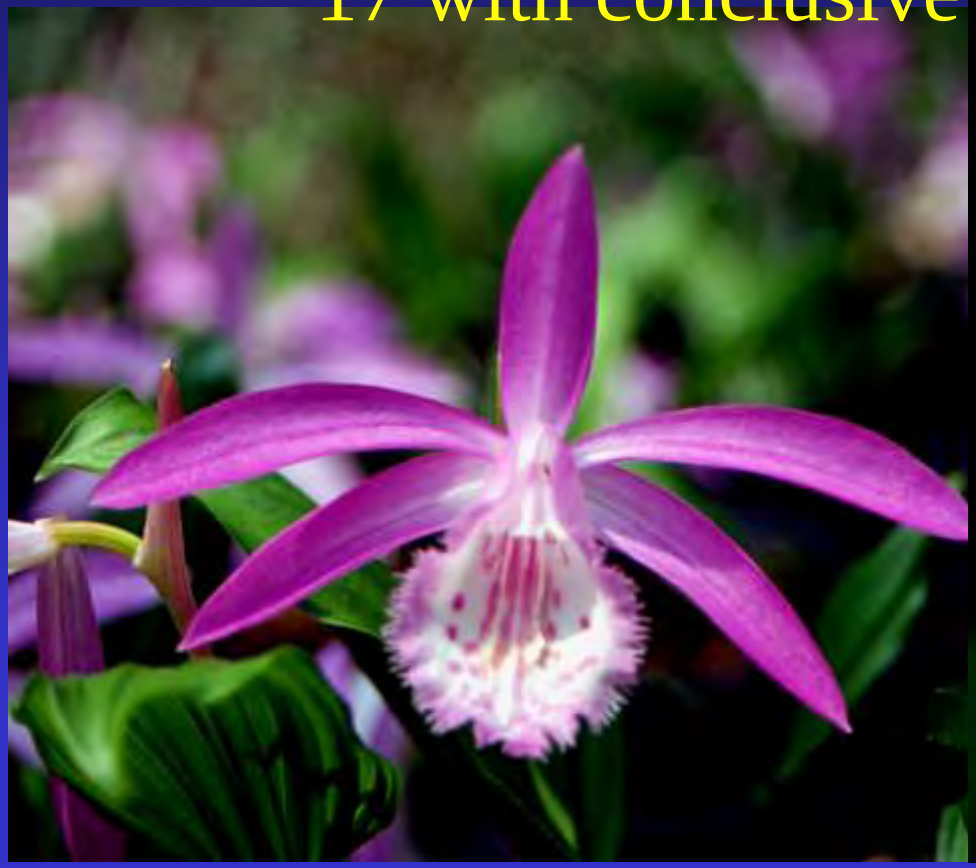
Reduce growth vigor and quality

Not curable once infected

Viruses known to infect orchids in the literature

In total, more than 30 viruses recorded in literature.

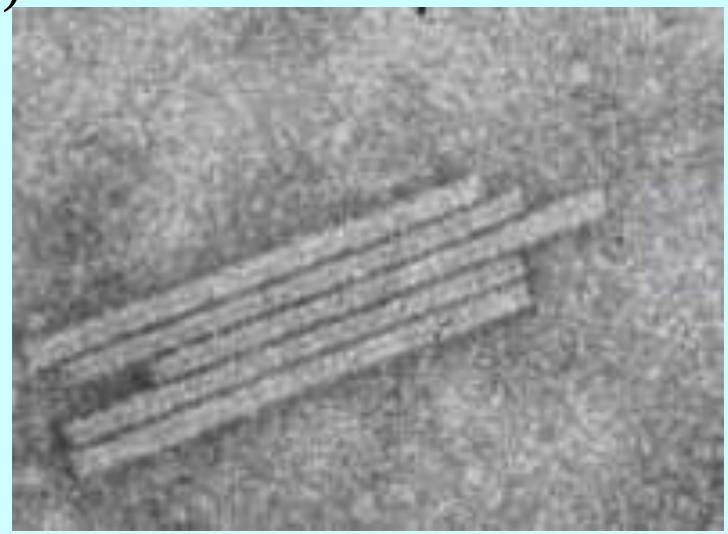
17 with conclusive taxonomic status.



Rod-shaped

Tobamovirus

- *Odontoglossum ringspot virus* (ORSV)
- *Tobacco mosaic virus*-orchid strain (TMV-orchid)



Potexvirus

- *Cymbidium mosaic virus* (CymMV)

Tobravirus

- *Tobacco rattle virus* (TRV)

Potyvirus

- *Bean yellow mosaic virus* (BYMV)
- *Dendrobium mosaic virus* (DenMV)
- *Clover yellow vein virus* (CYVV)
- *Turnip mosaic virus* (TuMV)
- *Vanilla mosaic virus* (VaMV)
- Phalaenopsis chlorotic spot virus (PhCSV)

Closterovirus

- *Dendrobium vein necrosis virus* (DVMV)

Isometric

Tombusvirus

Cymbidium ringspot virus (CyRSV)

Carmovirus

Carnation mottle virus (CarMV)

Cucumovirus

Cucumber mosaic virus (CMV)

Nepovirus

Tomato ringspot virus (TomRSV)

Tospovirus

Tomato spotted wilt virus (TSWV)

Impatiens necrotic spot virus (INSV)

Capsicum chlorosis virus (CaCV)

Unassigned

Orchid fleck virus (OFV)

Orchid viruses found in Taiwan

1. *Odontoglossum ringspot virus* (ORSV)
2. *Cymbidium mosaic virus* (CymMV)
3. *Cucumber mosaic virus* (CMV)
4. *Capsicum chlorosis virus*-phalaenopsis isolate (CaCV)
5. Phalaenopsis chlorotic spot virus (PhCSV)
6. *Bean yellow mosaic virus* (BYMV)
7. *Turnip mosaic virus* (TuMV)
8. *Carnation mottle virus* (CarMV)
9. *Orchid fleck virus* (OFV)

Odontoglossum ringspot virus (ORSV)

Tobamovirus, rigid rods, 300 nm in length

Heat stable, tolerant up to 95℃

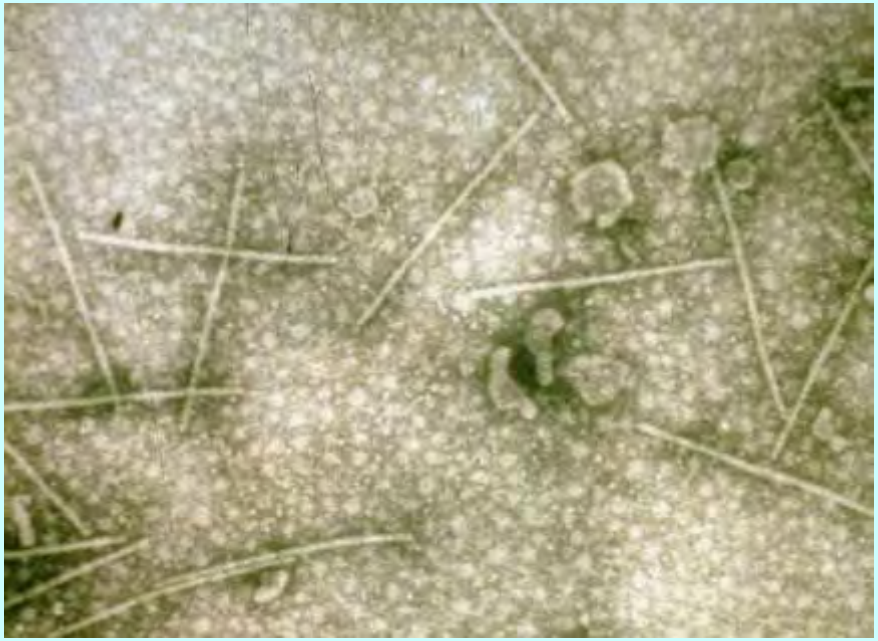
Highly stable in vitro, last over years (10 yrs)

