Differential Reaction of Citrus Species in Malaysia to Huanglongbing (HLB) Disease using Grafting Method

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Summary: Huanglongbing (HLB) is a phloem limited disease in citrus caused by a fastidious bacterium called Candidatus Liberibacter found in Africa, Asia and United States of America (USA). HLB can severely reduce vigor and yield or kill all citrus trees within 5 years. There is a need to screen and identify suitable rootstock for propagation of clean plan materials for citrus in the tropics. This study was conducted to detect the presence of HLB on 18 selected citrus species and to categorize the level of infection and susceptibility of citrus species to HLB.

Approach: Eighteen citrus species were assessed for susceptibility to HLB by graft transmission from source infection (Citrus reticulata).

Results: HLB was detected in 15 species 6 months after grafting using PCR test.

Conclusion: The species could be categorized in five groups: Severe group (72-58% severity) which includes Citrus reticulata, C. sinensis, C. reshni cv. cleopatra, moderate group (50-41% severity) includes Fortunella sp. cv. Kasturi Chinai, C. macrophylla, C. microcarpa, mild group (25-17% severity) which included C. medica, C. aurantifolia, Citrus sp. (natural biotype), C. jambhiri. The tolerant group which did not show any HLB symptoms but tested positive by PCR test includes C. aurantium and C. aurantifolia. The resistant groups which include C. grandis cv. Limau Bali, C. hysterix and Citrus sp. cv. Limau Tembikai showed no symptoms and were tested negative for HLB.

Key words: Huanglongbing, citrus greening disease, disease severity, citrus rootstock

INTRODUCTION

Citrus is believed to have originated from the region within Northeast India, South China, Indonesia and Peninsular Malaysia. It is an extremely important crop on a world basis and the total world production of citrus was estimated at over 73 million metric tons[1]. In Malaysia, citrus is grown in commercial orchards, backyard orchards and small holdings in various parts of the country. For conservation purposes citrus collections was established, which have notable genetic diversity, particularly of the pummelo and some of the related genera and appear to be fairly well maintained. Some are also observed in areas such as the Taman Negara National Park in Pahang and the Danum Valley in Sabah[2].

Huanglongbing (HLB), commonly known as citrus greening, is one of the most serious diseases that affect citrus fruit. HLB has destroyed an estimated 60 million trees in Africa and Asia[3-5] and occurs in more than 40 countries including Malaysia[6]. In Malaysia HLB was first detected in Cameron Highland in 1990. Survey done by Azizah and Zazali[6] revealed that approximately 70% of the cultivated area with citrus in Malaysia were affected by HLB disease[6]. In separate studies, it was shown that HLB was successfully experimentally transmitted from the infected citrus to periwinkle (Catharanthus roseus) and a non-rutaceous host by means of dodder (Cuscuta campestris)[7]. HLB is caused by an uncultured phloem limited bacterium that was first characterized in 1994 with the 16S rDNA sequence and classified to be a new genus in the α-Proteobacteria subdivision[8-9].

Polymerase Chain Reaction (PCR) for HLB detection was developed in 1996 based on the amplification of 16SrDNA fragments[9]. The pathogen
of HLB has many isolates in various hosts so far. It cannot be diagnosed easily by conventional procedures such as electron microscopic examination of ultra-thin sections, bioassay on indicator plants and Enzyme-Linked Immunosorbent Assay (ELISA) with polyclonal or monoclonal antibodies. However molecular tools such as PCR are a very effective, simple and sensitive tool for HLB detection. Therefore this study was conducted to detect the presence of HLB on 18 selected citrus species for rootstock through grafting and to categorize the level of infection and susceptibility of 18 citrus species to HLB in Malaysia.

**MATERIALS AND METHODS**

**Planting materials:** Seeds were obtained from the Malaysian Agricultural Research and Development Institute (MARDI) and the Department of Agriculture in Terengganu, Malaysia. Seeds of 18 citrus rootstocks were sown in seed trays using a soil mixture of soil, peat and sand (2:3:1). The seedlings were transplanted 15-20 days after germination into a 16 cm diameter pot. The seedlings were ready for grafting when they were 40-50 cm tall. All experimental plants were grown in an insect proof screen house.

