Research article

Controlling Citrus Huanglongbing (HLB) for the Rehabilitation of Citrus Orchards in Cambodia

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Abstract Citrus Huanglongbing **(**HLB) has been seriously damaging Cambodian citrus industry in terms of major yield loss, poor fruit quality, and shortened average lifespan of citrus tree. HLB is mainly transmitted by vegetatively propagated citrus seedlings and spread by psyllid vector in the field. HLB pathogen is a non-cultivable bacterium "*Candidatus* Liberibacter asiaticus*"*. Major strains of HLB pathogen have been investigated and the most virulent strain Type-II was commonly observed in Pursat sweet orange, mandarin and pummelo trees in Cambodia. Currently, there are no promising technologies and cultural practices to control HLB in citrus orchards in Cambodia. New health management practices of citrus HLB have been initiated in 2006 under the expert guidance of the National Taiwan University (NTU) and the Food and Fertilizer Technology Center in the Asian and Pacific Region (FFTC/ASPAC). Pathogen-free (PF) seedlings propagated in screen houses of the Royal University of Agriculture were transplanted in the demonstration orchard of Battambang and Takeo Provinces. The supply of the PF-seedlings of major citrus species cannot currently catch up with the demand of citrus growers. According to a preliminary survey, marcotted or grafted seedlings of sweet orange propagated by farmers transplanted in major citrus production areas such as Battambang, Pursat and Siem Reap Provinces were seriously infected by HLB. Up to now, fundamental knowledge and techniques such as identification of major strains of the HLB pathogen and establishment of disease indexing laboratory and pathogen-free nursery system have been conducted. In addition, a variety of relevant techniques and cultural practices to control HLB were transferred to Cambodia, such as technologies for the production of PF-seedlings and transplanting to demonstration farms for their health management, and so forth.

Keywords huanglongbing (HLB), health management, citrus production, pathogen-free citrus seedling

INTRODUCTION

Citrus greening was first reported in 1947 from South Africa, although a similar disease known as "Huanlongbing" (HLB, yellow shoot) was already known in 1943 in Southern China (Cheng, 1943). The HLB disease, locally called "Likubin," was first identified in Taiwan in 1951, six years after the end of World War II. The HLB inoculums might have been intentionally brought into Taiwan from southern China through some infected propagation materials such as citrus scions or seedlings. HLB was first considered as a kind of physiological disorder caused by nutrient deficiency, poor drainage, and so forth. However, HLB could not be controlled by the amendment of soil properties, and has spread rapidly all over Taiwan. Matsumoto and his coworkers initiated etiological studies on HLB in 1956, and they successfully demonstrated in 1961 that the so-called HLB was not a physiological disorder of citrus tree, but was caused by a virus-like microbe transmitted through grafting of diseased scion, and named it Likubin (decline) (Matsumoto, Wang and Su, 1961). This

destructive disease spread all over Southeast Asia during the 1960s, and was locally called leaf mottle yellows, citrus vein phloem degeneration (CVPD), and citrus dieback in Philippines, Indonesia, and India, respectively. In a short period of time, HLB became one of the most devastating diseases for citrus growing areas in tropical and subtropical Asia. Miyakawa and Tsuno (1989) first found HLB in Iriomote, the southernmost island of Okinawa, Japan in 1988. Afterwards, HLB was reported in Brazil (Lopes, 2006) and Florida (Bove, 2006) in 2004 and 2005, respectively. Etiological and epidemiological studies on HLB have been conducted in Taiwan in order to develop effective and efficient management strategy of HLB since 1955. HLB commonly occurs as a mixed infection with citrus tristeza and/or tatter leaf viruses, causing severe yellow mottling and tree decline, and ultimately death of citrus trees. These diseases are generally controlled by integrated control measures. Establishment of a pathogen-free nursery system is of primary importance for reducing prevalence of these diseases in the early stage of tree development. Combination of shoot-tip micrografting (STG) technique and heat-treatment has been successful in establishing pathogen-free foundation stock of citrus cultivars. Along with the said techniques, a precise and rapid indexing technique is indispensable for health management of production and cultivation of pathogen-free citrus seedlings.

Up to present, pathogen-free citrus foundation, nursery system, and disease indexing laboratory were established in RUA through the international collaboration project among RUA, NTU and FFTC/RDF. The on-going project aims to conduct etiological and epidemiological studies of HLB, and technology transfer of health management of PF-seedlings in the demonstration farms established in major citrus production areas in Cambodia.

CITRUS PRODUCTION IN CAMBODIA

Pursat sweet orange seemed to be introduced to Cambodia in Angkor period from China. Sweet orange has been grown only in the surrounding area of houses. After the French occupation in 1954, sweet orange cultivation has been spread nationwide, thanks to technical training on modern citrus cultivation for farmers.

In Cambodia, fruit crop production is the 2nd major crop next to rice production. Within fruit crops, citrus is the second important fruit crop next to mango according to the statistic acreage of fruit crops reported by MAFF, i. e. Areas (ha) of fruit crop, 2009: Mango, 23,734; Orange, 3,553; Custard apple, 3,213; Longan, 2,376; Sapodilla, 2,052; Guava, 1,745 and Milk fruit, 1,216 has. Citrus cultivation provides year-round harvest with suitable income for increasing rural economy from 1954 to 1967 before the civil war. The total growing areas of citrus was 3976 ha (Battambang, 2391 ha; Pursat, 345 ha; Kandal, 246 ha; Kampong Cham, 331ha; and other provinces, 663 ha). During 1968-1969, around 18000 tons of citrus fruits were exported abroad. In 1987 Cambodia was able to produce 40000 tons of citrus fruits.