Transmitted by mechanical contact, no vector known

Cymbidium mosaic virus (CymMV)

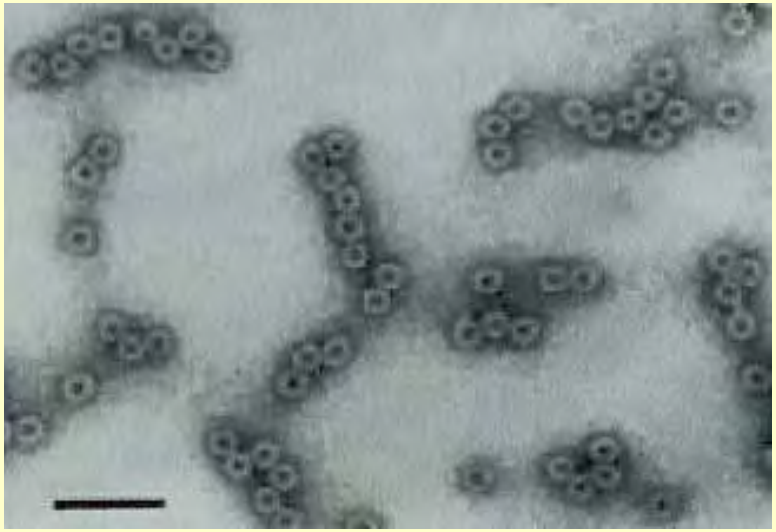
- *Potexviruses*, filamentous particles (450 nm in length)
- Transmitted by contact, no vector known.
- Not as stable in vitro as ORSV,

  but very stable compared to other plant viruses.



Cucumber mosaic virus (CMV)

- Symptoms: yellow striping on leaves.
- *Cucumovirus*
- Isometric particles
- Mechanically transmissible and also by aphids with non-persistent manner.
- Occurs more frequently on orchids grown in open fields



Capsicum chlorosis virus (CaCV)

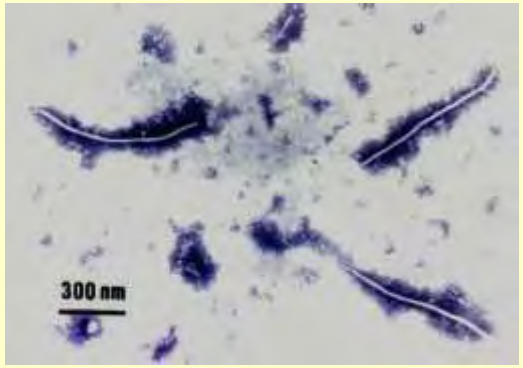
Incorrectly called “Taiwan virus”

- Symptoms: chlorotic ringspot on leaves.
- ***Tospovirus***
- Isometric enveloped particles.
- Serologically related to serogroup IV tospoviruses.
- Mechanically transmissible and also by thrips with persistent manner.
- Species of vector thrips is not known yet.

Phalaenopsis chlorotic spot virus (PhCSV)

or Basella rugose mosaic virus (BaRMV) ?

Potyvirus



Mechanical transmissible and also by aphid with non-persistent manner

Induce chlorotic spot symptoms on leaves

Need to confirm its taxonomic status.

Turnip mosaic virus (TuMV)

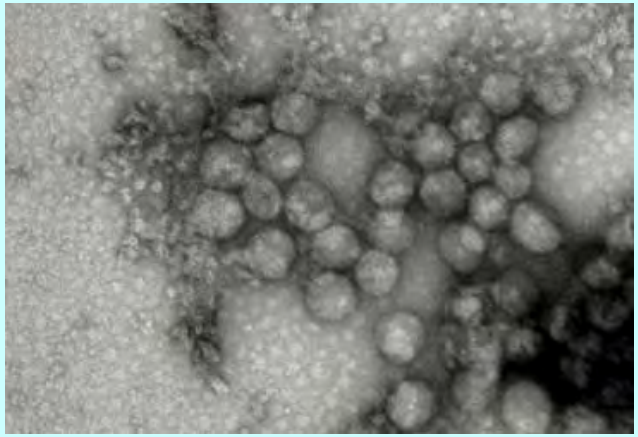
Potyvirus

Transmitted mainly by aphids with non-persistent manner.

Natural hosts for TuMV are cruciferous vegetables.

Found only once in Taiwan.

Carnation mottle virus (CarMV)



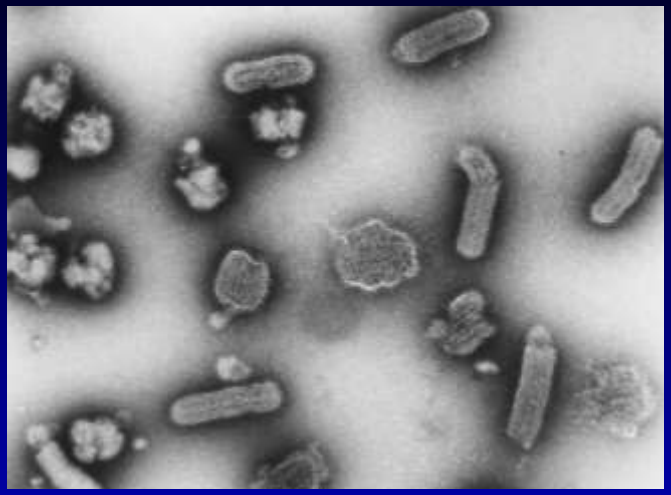
Carmovirus, Tombusviridae

Very stable in vitro and remains infective for long period of time.

Can easily contaminate working benches and laminar flow.

Possibly induce chlorotic spots on Phalaenopsis.

Orchid fleck virus (OFV)



Rhabdovirus The unassigned viruses

Bullet shaped virus particles

Transmitted by mites (*Brevipalpus californicus*)

First reported in Japan.