**Source inoculums and graft transmission success:** Source of HLB inoculums was collected from **Citrus reticulate** cv. Limau Madu which was obtained from University Putra Malaysia (UPM) and confirmed by PCR test. Infected scions were grafted on 18 citrus species (Table 1). Side grafting method was chosen to ensure high rate of HLB transmission via vector transmission using *Diaphorina citri* which has vector preference. Terminal shoots of 10 cm long were randomly collected from trees with typical symptoms. Twigs were then kept in the transparent plastic bags and placed in a cool box to maintain their freshness.

**Vegetative growth assessment of inoculated citrus seedlings:** The seedlings were washed thoroughly rinsed in tap water, followed by distilled water and then dried at 65°C for 4-5 days to measure the total dry weight in inoculated and non inoculated plants. Plant height and stem diameter of non inoculated and inoculated citrus species were measured. Data were collected, analyzed and means were separated using Duncan’s Multiple Range Test (DMRT).

**Disease severity:** Disease severity was determined according to alternative rating scale proposed by Bowen and Kranz. Base on the leaf symptoms, the scale includes: 0 = no symptom, 1 = Mild (blotchy mottling symptoms observed from 1-30% on seedlings canopy), 2 = Moderate (yellowing symptoms observed from 31-50% on seedlings canopy), 3 = Severe (blotchy mottling, midrib yellowing and twig dieback symptoms observed of more than 50% on seedling canopy). The disease severity was measured using the formula below:

\[
\text{Disease severity} = \frac{\sum (a \times b)}{N \times Z} \times 100\%
\]

Where:
- \(\sum (a \times b)\) = Sum of the symptomatic plant and their corresponding score scale
- \(N\) = Total number of sampled plant
- \(Z\) = Highest score scale

**DNA extraction of citrus tissues:** Leaf samples were collected for evaluation from seedlings which were inoculated by grafting method. DNA extraction from citrus tissues was prepared following the method described by Hung et al. DNA was extracted from HLB-infected tissue using Cetyl Trimethyl Ammonium Bromide (CTAB). The pellets were washed with 70% ethanol, dried and resuspended in 100 µL TE buffer.

**Polymerase chain reaction (PCR) conditions, primers and gel electrophoresis:** PCR was performed using 25 µL of reaction mixture containing 20 mM Tris-HCl (pH 8.0), 50 mM KCl, 4 mM MgCl₂, 0.2 mM of dATP, dTTP, dCTP and dGTP, 50 ng forward primer, 50 ng reverse primer, 0.75 units of Taq DNA polymerase and 200 ng genomic DNA. The thermal cycle condition was: 1 cycle at 95°C for 2 min 35 cycles at 95°C for 40 sec, 60°C for 1 min and 72°C 1 min then followed

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**Table 1: Percentage of grafting success of 18 selected citrus species**

| Sr. No | Scientific name | Local name | Grafting success (%) |
|--------|-----------------|------------|----------------------|
| 1      | *C. jambiri*    | Rough lemon | 80                   |
| 2      | *C. grandis*    | Limau Bali | 78                   |
| 3      | *C. aurantium*  | Limau Samur | 75                   |
| 4      | *C. reticulate* | Limau madu | 75                   |
| 5      | *C. medica*     | Limau susu | 70                   |
| 6      | *C. microcarpa* | Limau kasturi | 69                   |
| 7      | *C. sinensis*   | Limau lankat | 68                   |
| 8      | *C. aurantifolia* | Limau nipsi | 65                   |
| 9      | Citrus sp.      | Limau naga | 65                   |
| 10     | *C. reshni*     | Cleopatra mandarin | 65                   |
| 11     | *C. aurantifolia* | Limau (Mexican lime) | 62                   |
| 12     | Citrus sp. *natural biotype* | 60                   |
| 13     | *C. hystrix*    | Limau purut | 60                   |
| 14     | Fortunella sp.  | Limau kasturi chini | 58                   |
| 15     | Citrus sp. *natural biotype* | 55                   |
| 16     | Citrus sp. *natural biotype* | 55                   |
| 17     | *C. macrophylla* | Machrophylla | 50                   |
| 18     | Citrus sp.      | Limau tembikai | 35                   |

*: Unknown citrus species in Malaysia
by a 72°C extension for 10 min. Significant primers pair, composed of the forward primer of O11 (5'-GCCTG GTG ATG CAA TAC GAG CGG CA-3') and reverse primer of O12c (5'-GCT CGC GTA CTT CGC AAC CCA T-3'), was used to amplify the 16S ribosomal DNA fragment. Amplification of DNA were determined by electrophoresis on 1.2% agarose gel for about 30-45 min and visualized by ethidium bromide staining[13].

