

## THE MOLECULAR DETECTION OF CITRUS VEIN PHLOEM DEGENERATION (CVPD) PATHOGEN (*Liberobacter asiaticus*) IN *Diaphorina citri* KUWAYAMA (HOMOPTERA : PSYLLIDAE) AND OTHER INSECTS ASSOCIATED WITH CITRUS PLANT

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### ABSTRACT

The research was conducted at Laboratory of Biotechnology Faculty of Agriculture Udayana University. The purpose of this research were to investigate molecular detection of CVPD pathogen in its vector, other phytophagous insects on citrus cv, Siam and kemuning (*Murraya paniculata* L. Jack.). The result of this research showed that adults and nymphs of *Diaphorina citri* Kuw. could transmit CVPD disease, but the pathogen of CVPD could not be transovarially transmitted. As a host of CVPD disease, kemuning could be source of infection. The adult of *Toxoptera citricidus* Kirkaldy and *Maleuterpes dentipes* Hell. could not potential transmitted CVPD disease.

*Keywords:* PCR, CVPD, *D. citri*, *T. citricidus* and *M. Dentipes*

### INTRODUCTION

Citrus are priority horticulture commodity for cash crop development due to their good taste and nutrition contents. Annual citrus productivity in Indonesia at the moment is still relatively low. i.e. 8.6 – 15 tons per ha (Anonimus, 2012). This has been caused by CVPD by CVPD attack (Wirawan dkk., 2003). CVPD is the most important disease and a major cause of yield loss citrus plantations in almost all countries, especially Asia and Africa. In 1965 in Africa reduced citrus crops from CVPD diseases between 30 % - 100%. In Thailand damage of plants more than 95%, while in Indonesia approximately three million damaged crops

between the years 1960 – 1970 ( Julyasih, 2009). CVPD is a disease transmitted through a certain kind of insect known as *D. citri* (Tirtawidjaja an Suharsono, 1990). Recent studies indicated that CVPD pathogens are bacteria called *Liberobacter* that can be detected using 16S rDNA (Bove *et al.*, 1996). The transmission of CVPD in Bali has been rapid while the population of *D. citri* is very small. Other factor are considered contributive to the spread of CVPD ( Wijaya, 2003). The purposes of this research were to undertake molecular detection regarding *Liberobacter asiaticus* among *D. citri* insects as well other insects associated with citrus plant.

## MATERIALS AND METHODS

The research was conducted in Biotechnology Laboratory Faculty of Agriculture, Udayana University. The research was initiated by collecting insects associated with citrus plant found in the field. Molecular analysis was then conducted on the insect collection which consisted of such species *D. citri*, *Toxoptera citricidus* Kirkaldy and *Maleuterpes dentipes* Hell. Each of these insects was put into different ependorf tubes. CTAB extract was then added and placed in a temperature room. The insect were incubated for 20 minutes at a temperature of 65°C. Then, it was centrifuge at 6800 rpm for five minutes. The resulted in two layer, i.e. the lower part consisting of organic substance and upper layer consisting of solution. This solution as then taken out for 90 ml and was transformed to the new ependorf tube. A total of 10 ml NaOAc and 200 ul ethanol with absolute freezing temperature was added. Then it was kept in freezer for 30 minutes. The second centrifuged was undertaken at 11500 rpm for 15 minutes. Then result was pellet and supernathan. The pellet was cleaned using 200 ul ethanol 70% centrifuged at 11500 rpm for two minutes and the supernathan formed was discarded. The pellet was later dried for 15 minutes and 20 ul TE buffer (sterile distilled water). The isolated DNA was then suspended in buffer

TE solution which was then analysed using PCR. Finally, electroforesis were undertaken using agarose gel 1%.