Occurring also in Brazil,
Germany, USA and Australia

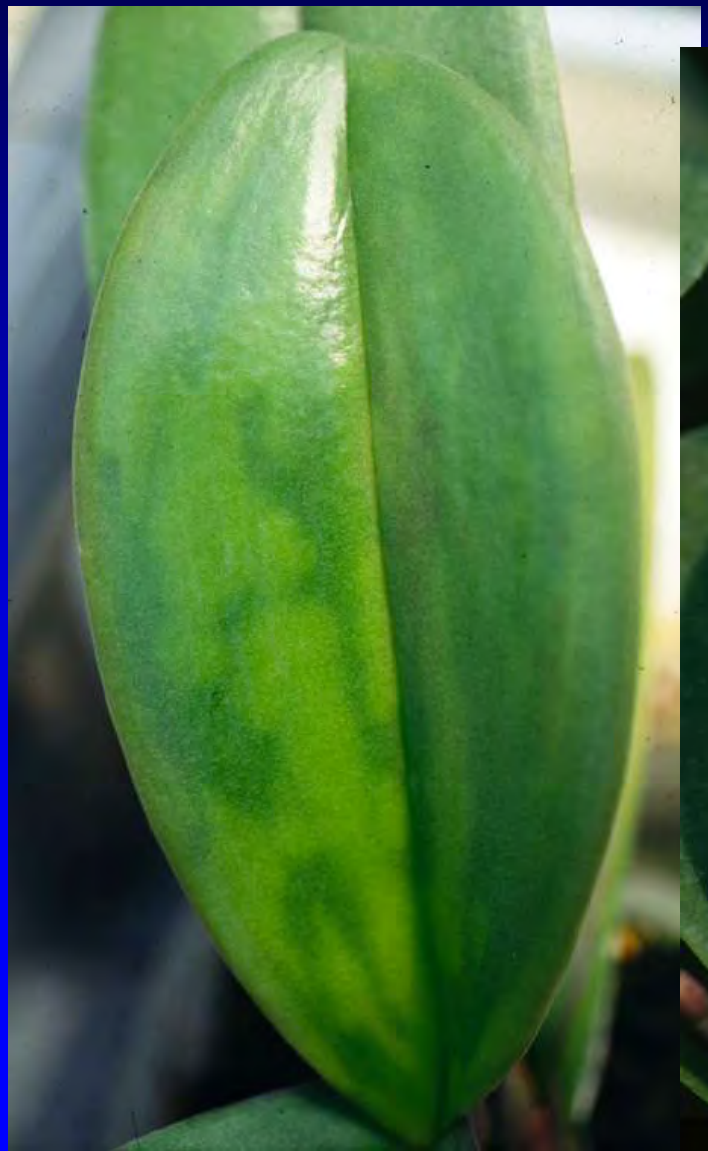
OFV has been eradicated since 2005

Table. Result of surveillance of *Orchid fleck virus* in Taiwan since 2005 to 2007.

Source	Species	No. of sample	Detection method	Date	Result	
					RT-PCR	ELISA
Lu-ku	cymbidium	37	ELISA	94.12.08	-	0/37
Yu-chi	cymbidium	>5000	V. I.	95.10.07	0	0
Chai-yi	phalaenopsis	2	RT-PCR	95.10.23	0/2	-
TDAIS	cymbidium	3	RT-PCR, ELISA	96.01.26	0/3	0/3
TDAIS	cymbidium	16	RT-PCR, ELISA	96.01.26	0/16	0/16
Yu-chi	cymbidium	>5000	V. I.	96.01.26	0	0
Pin-tung	phalaenopsis	1	RT-PCR	96.04.10	0/1	-
Hou-Li	oncidium	5	RT-PCR	96.08.22	0/5	-
Pu-Li	oncidium	1	RT-PCR	96.09.28	0/1	-

V.I. : Visual inspection

Symptoms induced by ORSV on *Phalaenopsis* spp.



ORSV symptoms on oncidiums

ORSV symptoms on other orchids

CymMV symptoms on *Phalaenopsis* spp.