RESULTS

Graft transmission success: All 18 citrus species were grafted using infected scions of Citrus reticulata. The performance of citrus plants varied in terms of grafting success. Result in Table 1, shown the highest grafting success was observed on Citrus sp. cv. Mexican Lime and Citrus sp. cv. Limau Tembikai (from 70-80%). The scion used for grafting on these rootstocks grew and was normal growing development. Low grafting success was observed on three citrus which are the natural biotypes (Citrus sp.), C. macrophylla and Citrus sp. cv. Limau tembicai (from 35-85%).

Effects of HLB on vegetative growth: Mean comparison of total dry weight, plant height and stem diameter of none inoculated, inoculated and reduction rate of citrus species were measured six months after inoculation. There were significant difference at probability statistic level of p=0.05. Total dry weight, plant height and stem diameter of inoculated citrus species were significantly higher on C. hystrix, C. grandis, C. aurantium and C. aurantifolia than on C. reticulata cv. Limau Madu, C. sinensis cv. Limau Lankat, C. microcarpa cv. Limau Kasturi and Citrus sp. (natural biotype) (Table 2). Mean comparisons revealed the highest total dry weight, plant height and stem diameter in non-infected than infected citrus species.

In inoculated citrus species, mean comparisons of total dry weight, plant height and stem diameter were significantly different at probability statistic level of p<0.05. Total dry weight, plant height and stem diameter of seedlings were significantly different on C. jambhiri, C. aurantium, C. macrophylla and citrus sp. cv. Coleopatra than on Citrus sp.cv. Limau Tembikai, Citrus sp. (natural biotype) and C. aurantifolia cv. Mexican Lime. Stem diameter of citrus species was also significantly different at probability statistic level of 5%. The highest stem diameters were observed on C. jambhiri, C. aurantium and C. aurantifolia than other species such as Citrus sp. (natural biotype) and Citrus sp. cv. Limau Tembikai (Table 2).

Table 2: Comparison between mean of total dry weight, plant height, stem diameters and reduction rate on inoculated and non inoculated of citrus species six months after inoculation

| Citrus species | Inoculated | Non inoculated | Reduction rate (%) |
|----------------|------------|---------------|--------------------|
| Scientific name | Local name | Total dry weight (g) | Plant Height (cm) | Stem diameter (mm) | Total dry weight (g) | Plant Height (cm) | Stem diameter (mm) | Total dry weight (g) | Plant Height (cm) | Stem diameter (mm) |
| C. grandis | Limau bali | 71.40a | 72.50a | 19.3 | 71.00 | 72.50 | 19.3 | 6.05 | 3.00 | 5.00 |
| C. hystrix | Limau purut | 67.50ab | 68.20bc | 17.6 | 67.10 | 68.20 | 17.6 | 4.17 | 2.00 | 7.36 |
| C. medica | Limau suru | 65.42ab | 64.90bc | 16.7 | 65.00 | 64.90 | 16.7 | 2.17 | 1.00 | 1.17 |
| C. aurantium | Limau samur | 68.40ab | 69.40ab | 19.0 | 73.70 | 75.80 | 23.5 | 4.05 | 2.00 | 7.05 |
| C. aurantifolia | Limau nipsis | 65.20ab | 69.00ab | 14.6 | 78.10 | 77.00 | 17.5 | 0.00 | 0.00 | 1.00 |
| C. macrophylla | Macrophila | 62.00bc | 64.20bcdef | 16.0 | 79.12 | 78.00 | 20.0 | 3.00 | 1.00 | 2.00 |
| C. aurantifolia | Mexican lime | 56.80de | 59.09 | 18.6 | 69.20 | 66.50 | 21.0 | 2.17 | 1.00 | 1.17 |
| Citrus sp. | Limau tembikai | 59.06de | 61.20defg | 15.3 | 61.00 | 66.00 | 16.5 | 3.00 | 1.00 | 2.00 |
| C. reticulata | Coleopatra mandarin | 57.80 | 60.00 | 14.0 | 78.00 | 77.00 | 18.0 | 3.00 | 1.00 | 2.00 |
| C. jambhiri | Rough lemon | 66.70ab | 68.00bc | 19.3 | 81.70 | 80.00 | 24.0 | 4.00 | 2.00 | 2.00 |
| F. reticulata | Kasturi chin | 55.10de | 59.50 | 15.5 | 75.00 | 74.00 | 19.5 | 3.00 | 1.00 | 2.00 |
| Citrus sp. | Limau naga | 56.40de | 61.00de | 17.2 | 69.10 | 69.00 | 19.5 | 4.00 | 2.00 | 2.00 |
| Citrus sp. | Limau 3 | 65.60de | 60.60 | 16.6 | 66.50 | 67.00 | 19.5 | 4.00 | 2.00 | 2.00 |
| Citrus sp. | lima1 | 58.90de | 61.40defg | 14.0 | 66.50 | 66.00 | 16.0 | 4.00 | 2.00 | 2.00 |
| Citrus sp. | lima2 | 58.20de | 60.80 | 15.3 | 68.50 | 67.00 | 17.5 | 5.00 | 3.00 | 5.00 |
| C. microcarpa | Limau kasturi | 54.60de | 59.60 | 15.2 | 75.70 | 73.50 | 19.0 | 5.00 | 3.00 | 5.00 |
| C. sinensis | Limau lankat | 48.32 | 56.00 | 13.2 | 76.50 | 76.00 | 19.0 | 4.00 | 2.00 | 2.00 |
| C. reticulata | Citrus madu | 43.70 | 50.40 | 12.5 | 73.50 | 70.0e | 18.0 | 5.00 | 3.00 | 5.00 |

