



### Special project

## A rapid, less-costly and accurate detection of citrus greening (HLB) pathogen in the ASPAC region (Year 1)

**IN RECENT DECADES, CITRUS GREENING DISEASE OR HUANGLONGBING (HLB)** has been causing major damage and economic losses to the citrus industry in the Asian and Pacific (ASPAC) region. The identification of HLB disease infection is extremely difficult because of its similarity to the symptoms of such nutrient deficiencies of Zn and Fe, and its pathogen, *Diaphorina citri*, cannot be cultivated on an artificial culture medium. In addition, no effective agrochemicals to control this disease are yet available. The only countermeasure to cope with HLB infection is to cut down the infected citrus trees. Therefore, early detection is very important to mitigate the damage caused by HLB infection. In view of the urgency and seriousness of this problem, FFTC implemented a research project to develop a rapid, less-costly and accurate detection of the HLB pathogen in partnership with the National Taiwan University (NTU) and the National Institute of Fruit Tree Science (NIFTS), Japan.

Polymerase chain reaction (PCR), which is sensitive and reproducible, is the most common method used for detection of HLB pathogen. However, this method requires a thermal cyclor and other basic apparatus for molecular biological experiments, as well as well-trained personnel. Meanwhile, the loop-mediated isothermal amplification (LAMP) is a newly developed method for DNA amplification in Japan. This method is considered to have the following advantages over PCR: 1) DNA amplification is catalyzed by only one enzyme; 2) Highly efficient amplification (within 30 minutes using a simple, affordable water bath); 3) Highly specific detection of HLB pathogen by four different kinds of primer; and 4) Large quantity of the product amplified suitable for under-equipped laboratories of extension centers and local quarantine offices. In the first year's trial of this collaborative research project, the following activities were undertaken: 1) Selection of promising four-primer-combination for the detection of the Japanese HLB isolates; 2) Test for applicability of the primer-combinations selected for HLB isolates collected from Taiwan and other ASPAC countries; 3) Comparison between LAMP and PCR for their advantages and disadvantages; and 4) On-site demonstration of LAMP method by Japanese scientists.

In the laboratory test of the LAMP method using Taiwanese HLB infected leaf samples, the following results were obtained for comparison with PCR.

- 1) The four primer combination, which detected all the Japanese HLB pathogens, could also be applicable to the Taiwanese pathogens without modification.
- 2) LAMP method proved to be more rapid and simpler than PCR in terms of HLB-detection.
- 3) The sensitivity of the LAMP method was almost the same



Participants of the LAMP workshop with Prof. Hong-Ji Su (rightmost), FFTC Consultant and Professor Emeritus, NTU.

as that of PCR, but slightly inferior to the latter. When low titer of HLB pathogen existed in the infected leaves, there was no change in the solution to indicate presence of pathogen in the LAMP method, while PCR was able to detect the pathogen.

- 4) More efficient and reliable extraction method of HLB pathogen from infected leaves should be developed to increase the sensitivity of the LAMP method.

A one-day workshop on "Application of LAMP method for detection of HLB (citrus greening disease) pathogen" was also held at the National Taiwan University (NTU) as part of the collaborative research project. Japanese scientists from the National Agricultural Research Center for Kyushu Okinawa Region (KONARC) and the NIFTS were invited to give a lecture and laboratory demonstration on Loop-mediated isothermal amplification (LAMP) for HLB pathogen detection. Under the coordination of Prof. Hong-Ji Su, FFTC Consultant and NTU Professor Emeritus, more than 10 scientists engaged in HLB research all over Taiwan participated in the workshop.

### International Collaborative Project on "A rapid, less-costly and accurate detection of citrus greening (HLB) pathogen in the ASPAC region" (Year 1)

This two-year (April 2007 - March 2009) collaborative research project is being carried out in Taiwan ROC and Japan.

Co-sponsors:

National Taiwan University (NTU), Taiwan  
ROC

National Institute of Fruit Tree Science  
(NIFTS), Japan

National Agricultural Research Center for  
Kyushu Okinawa Region (KONARC)

Okinawa Prefectural Agricultural Research  
Center (OPARC)

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