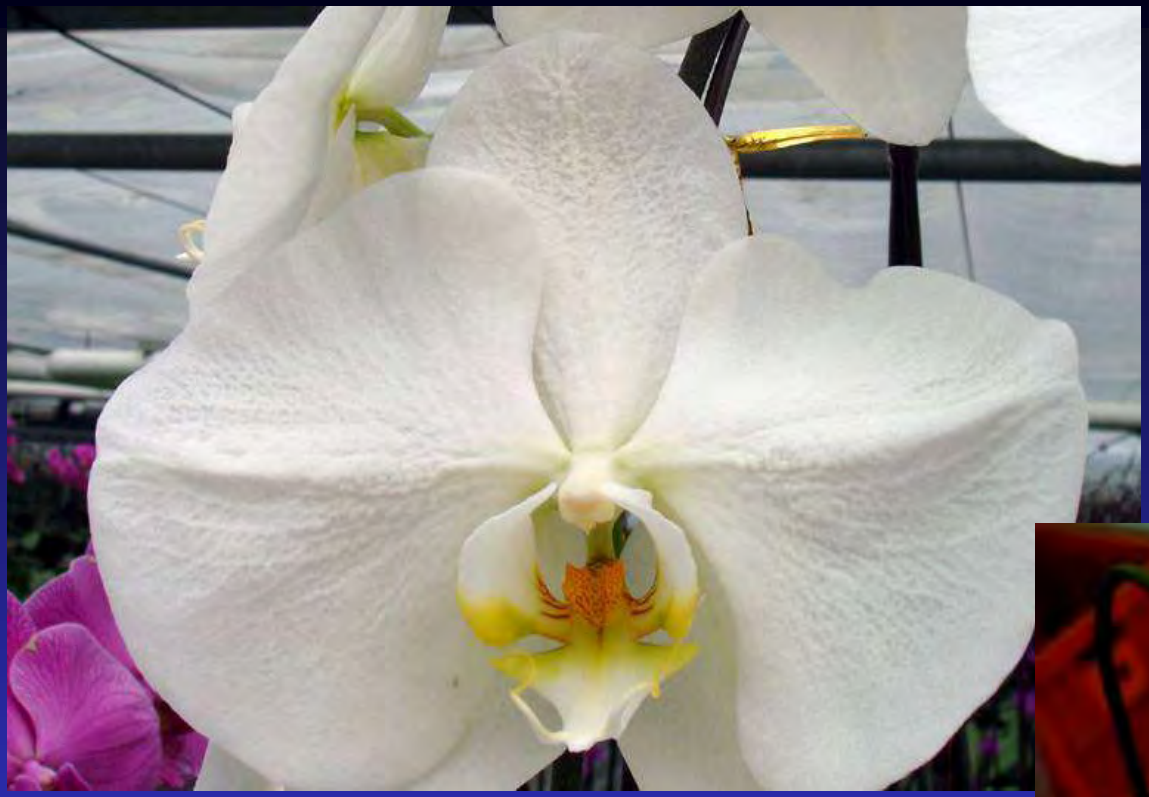
CymMV symptoms on Oncidiums

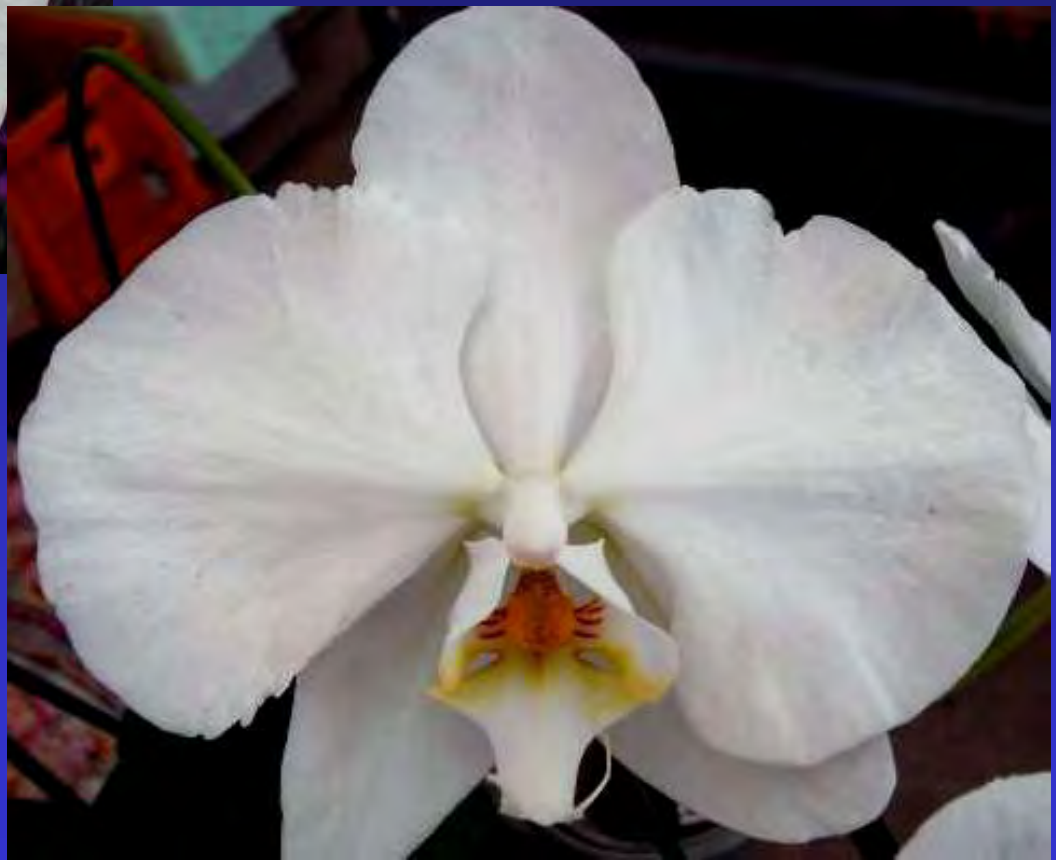
CymMV symptoms on other orchids

Symptoms on *Phalaenopsis* induced by dual infection of ORSV and CymMV

ORSV

CymMV



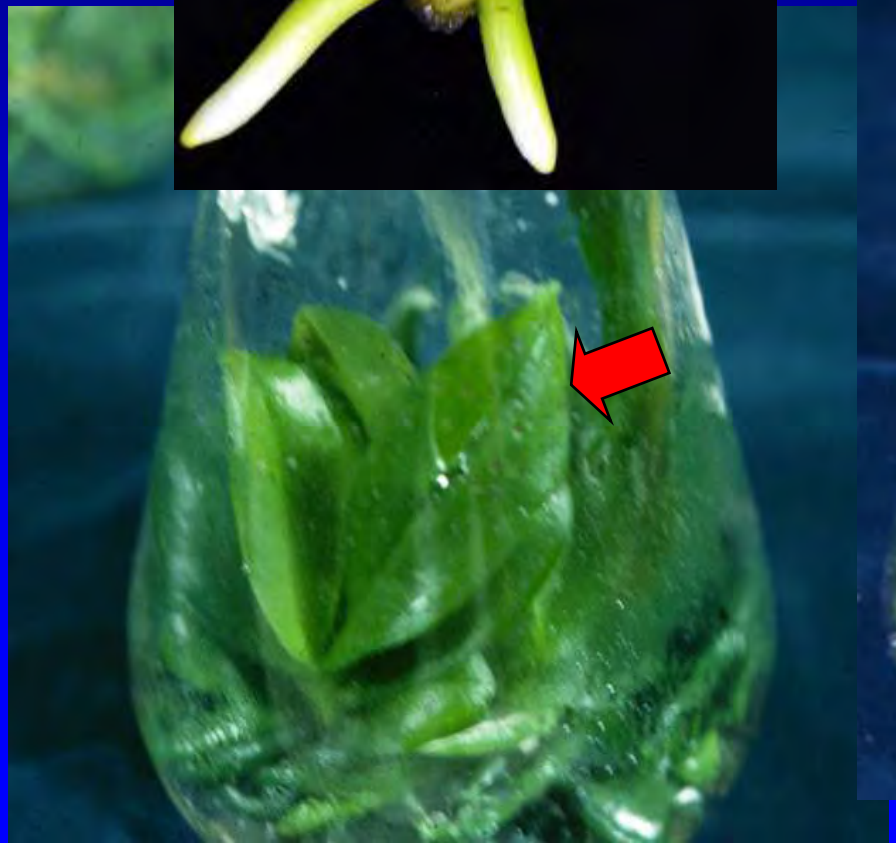


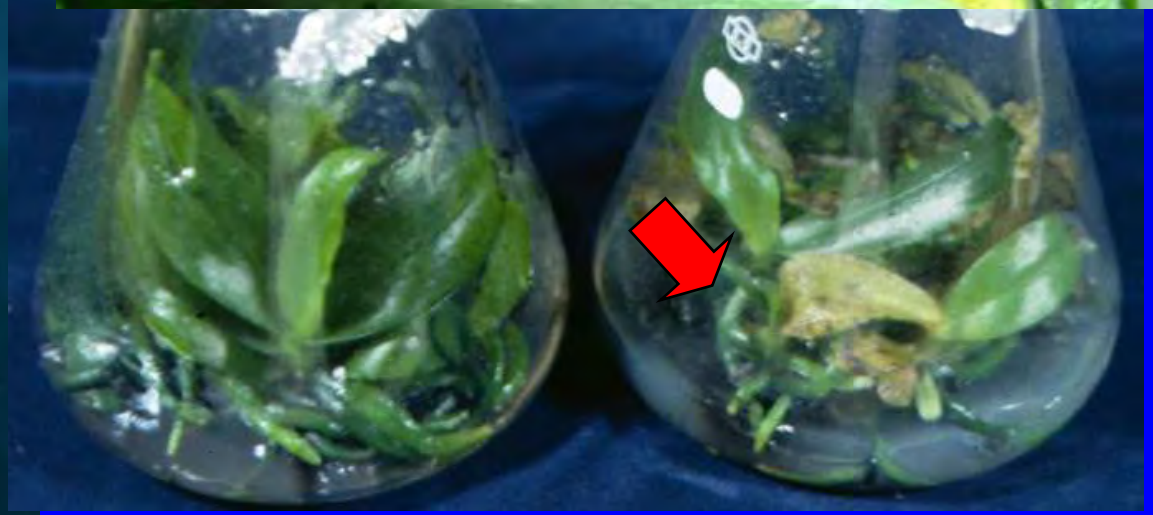
Symptom expression:

1. Type of infected virus?
2. Single or mixed infection?
3. Natural resistance of cultivar?
4. Age of plant?
5. Environment factor?
6. Stress?