Means within column followed by the same letters are not significant at p = 0.05
Fig. 1: Symptoms of HLB observed on different citrus species six months after graft inoculation; (a): *C. reticulate*; (b): *C. reshni* cv. Cleopatra; (c): *C. sinensis*, (d): *C. macrophylla*; (e): *C. aurantifolia* cv. Limau Nipis; (f): *C. microcarpa* cv. Limau Kasturi; (g): *Fortunella* sp.; (h): *Citrus* sp.; (i): *Citrus* sp.; (j): *Citrus* sp.; (k): *C. jambhiri*; (l): *C. medica*; (m): *Citrus* sp.; (n): *C. aurantifolia*; (o): *C. aurantium*; (p): *C. grandis*; (r): *C. hysterix*; (s): *Citrus* sp. cv. Limau tembikai

**Symptom expression of HLB:** Symptoms expression is shown in Fig. 1. It was observed that *C. grandis* cv. Limau Bali, *C. hysterix* cv. Limau purut and *Citrus* sp. cv. Limau tembikai showed no symptom of HLB after 6 months of inoculation and leaves remained green. Also no symptom of HLB occurred on *C. aurantifolia* cv. Mexican Lime and *C. aurantium* six months after graft inoculation. *C. sinensis* cv. Limau Lankat, *C. reticulata* cv. Limau Madu and *C. reshni* cv. Cleopatra showed severe symptoms of HLB at the sixth month after inoculation. *C. reticulata* showed midrib yellowing, yellowing and mild twig dieback six months after graft inoculation. *C. sinensis* and *C. reshni* cv. Cleopatra showed blotchy mottling and yellowing on leaf. On *C. macrophylla* fertilizer deficiency symptom was observed, but *C. aurantifolia* cv. Limau nipis showed mild blotchy mottling and midrib yellowing. *C. microcarpa* cv. Limau kasturi and *Fortunella* sp. were able to show blotchy mottling and mild yellowing on main veins. Mild midrib yellowing were observed on tow *Citrus* sp. (citrus natural biotype), *C. jambhiri* and *C. medica*, but on other citrus natural biotype and *Citrus* sp. cv. Limau Naga, mild blotchy mottling were observed.

**Disease severity of HLB on citrus species:** Disease severity was evaluated on individual inoculated seedlings. There were significant differences of disease severity observed among the 18 citrus species. *C. reticulata*, *C. sinensis* and *C. reshni* cv. Cleopatra showed high level of severity with value of 94.45-50% respectively. The symptom of HLB started to show 8 weeks after inoculation. This species showed severe symptom of HLB after 24 weeks (6 months) of inoculation (Fig. 2). *Fortunella* sp., *C. mycrophylla* and *C. microcarpa* showed moderate symptom of HLB with the value of 41-50% respectively.