In recent years, areas of citrus production decreased significantly due to HLB occurrence, i.e. 2556 ha in 2007 (Table 1) decreasing from 3976 ha in 1967. Battambang province holds the highest growing area at 1865 ha, decreasing from 2391 ha in 1967, while Kampong Cham still holds 311 ha without decrease, coming to the second. Citrus areas in Pursat province decreased drastically from 345 ha in 1967 down to 59 ha in 2007 due to HLB epidemic.

Table 1 Production areas of citrus in Cambodia (2007)

Province	Area (ha)
Siem Reap	145
Battambang	1865
Pursat	59
Kampong Thom	74
Kampong Cham	311
Kampot	102
Total	2556

Source: Provincial Agriculture Department of Battambang.

The major citrus species cultivated in Cambodia are sweet orange, mandarin, pummelo, lime and Kaffir lime (Martin, 1971). Pursat sweet orange has been most commonly cultivated. The HLB disease has commonly affected all cultivars.

HLB SITUATION

Citrus trees grown in Cambodia have been affected by many different diseases which are causing considerable damage to citrus production. The most serious threat has been caused by Huanglongbing (HLB) which is known as " Slek Prak " in Khmer language. At present there is not enough technical disease control or prevention capacity for this kind of disease. The HLB disease has been commonly occurring in citrus-growing areas in Cambodia since 1980s. The common symptoms caused by HLB pathogen include yellowing of the veins and adjacent tissue, followed by yellowing with pale-green mottling of entire leaf. With ageing, the diseased leaves become hard, curling outwards and occasionally develop vein corking. The typical symptoms produced on different citrus cultivars e. g., Pursat sweet orange, mandarin and pummelo, and common root-stock cultivar, Rangpur lime, are shown in Fig. 1. However, the symptoms vary with citrus variety, strains of the pathogen, and environmental conditions.

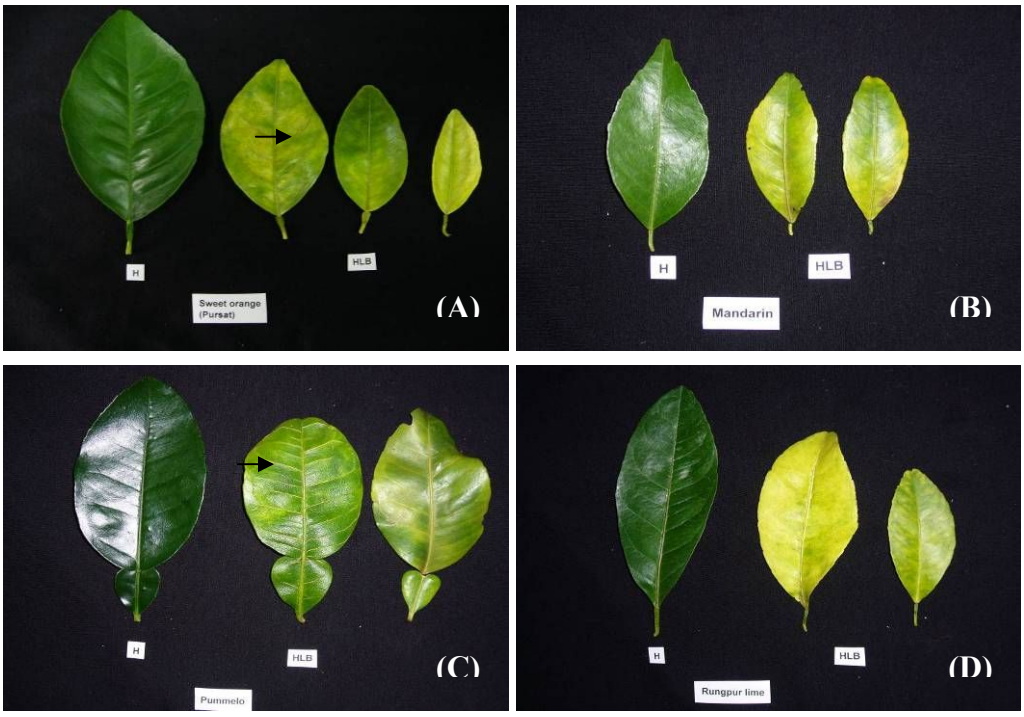

Fig. 1 Symptom expression of HLB in common citrus cultivars, and root-stock in Cambodia

(A) Leaf symptoms of HLB-affected Pursat sweet orange, showing yellowing with pale green mottling of mature leaves (second from left) with vein corking (→) and a newly grown small and slender leaf with yellow chlorosis (rightmost), and one healthy leaf (leftmost); (B) Leaf symptoms of diseased mandarin, showing yellow mottling of mature leaves (right and center) with curling; (C) Leaf symptoms of diseased pummelo, showing yellow mottling on mature leaves (right and center) with curling and severe vein corking (→) on the center leaf; (D) Leaf symptoms of diseased Rangpur leaf (right and center) showing yellow mottling, and a healthy leaf on the left.

The pathogen of the HLB disease in the above-mentioned major citrus cultivars and Rangpur root-stock were detected by polymerase chain reaction **(**PCR) test followed by electrophoresis analysis with primer pairs specific to HLB pathogen (F: CAC CGA AGA TAT GGA CAA CA; R: GAG GTT CTT GTG GTT TTT CTG). The protocol of HLB detection was described in detail in the former publications (Hung *et al.*, 1999; Su, 2008). HLB-infected leaves with typical yellow mottling symptoms, and those without any symptoms were collected from Pursat sweet orange, mandarin, pummelo and Rangpur lime trees for the infected and the control, respectively (Fig. 2A).