Mode of transmission and virus sources

1. Transmission from infected mother plants by vegetative propagation including tissue culture.





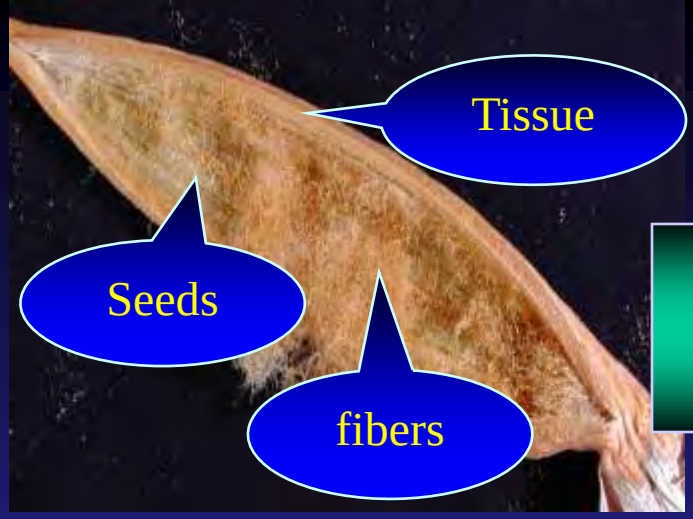
Mode of transmission and virus sources

2. Seed-borne transmission

Survey of virus infection in Phalaenopsis seedlings

Virus infection rate of Phalaenopsis seedlings propagated from tree seeds

Sources of flask	No. of flask	Virus infection rate ORSV	CyMV	O+C	(%)
A-W	9	1	0	0	0.7
B-6106	10	3	3	1	4.7
C-00110	10	7	6	3	10.7
D-34010	10	11	58	7	50.7

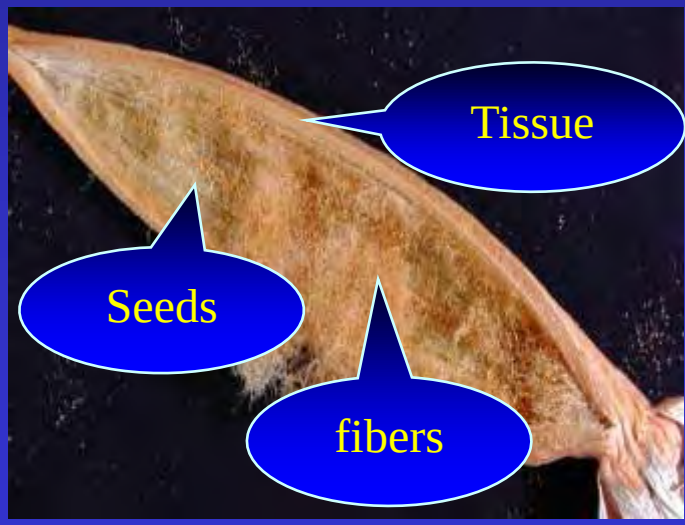


Virus concentration in different parts of *Phalaenopsis* fruit capsule assayed by ELISA

	A		B		C		D	
	ORSV	CyMV	ORSV	CyMV	ORSV	CyMV	ORSV	CyMV
Capsuls	0.065	*	*	*	0.315	1.108	0.589	0.060
Cotton fiber with seeds	0.112	*	*	*	0.071	1.701	*	0.043
Cotton fiber	0.148	*	*	*	0.086	0.045	*	0.033
Seed wash 1 time with ddH_2O	0.077	1.574	0.287	2.315	0.108	0.049	0.335	0.085
Seed wash 2 times with ddH_2O	0.049	2.545	0.260	0.592	0.142	0.054	0.569	0.058
Seed wash 3 times with ddH_2O	0.024	1.791	0.421	0.521	0.098	0.058	0.178	0.072
Seed wash 4 times with ddH_2O	0.063	2.955	0.516	0.276	0.096	0.102	0.047	0.076
Seed wash 5 times with ddH_2O	0.091	1.598	1.103	0.496	0.025	0.101	0.080	0.060

Mechanisms for seed transmission of Phalaenopsis orchids

1. ORSV and CyMV are only contaminating on the surface of seeds instead of infecting the seeds.
2. Virus infected mother plants will produce infected capsules with high concentration of viruses on the seeds and the fibers.
3. Seeding the infected fibers together with the seeds will eventually result infected seedlings.
4. Infection rate of the seedlings is variable depending of the workers during subculture of the seedling.



Mode of transmission and virus sources

3. Virus contamination during tissue culture passages.

Mode of transmission and virus sources

4. Virus contamination during transplanting of plantlets

Mode of transmission and virus sources

5. Transmission by mechanical contact during culturation.

Mode of transmission and virus sources

7. Transmission by insect or other pests.

How to produce virus clean orchid plantlets

1. Screening for non-infected plants before mericloning propagation

a. Collect true to type and symptomless plants.
b. Maintain in a isolated house and keep them well separated.
c. Apply virus indexing tests such as ELISA or RT-PCR on the plants.
d. Eliminate any detected plants from the isolated house.
e. Perform virus check again after at least one month later.
f. Conduct mericloning on the doubly checked plants.
g. Keep the doubly checked plants as mother stocks and apply virus check at least once every year.

Special concerns:

1. **Apply sensitive, dependable and reproducible virus indexing techniques.**
   ELISA, RT-PCR, Biochips, Immuno-strips (Rapid test), etc.