This symptom started on week 10 after inoculation and showed severe symptom six months after inoculation (Fig. 3). *C. medica*, *C. aurantifolia*, *Citrus* sp. *C. jambhiri* and three citrus biotype (*Citrus* sp.) showed mild symptom of HLB with the value of 17-25% starting 14 weeks after inoculation and showed the highest symptom also 6 months after inoculation (Fig. 4). *C. aurantifolia*, *C. aurantium*, *C. grandis* and *C. hysterix* did not show symptom of the HLB 6 months after inoculation.
Fig. 4: Progress of HLB disease severity on mild group of citrus species

Fig. 5: 116s rDNA fragments with molecular weight of 1160 bp were successfully amplified from infected; (a): M. Marker (1): Water; (2): Citrus sp. cv. Limau Tembikai; (3): C. grandis cv. Limau Bali; (4): C. medica; (5): Citrus sp. cv. Limau Naga; (6): Citrus sp. (natural biotype); (7): Citrus sp. (natural biotype); (b): M. Marker (1): Water; (2): C. reticulate; (3): C. sinensis; (4): C. microcarpa cv. Limau Kasturi; (5): C. aurantifolia cv. Limau Nipis; (6): C. reshni cv. Cleopatra; (7): C. macrophylla; (c): M. Marker (1): Water; (2): C. hysterix cv. Limau Purut; (3): C. aurantifolia cv. Mexican Lime; (4): C. aurantium; (5): Citrus sp. (natural biotype); (6): C. jambhiri; (7): Fortunella sp.

Table 3: Percentage of positive PCR test of citrus species

| Citrus species | Local name         | Positive PCR test (%) |
|----------------|--------------------|-----------------------|
| C. reticulata  | Limau Madu         | 100.00                |
| C. sinensis    | Limau Lankat       | 83.30                 |
| C. reshni      | Cleopatra          | 66.67                 |
| C. microcarpa  | Limau Kasturi      | 66.67                 |
| Citrus sp.     | Limau Naga         | 66.67                 |
| C. macrophylla | Machrophylla       | 66.67                 |
| Fortunella sp. | Limau kasturi chini| 66.67                 |
| C. aurantifolia| Limau Nipis        | 50.00                 |
| C. medica      | Limau susu         | 50.00                 |
| C. jambhiri    | Rough lemon        | 50.00                 |
| Citrus sp.     | natural biotype    | 33.34                 |
| Citrus sp.     | natural biotype    | 33.34                 |
| Citrus sp.     | natural biotype    | 33.34                 |
| C. aurantium   | Limau Samur        | 16.00                 |
| C. aurantifolia| Mexican lime       | 16.00                 |

Detection of HLB pathogen on the species using PCR test: The amplified PCR product is 1160bp which is the targeted 16S rDNA gene sequence region of the HLB pathogen amplified by the OI1 and OI2c primer sets. According to the result HLB was detected on fifteen citrus species (Table 3 and Fig. 5). According to the PCR test HLB was not present on 3 of the species including C. grandis, C. hysterix and Citrus sp.cv. Limau Tembikai (Fig. 5) and also the seedling did not show symptoms of HLB 6 months after graft inoculation. Results of PCR test show HLB was present on C. reticulata and C. sinensis (Fig. 5) and showed high infection with the value of 83.3-100% respectively among the citrus species. Results in Table 2 also showed that HLB was present on C. microcarpa cv. Limau Kasturi, C. reshni cv. Cleopatra, Citrus sp. cv. Limau Naga, C. macrophylla and Fortunella sp. (Fig. 5) and infection rate were 66.67%.

HLB was also detected on C.aurantifolia cv. Limau Nipis, C. medica and C. jambhiri. The results of PCR test (Table 3) has shown 50% rate of infection. HLB was present on 3 Citrus sp (natural biotype) with 33.3% rate of infection. C. aurantifolia and C.aurantium showed lowest infection (16% positive PCR test of each species), but on symptom expression these species did not show any symptom of HLB. However HLB was present on 15 citrus species which were tested in the experiment and HLB was not present on 3 species including C. grandis, C. hysterix and Citrus sp. cv. Limau Tembikai. PCR is certainly a very effective, simple and sensitive tool for HLB detection. However, Candidatus Liberibacter is very low in concentration and unevenly distributed in its natural hosts.

