Development of Simple and Rapid Detection Methods for Accelerating the Eradication Measures of Citrus Greening Disease

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Citrus greening (huanglongbing; HLB) is a devastating disease of citrus trees with high economical costs to the worldwide citrus industry [1]. This disease is caused by phloem-limited fastidious bacterium, '*Candidatus* Liberibacter asiaticus' (Las) in Japan and is transmitted by grafting and by the sap-sucking psyllids *Diaphorina citri*. When infected trees develop the symptoms, the tree vigor declines rapidly, followed by subsequent death. All major commercial citrus cultivars are susceptible to the bacterium, and no effective control is known other than the removal of infected trees. Therefore, in areas in which greening has not become established, rapid identification and culling of infected trees and quarantine practice on budwoods are the most important control measures. Various DNA amplification methods, including polymerase chain reaction (PCR) have been used to test greening-infected plants. However, in these methods, total DNAs from suspected plants have to be extracted by using DNA extraction kits or reagents before DNA amplification. Therefore, when a large number of suspected trees is investigated, the time and cost for DNA extraction become a practical limitation. To cope with this problem, it is imperative to make the procedures for DNA extraction as simple as possible.

At first, we found a Las-specific sequence region and designed new primers with high sensitivity and accuracy. PCR using the new primer set resulted in more sensitive detection than any other known primer sets did, and high specificity for Las was also confirmed [2]. Next, to facilitate detection of the pathogenic bacterium by PCR using the new primer set, we tried to develop a direct-PCR method that does not require DNA extraction from infected trees. Since the pathogenic bacteria are localized in the phloem of infected trees, it was possible to gain extracts of the pathogenic bacteria by disruption of the midribs of the infected leaves and centrifugation these solutions. Thus, when we performed PCR with the extract solutions as templates, it was possible to detect pathogenic bacteria with sufficiently high sensitivity, in spite of extremely easy method [3]. Moreover, we also develop some new methods (ex. method of multiplication of pathogen *in planta*, method of cultivation selectively of pathogen, and method of finding disease-specific biomarker in infected plants). These research and development will contribute to accelerating the eradication measures of this disease.

References

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