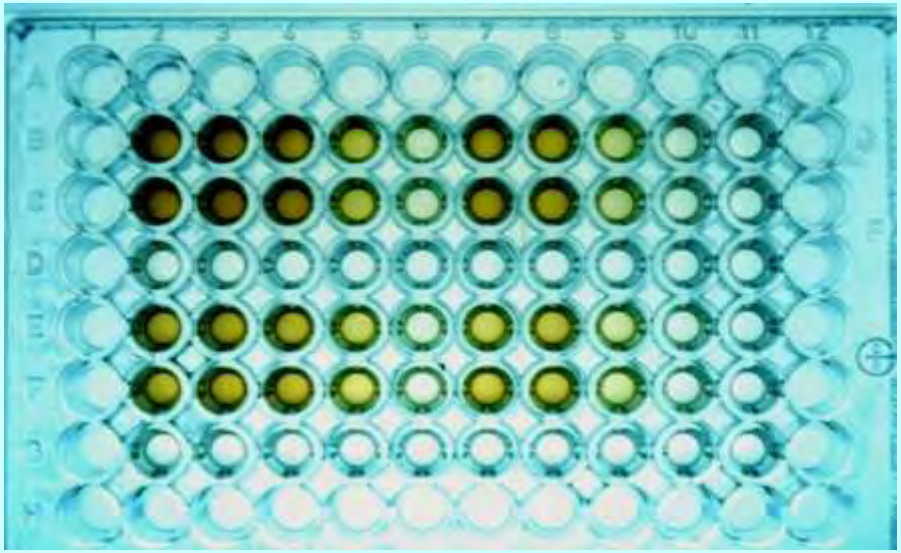
2. **Use the right sampling method.**

3. **Repeats and alternatives.**

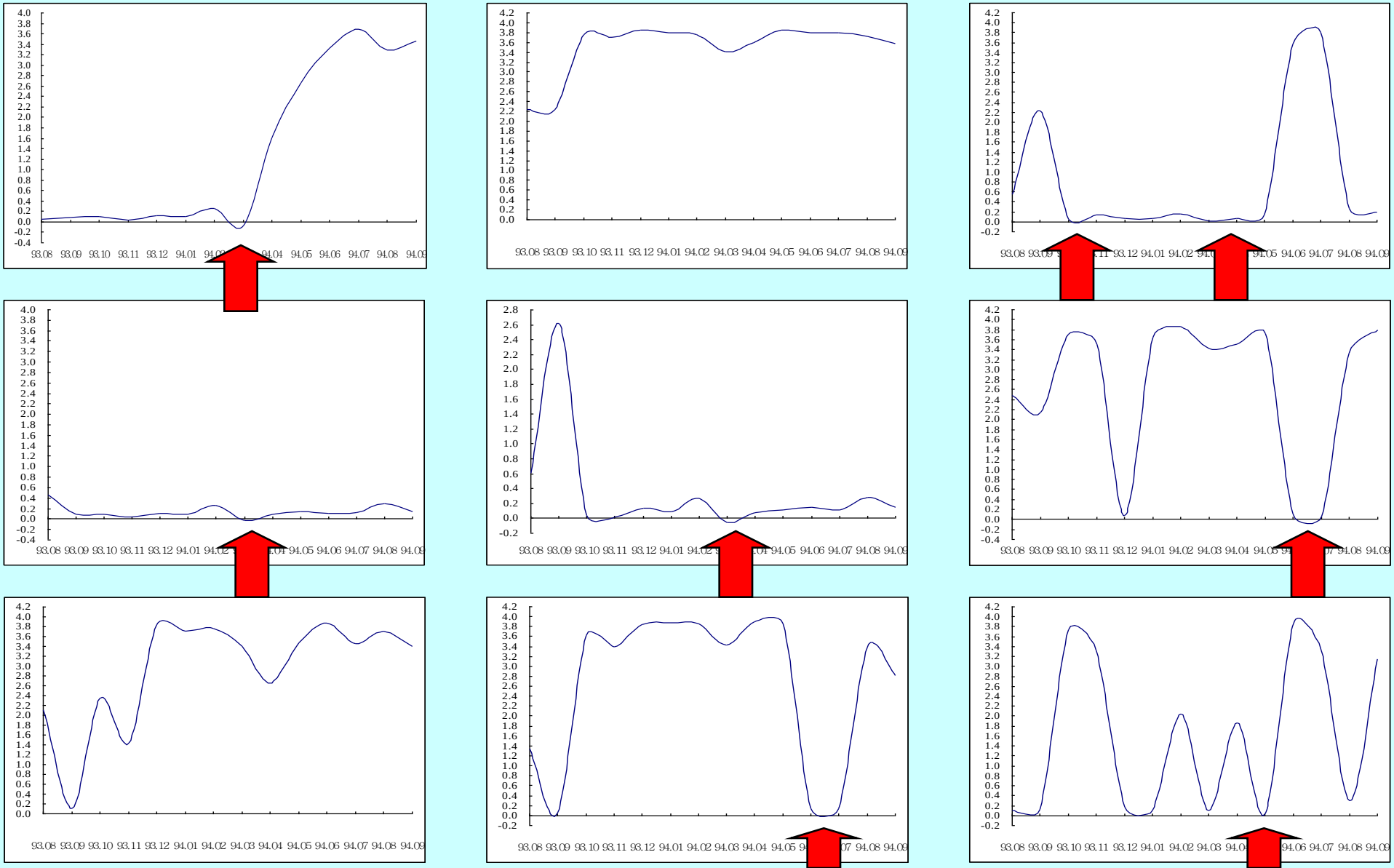
(Enzyme linked immuno-sorbent assay, ELISA)

- The most widely used plant virus indexing technique since 1977.

1. Sensitive
2. Specific
3. Efficient
4. Reproducibe
5. Objective
6. Cost effective



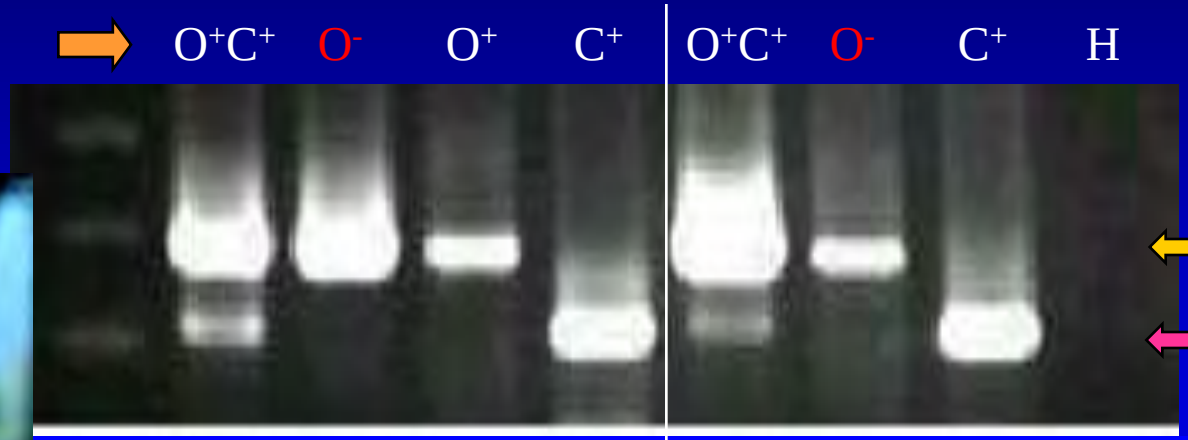
Fluctuation of virus concentration in orchid tissue as determined by ELISA within one year.



