Note

DISTRIBUTION OF CVPD^r GENE AMONG SOME CITRUS PLANTS IN BALI

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ABSTRACT

Citrus Vein Phloem Degeneration (CVPD) is the main disease of citrus plant caused by a Gram negative bacteria, Candidatus *Liberobacter asiaticus*. The disease is also called citrus greening disease or recently called citrus *huanglongbing*. The CVPD^r gene was firstly found in *Triphacia trifoliata* a citrus relative plant which considered to be resistant to CVPD disease. The distribution of the gene among the citrus relative plants in Bali were studied. The result of this study so far, CVPD^r gene were found in the seedless lime (*Citrus aurantiifolia*) and *Triphacia trifoliata* which are known as a resistant or tolerant to CVPD disease, however, *Citrus maxima*, *Citrus nobilis*, *Citrus reticulate*, and *Citrus amblycarpa* are all sensitive plants but their harboring the CVPD^r gene. This result indicated that CVPD^r gene was not give a resistant to the disease in these plants. This results suggested that other/s gene is needed to give a resistant or tolerant in these plants.

Citrus Vein Phloem Degeneration (CVPD) disease or huanglongbing is the main disease of citrus plantation in everywhere in the world. The causative agent of the disease is Candidatus *Liberobacter asiaticus* and it were spreaded by insect vector *Diaphorina citri* or through aculus bud (Tirtawijaya S., 1981, Sandrine et al, 1996, Wirawan, et al, 2004, Secor G.A., 2009). The whole genome of *L. asiaticus* strain A4 was sequenced by Zeng *et al*, and reported in 2014. The bacteria was unable to be cultured *in vitro* and its was present in the phloem of the citrus plants (Sandrine et al, 1996). Many study tried to find how to control the pathogen and to control the insect vector, including the use of gene resistant to the disease. Gene resistant to CVPD disease or huanglongbing has been reported by (Nariani T.K. 1981,). Our previous study on *Triphacia trifoliata* a citrus relative which considered to resistant to the disease, found harboring the *candidatus* gene that resistant to CVPD disease (Wirawan, *et al*, 2004, Wirawan I G.P. and Ketut Sri M. J., 2015). The gene is called CVPD^r gene was isolated by *in vitro* plant mutation. One mutant among almost 7000 *Triphacia trifoliata* mutants found to be sensitive to CVPD disease. Using this mutant the CVPD^r gene was isolated by using IPCR technique. This study tried to see the distribution of CVPD^r gene among citrus plants or their relative. The plants used in this study were *Triphasia trifoliate*, *Citrus aurantiifolia*, *Citrus maxima*, *Citrus nobilis*, *Citrus reticulate*, and *Citrus amblycarpa* . The DNA was isolated from the leaves of citrus plants studied, and the DNA isolation was using NucleoSpin®Plant II

(Macherey-Nagel). The total DNA from those plants were then subjected to a polymerase chain reaction (PCR) to amplify the fragment CVPD^r gene using a AmpliTaq Gold®360 Master Mix from Applied Biosystems. The results of this study show that CVPD^r gene were shown in almost all plant studied (Fig. 1). The result of this study showed that CVPD^r gene were found in all plant tested except in one plant, *Murraya sp.*(a negative control plant). The result indicated that the gene was not fully resistant to the CVPD disease in *Citrus maxima*, *Citrus nobilis*, *Citrus reticulate*, and *Citrus amblycarpa* . The plants that showing resistant to the disease were seedless lime (*Citrus aurantiifolia*) and *Triphacia trifoliata*. We do not know yet why, it could due to the polymorphism of the gene or to be resistant need other/s gene to involve in those plants. The comparison of the CVPD disease symptom among plants studied were also shown in Fig. 2. The rate of the symptom observed in this study were varied among the plants, it could be due to the polymorphism of the gene as well. So that, for the further study we will study these all possibilities.

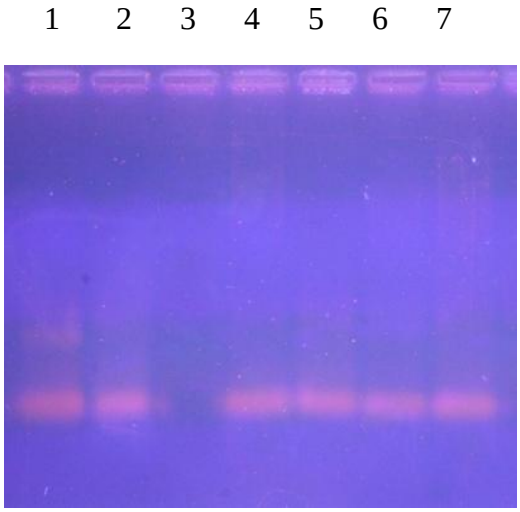

Fig. 1. Distribution of CVPD^r gene among various citrus plants. Lanes 1. *Triphacia trifoliate*, 2. Seedless lime (*Citrus aurantiifolia*), 3. *Murraya sp*.(as a negative control), 4. *Citrus maxima*, 5. *Citrus nobilis*, 6. *Citrus reticulate*, 7. *Citrus amblycarpa*

Fig. 2. Various CVPD disease sysmptom in the citrus plants. A. *Triphacia trifoliata*, B. Seedless lime (*Citrus aurantiifolia*), C. *Citrus maxima*, D. *Citrus reticulate*, E. *Citrus nobilis*, F. *Murraya sp*, G. *Citrus amblycarpa*

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