A sensitive DNA microchip array for simultaneous detection of two orchid viruses

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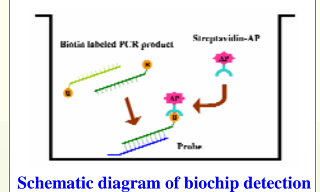
ABSTRACT

A multiplex RT-PCR protocol and a DNA microchip array system, both simultaneously detect two most economically important orchid viruses, *Odontoglossum ringspot virus* (ORSV) and *Cymbidium mosaic virus* (CymMV) were developed and their sensitivities evaluated. The latter approach is based on specific capture of RT-PCR amplified products with oligonucleotide DNA probes immobilized on a microchip. Two oligonucleotide probes, 20-30 mer in size, were designed for each virus based on their sequences of DNA products amplified by multiplex RT-PCR. Two primer pairs designed for specific amplification of ORSV and CymMV were combined in the one-step multiplex RT-PCR amplification test. Four primers pre-labeled with biotin in their 5'-ends facilitating the detection of the captured PCR products by streptavidin conjugated-alkaline phosphatase were used in DNA microchip array assay. Test of 106 infected orchid plants by multiplex RT-PCR and DNA microchip array showed that 80 and 97% specimens, respectively, were infected with ORSV, and 76- 98%, respectively, with CymMV. About 17-22% orchid samples that were not detected by multiplex RT-PCR were positively identified by DNA microchip method. The DNA microchip method is more sensitive than the multiplex RT-PCR, and is a better choice for routine virus indexing during orchid seedling production.

OBJECTIVES

To develop a method that can confirm PCR result by capturing the PCR products with specific DNA probes immobilizing on a DNA microchip. The captured PCR product will then be detected colormetrically by the use of biotin-strepavidin enzyme conjugated system.

Some symptoms cuased by ORSV and CymMV



Schematic diagram of biochip detection

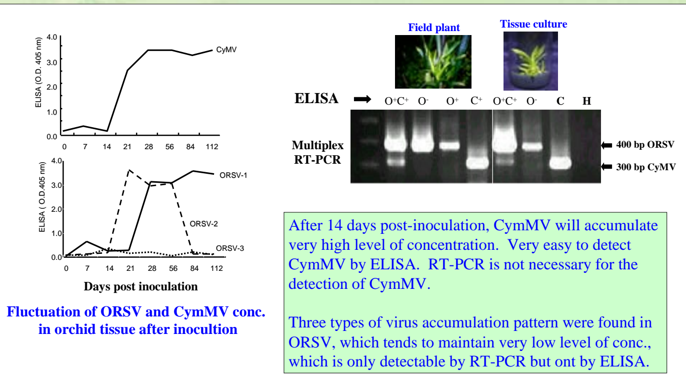
BACKGROUND

1. Orchids are popular and economically important ornamental crop for many countries around the world.
2. Most orchid species are frequently infected by *Odontoglossum ringspot* virus (ORSV) and *Cymbidium mosaic virus* (CymMV), which induce symptoms on the foliar or floral parts that may jeopardize the growth vigor and value of orchids.
3. Vegetative mericloning tissue culture technique is the major propagation means for commercial production of orchid plantlets, therefore screening for specific virus(es)-free mother stocks before mericloning has become a necessary process to prevent virus spread in orchid plantlets.
4. Currently, serological techniques such as ELISA are the most widely used methods for orchid virus indexing. However, our recent study has shown that some infected orchids may escape from the detection by ELISA. For this reason, we developed a highly sensitive multiplex RT-PCR protocol that can simultaneously detect ORSV and CymMV.
5. However, to apply RT-PCR in routine virus check in the real-life of orchid industry is still not practical for reading the PCR result after electrophoresis is subjective, experience dependent and sometimes the results are not reproducible.

RESULT

1. Oligonucleotide probes (20-30 mers in size) specific to the PCR products of ORSV and CymMV amplified by our one-step multiplex RT-PCR system were designed and tested for their reactivities and feasibilities serving as DNA array probes to capture the PCR products in biochip system. Two specific probes were selected for each viruses and immobilized on the surface of biochips. Different probes will be immobilized as specific spots on the biochips.
2. Two primer pairs selected for the amplification of ORSV and CymMV in one-step multiplex RT-PCR were pri-labeled with biotin in their 5'-end. After amplification the RT-PCR products will become biotin-labeled. The biotin-labeled PCR products will be captured specifically by the immobilized DNA probes for ORSV and CymMV. By the use of strepavidin conjugated with alkaline phosphatase (AP), the immobilized biotin-labeled PCR products will be further reacted and the AP enzyme will develop color reaction with their substrate NBT-BCIP.
3. Based on the coloration in the specific spot of ORSV or CymMV, the detection result is determined visually.