One-tube multiplex RT-PCR assays

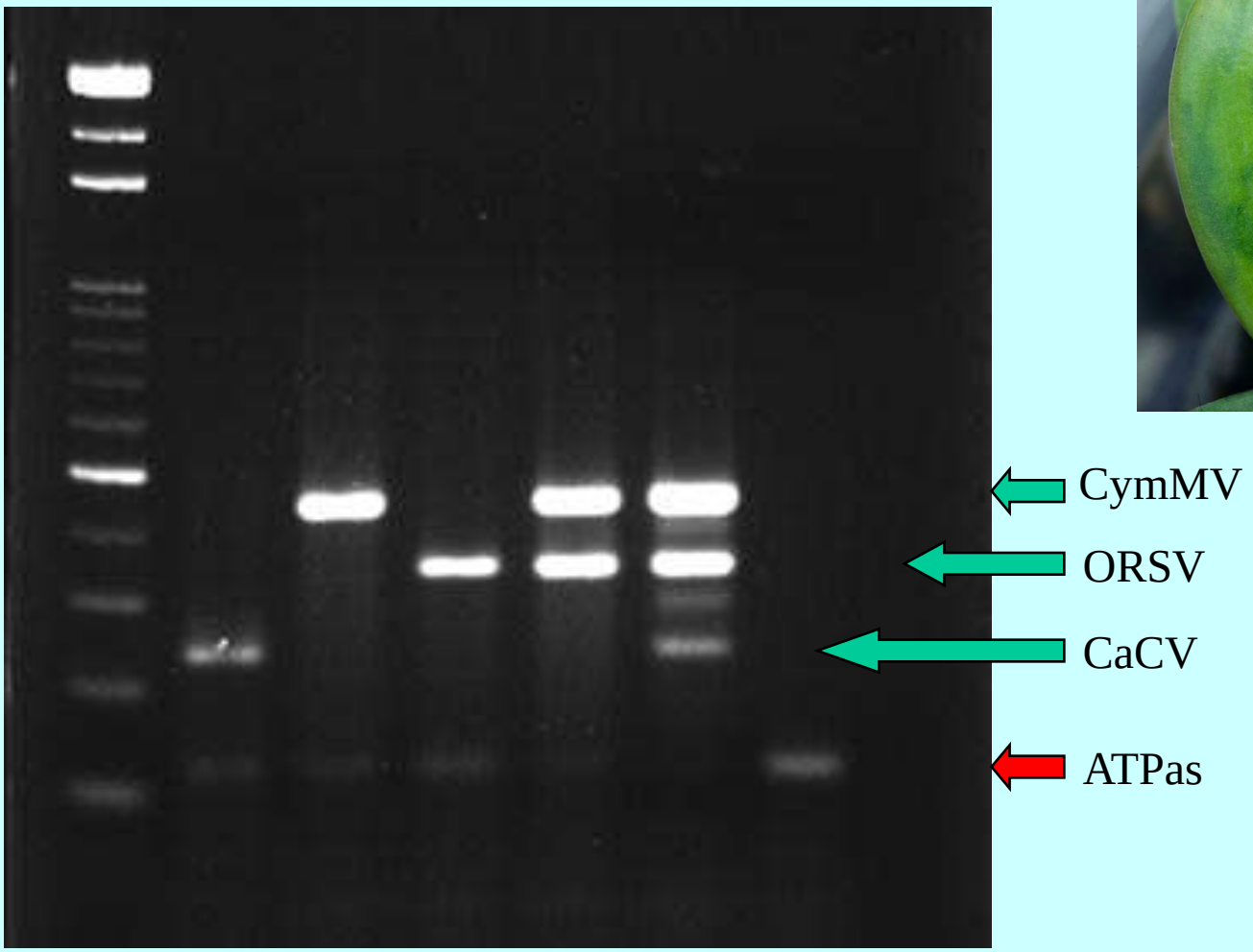
Detect CymMV and ORSV simultaneously

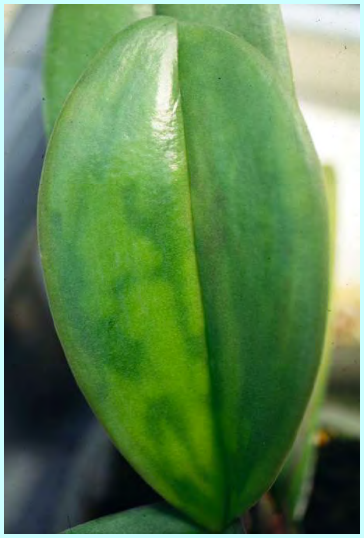
ELISA ➡



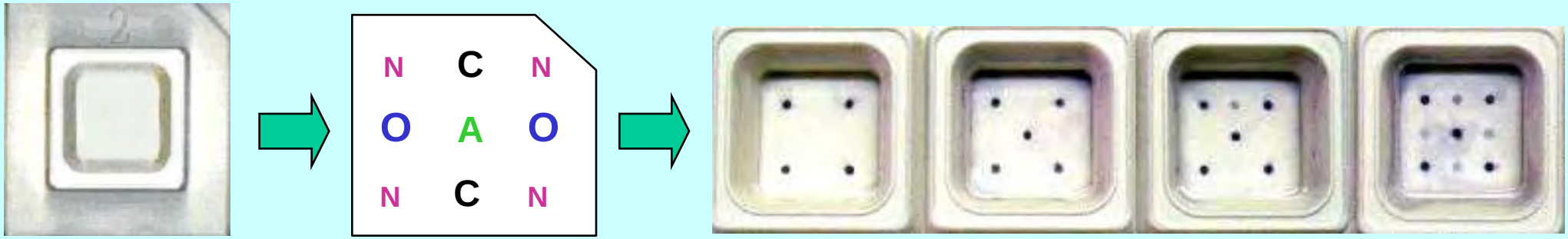
Field plant

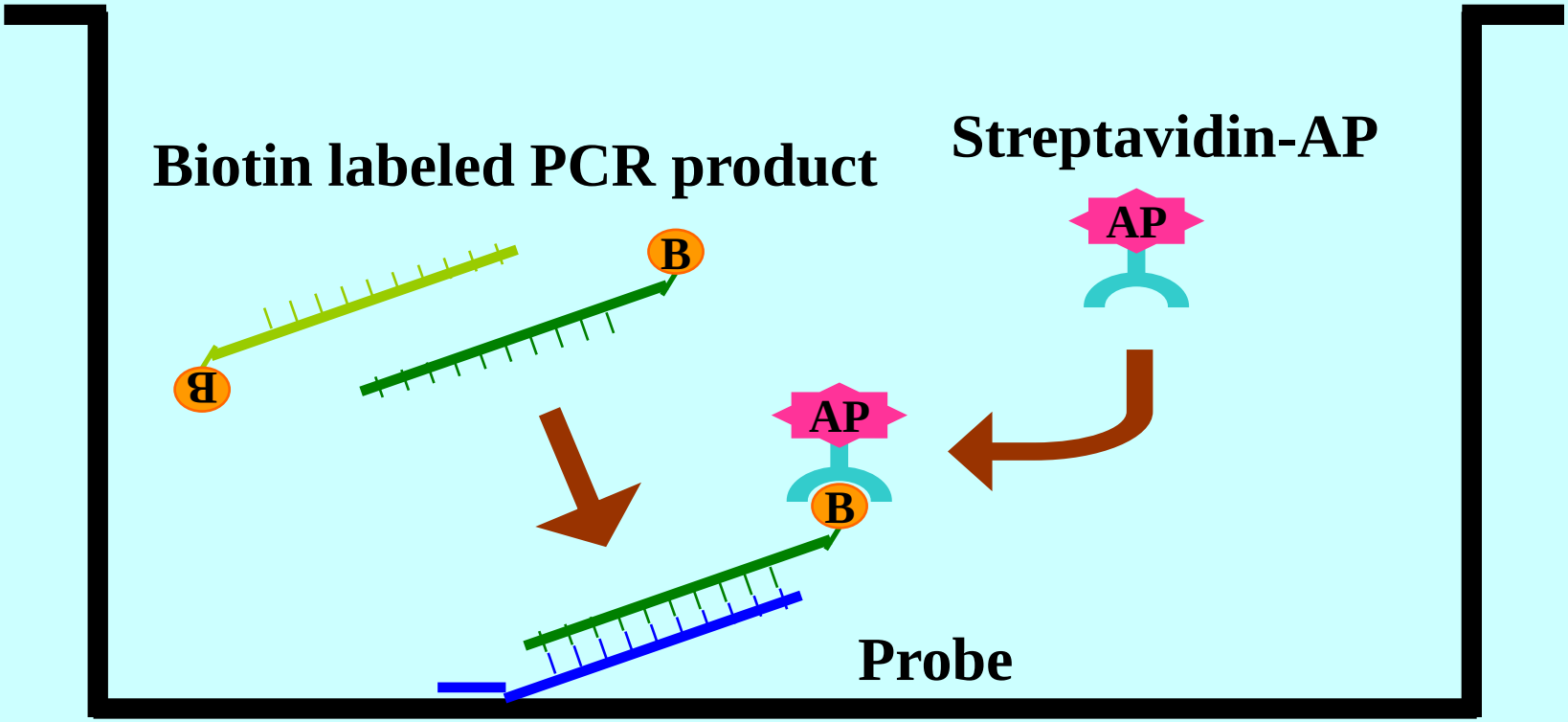
One-step multiplex RT-PCR for simultaneous detection of ORSV, CymMV and CaCV





Biochip detection technology





Biochip system for simultaneous detection of ORSV and CymMV.