**DISCUSSION**

Transmission of citrus greening occurs primarily via infective citrus psyllids, grafting and it is transmissible experimentally through dodder. However psyllid species feed and survive on citrus and citrus relatives. The side grafting method was chosen to transmit the HLB on species on high rate of inoculation. This study demonstrated high infection of HLB using grafting method. The scion used for grafting on these rootstocks grew and was normal growing development. C. reticulata and Limau Tembikai were less compatible with infected scion and the growth was disrupted. In this case scion survived and grew 3-5 cm after 6 months and the success rate of grafting was 35%. When the
scion and rootstock are from the same species grafting was be high successful. A successful graft union depends on good contact between the cambium of the rootstock with the cambium of the scion. The bacteria can be transmitted in orchards or nurseries by grafting and experimentally by several species of dodder (Cuscuta spp.)19. Reduction rate of total dry weight, plant height and stem diameters on seedlings which showed severe symptoms of HLB were higher than the species which showed less HLB symptom, tolerant or resistance species too. Because the seedlings infected with HLB are usually stunted and it will be unproductive 4-5 years after planting. The infected seedlings to HLB was showing low rate of growing. Ahmad evaluated the effects of HLB infection on growth performance on C. reticulata based on the percentage reduction rate of total biomass and plant height. It was observed that high reduction rate of total biomass and plant height with the value of 56.2% and 39.4% respectively on infected honey mandarin2. Citrus species was showing different symptom of the HLB. Seedlings which become infected by HLB usually developed one or more yellow shoots. Leaves become thicker, with enlarged and corky veins and green areas are lacking. In later stages, zinc-like deficiency symptoms developed, followed by leaf drop and twig dieback16. Ahmad was not able to detect HLB on C. grandis in green house after six months of infection3. These results also agree with the results which were reported by Manicom and Vuuren16, where they reported HLB symptoms on C. reticulata and C. sinensis (sweet orange) are more severe but lemon and grapefruits are tolerant. They also reported that C. aurantifolia and C. grandis are more tolerant to HLB. Some species and cultivars of citrus are somewhat tolerant to HLB. Most of the sweet orange trees become infected with the HLB pathogen and subsequently declined, while grapefruit was more tolerant15. In general, sweet oranges, mandarins and tangelos are most susceptible, grapefruit and lemon are more resistant and limes, Poncirus trifoliata and citranges are the most tolerant17. Ahmad in Malaysia also reported that no HLB symptoms were observed on pummelo but C. reticulata cv. Honey Mandarin, C. madurensis cv. Calamondin and C. aurantium show severity with value of 75%, 65% and 50% respectively, but he transferred HLB pathogen to that species using Diaphorina citri vector3. Infected orange (C. sinensis), mandarin (C. reticulata) and tangelo (C. reticulata x C. paradise) produce the most severe symptoms and infected trees die within 3-5 years18. PCR test on this study also demonstrated that HLB was absent on C. grandis, C. hysterix, Citrus sp. cv. limau Tembiki. These species were shown a normal growing during six months after inoculation. PCR is certainly a very effective, simple and sensitive tool for HLB detection. However, Candidatus Liberibacter is very low in concentration and unevenly distributed in its natural hosts. The PCR based assay detected almost all Asian HLB strain collected from different countries in Asia such as Malaysia13. Based on this study, it can be concluded that HLB can be identified on fifteen citrus species six months after graft infection. The species that were infected include: C. reticulata, C. sinensis, C. reshni cv. Cleopatra, C. microcarpa, C. medica, Citrus sp. (Natural Biotype), Citrus sp. (natural biotype), Citrus sp. cv. Limau Naga, Citrus sp. (natural biotype), C. jambhiri, Fortunella sp., C. aurantifolia and C. aurantium. But symptom expression and severity of HLB were different between species. However, C. grandis cv. Limau Bali, C. hysterix cv. Limau Purut Citrus sp. cv. Limau Tembiki showed negative reaction of HLB by PCR test and on these species no symptom showed six months after graft inoculation. According this study C. aurantifolia and C. aurantium was without symptom and showed 16% positive of HLB. However according to this study citrus species could be categorized as severe, moderate, mild, tolerant and resistant. Severe citrus species on HLB include C. reticulata, C. sinensis, C. reshni cv. Cleopatra. Moderate species include Fortunella sp., C. macrophylla and C. microcarpa cv. Kasturi. Mild species include C. medica, C. aurantifolia cv. Limau Nipis, Citrus sp. (natural biotype) and C. jambhiri. Tolerant species include C. aurantium and C. aurantifolia. Finally, resistant species include C. grandis, C. hysterix and Citrus sp. cv. Limau Tembikai. Ahmad used graft and insect vector to transmit HLB to evaluate citrus species against HLB. He reported that jasmine orange and pummelo were resistant to infection and no HLB symptom was observed even 6 months after inoculation. HLB isolate was also not detected in leaf tissue by PCR test2.
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