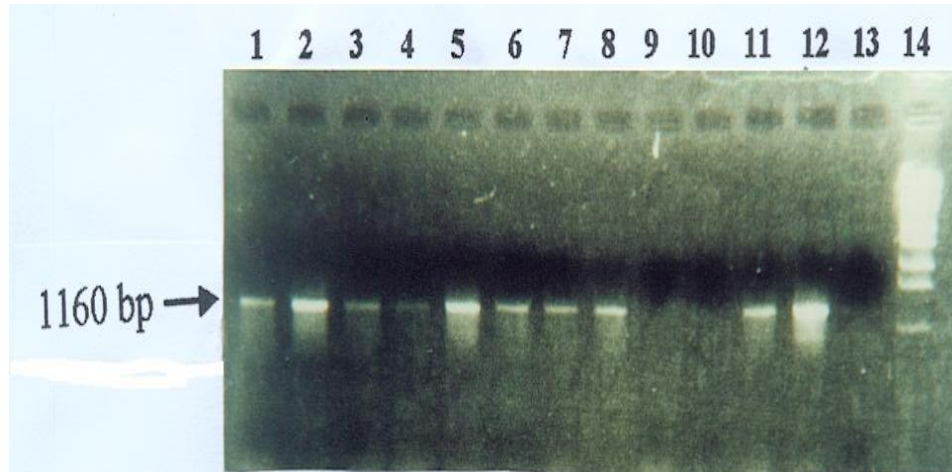
## RESULTS AND DISCUSSION

### The Existance of CVPD Pathogen in *Diaphorina citri*

The result of CVPD molecular pathogen detection on *D. citri* indicated that adult and nympha *D. citri* positive contained *Liberobacter* (Figur 1.). This means that adult and nympha *D. citri* could be a vector of CVPD. *D. citri* insect had a pointed sucking stylet. When the insect tried suck the sap from a plant, some bacteria entered into the insect body undergoing process due to chemical substance in found in the insect body. When *D. citri* which contained this *Liberobacter* sucked the sap of a healthy citrus plant the bacteria were expelled at though its saliva. The spread of the disease occurs primarily by insect vector *D. citri* Kuwayama. Tipe spread of the disease can also be caused by the spread of citrus plant seeds that have been infected by pathogens CVPD disease (Capoor *et al.*, 1974 in Mead, 1998). Typical symptoms of disease are the leaves become yellow, bones of leaves dark green, the leaves become more rigid and thicker than the healthy leaves and small (Mead, 1998 ; Knapp *et al.*, 1999). While the fruits becomes small and hard (Wirawan *et al.*, 1998). CVPD affected plants leaves

undergo chlorosis, the symptoms resemble nitrogen deficiency, zinc, manganese and iron (Tirtawidjaja, 1983). CVPD disease is causing gram negative bacteria named *Liberobacter* (Sandrine *et al.*, 1996). Pathogens can not be cultured in vitro, but

can be detected by PCR on 16S rDNA and electron microscopy (Hoy, 1998). African citrus psyllid, *Trioza erytreae* (del Guercio) that vectors CVPD disease in Africa, *Liberobacter africanum* (Subandiyah *et al.*, 2000).



**Fig. 1. Amplification Pathogen CVPD in *D. citri*, *T. citricidus*, *M. dentipes*, Leaves of Citrus and Kemuning by PCR Method.** 1. Leaves of citrus in the field, 2. Leaves of citrus at laboratory, 3. Flash of citrus in the field, 4. Flash of citrus at laboratory, 5. Adult *D. citri* in the field, 6. Adult *D. citri* at laboratory, 7. Nymph *D. citri* in the field, 8. Nymph of *D. citri* at laboratory, 9. Adult of *T. citricidus*, 10. Adult of *M. dentipes* in the field, 11. Citrus seedling, 12. Leaves of *Murraya paniculata*, 13. Eggs of *D. citri*, 14. DNA marker

### The Existance of CVPD Pathogen in *T. citricidus* and *M. dentipes*

The result of CVPD molecular detection on imago *T. citricidus* and *M. dentipes* showed the two species of insects did not contain *Liberobacter* (Figur 1). Consequently the two species of insects were not able to function as a CVPD vector. *T. citricidus* was in insect sucking the young leaves of citrus plant and they cannot act as a vector. This could happen because when the insect penetrated the plant using their stylet, it did not reach the layer where *Liberobacter* lived. On the other hand *M. dentipes* is an

insect having their own mouth used to bite and chew and their saliva was not extracted on to the leaves when eating them.

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