A



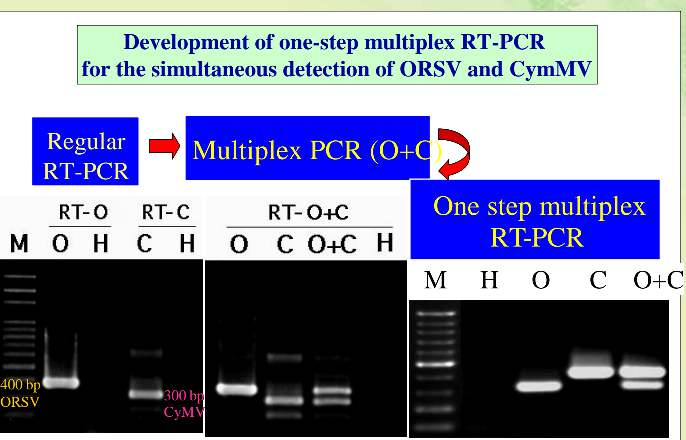
Fluctuation of ORSV and CymMV conc. in orchid tissue after inoculation

After 14 days post-inoculation, CymMV will accumulate very high level of concentration. Very easy to detect CymMV by ELISA. RT-PCR is not necessary for the detection of CymMV.

Three types of virus accumulation pattern were found in ORSV, which tends to maintain very low level of conc., which is only detectable by RT-PCR but ont by ELISA.

B

Development of one-step multiplex RT-PCR for the simultaneous detection of ORSV and CymMV



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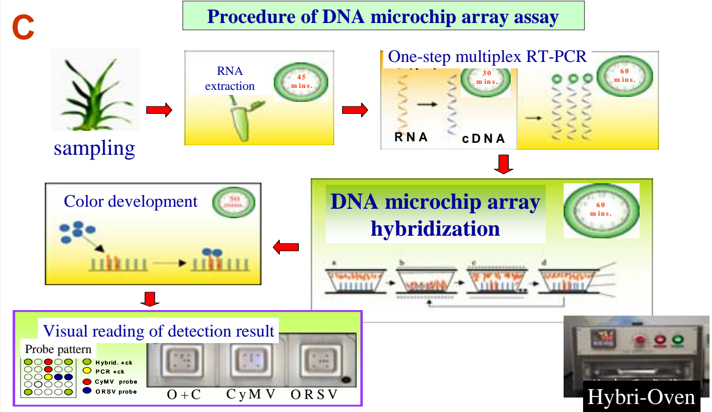

	CK	H. CK	1	2	D. CK
biochip					
Multiplex RT-PCR					

ELISA				
CymMV	0.165	0.005	0.109	0.219
ORSV	0.099	0.024	0.069	1.951

Comparison of orchid samples detected by biochip, multiplex RT-PCR, and ELISA. Note that sample No. 1 is not detected by ELISA and only faintly by RT-PCR, but it is detected as CymMV positive in biochip system. While sample No.2 is negative in ELISA, positive for ORSV and faintly for CymMV, but is confirmed to be dual infection by biochip system. Arrows indicate the faint CymMV bands.

% of detection	ORSV	CymMV
ELISA	65.0	70.0
RT-PCR	80.0	76.2
DNA microchip	96.5	97.6

Comparison on the percent of detection as determined by ELISA, multiplex RT-PCR and DNA biochip system for the detection of ORSV and CymMV in 106 orchid samples.

CONCULSION

1. Our result shows that the developed DNA biochip method is more sensitive than the multiplex RT-PCR. It can detect the two most economically important orchid viruses (i.e. ORSV and CymMV) simultaneously. This method has potential to include more other viruses in the same detection system without investing extra costs.
2. The complete procedure takes only about 6 hours, which is less than the traditional RT-PCR protocol.
3. The procedure has potential to be applied in the routine virus indexing especially in the screening of virus-free mother orchid stocks before mass production of tissue culture plantlets.