Biochips

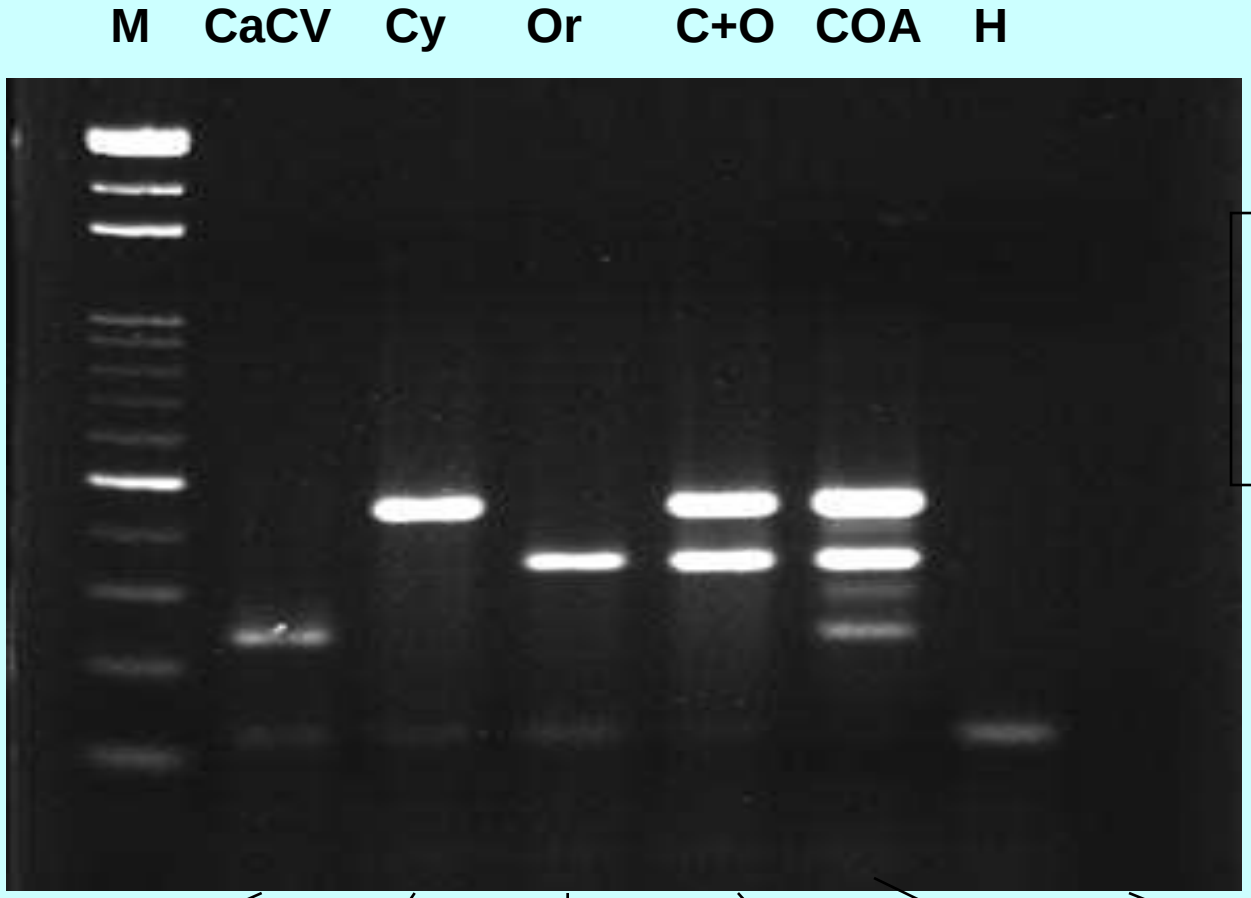
N C N
O A O
N C N

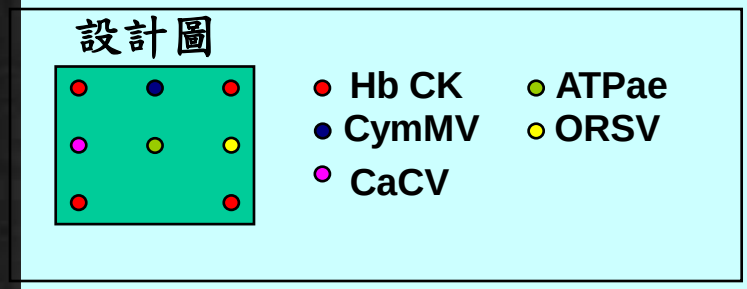
RT-PCR

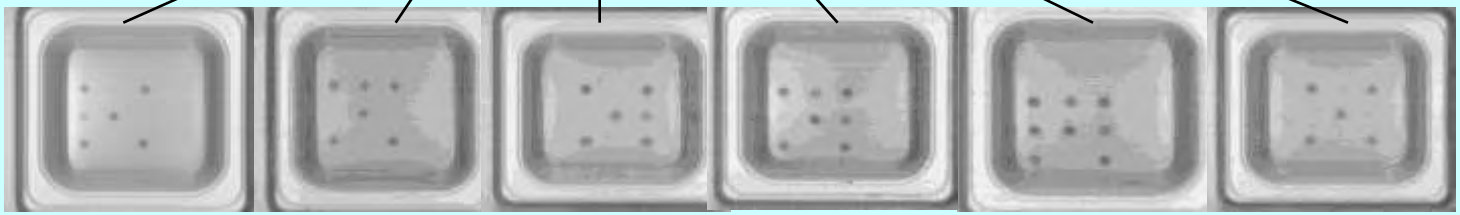
ELISA

	H CK	B	C	D. CK
CyMV	0.165	0.005	0.109	0.219
ORSV	0.099	0.024	0.069	1.951

Biochip system for simultaneous detection of ORSV, CymMV and CaCV







Special concerns:

1. Apply sensitive, dependable and reproducible virus indexing techniques.

2. **Use the right sampling method.**

   Leaves, roots, or spikes.
   and How to sample.

3. **Repeats and alternatives.**

   Do not depend on one single test.
   Use different techniques to confirm.

Timing and techniques for Phalaenopsis virus indexing in Taiwan.

Mother stocks

Mother flasks

Tissue cultured plantlets

Young plantlets

ELISA

PCR

PCR

PCR / ELISA

ELISA

Special concerns:

2. Take special precaution to prevent clean mother stocks from re-infection by viruses.

Maintaining clean mother stocks.

Executing sanitation procedures.

Cleaning of growing environment, tools, clothes and worker's hands appropriately

Maintaining in isolated clean room

Concluding remarks:

1. Producing virus clean orchids is a win-win situation for propagators and finished plants providers.

2. All partners in the orchid production chain should implement serious sanitation and strict production procedures to prevent virus spread.

3. However, it is difficult for nurseries to guarantee absolutely clean flowering orchids considering the high possibilities of re-infection during lengthy growing period.

4. Orchid consumers or down stream growers should compromise and accept the reality that certain percentage of virus infection in the finished flowering plants would not affect their mutual benefits.

Concluding remarks:

5. This understanding and compromise will certainly help the harmony of international orchid trading.
6. It should be (THANK YOU screening of virus-free stc for all orchid propagators.
7. No companies can overlook this common demand and still maintain their competitiveness in the orchid industry around the world.