Potential use of selected citrus rootstocks and interstocks against HLB disease in Malaysia

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ABSTRACT

Huanglongbing (HLB), also known as citrus greening disease, has spread to and is seriously threatening the citrus industry in more than 40 countries. The HLB pathogen is a phloem limited bacteria, Candidatus Liberibacter spp. There is little information pertaining to the effects of different citrus rootstocks and interstocks against HLB in Citrus reticulata. The objective of this study was to evaluate the beneficial effects of different combinations of citrus rootstocks and interstocks against HLB using side grafting. There were no symptoms of HLB when C. grandis was used as rootstock with C. hystrix as the interstock and vice versa six months after inoculation. However, C. Liberibacter asiaticus was detected in the scion using second PCR amplification. A high rate of disease severity was observed when C. aurantium was used as rootstock and C. aurantifolia as the interstock and vice versa. This study showed that C. Liberibacter asiaticus can be detected by conventional PCR and characteristics of their detrimental effects include low rate of vegetative growth and reduction of dry matter, root dry matter, plant height and stem diameter.

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1. Introduction

Candidatus Liberibacter is the causal agent for the Asian Huanglongbing (HLB), which is also known as citrus greening disease. This destructive disease is transmitted by the psyllid vector Diaphorina citri in Asia (Zhao, 1981) and Trioza erytreae in Africa (Graca, 1991). Graft transmission was first reported by Lin in China in 1950s (Hung et al., 2000). The variability in graft transmission of Ca. Liberibacter depends upon the plant part used for grafting, the amount of tissue used and the pathogen isolate with single buds (Batool et al., 2007). Graft transmission of the African greening varied from 0 to 50%, depending upon the isolate used. Side grafts with twigs were even more efficient in transmitting the pathogen. Seven months after grafting diseased buds on healthy rootstocks, 58% of the grafts survived, and out of those, 20% showed citrus greening symptoms. This author also reported that 10–16% of grafts with buds from asymptomatic branches on diseased trees developed symptoms, while 40% of grafts from symptomatic branches developed symptoms of citrus greening in 3–9 months.

Three types of C. Liberibacter have been reported: C. Liberibacter asiaticus in Asia, C. Liberibacter africanus in Africa and C. Liberibacter americanus in Brazil (Garnier et al., 2000, Teixeira et al., 2008, Teixeira et al., 2005). HLB can affect most citrus cultivars and relatives. HLB has been reported on Microcitrus australasica, Swinglea glutinosa, Annona squamosa, Clausena indica, Limo尼亚 acidissima, Balsamocitrus dawei, Aeglopsis chevalieria, Severinia buxifolia and Muraya paniculata. In addition to these non-Rutaceous hosts, only Catharanthus roseus (periwinkle) and Nicotiana xanthii (tobacco) have been reported as hosts for the pathogen. However, these two plant species were infected only under laboratory conditions and acted as indicator plants (Knapp et al., 2004).

Symptoms of HLB can be confused with stubborn disease, CTV infection, Phytophthora infection, citrus blight or certain nutrient deficiencies. This makes HLB difficult to identify in the field. However, a yellowing canopy, blotchy mottled leaves and small lopsided fruits with aborted seeds are good indications of HLB. Diagnosis must then be confirmed through the use of electronic microscopy, DNA hybridization or PCR to identify the bacterium. The HLB bacteria are restricted to phloem sieve tubes in affected plant cells and are transmitted through the use of electronic microscopy, DNA hybridization or PCR to identify the bacterium. The HLB bacteria are restricted to phloem sieve tubes in affected plant cells and are transmitted through the use of electronic microscopy, DNA hybridization or PCR to identify the bacterium. 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removing sources of inoculums. According to Kapur et al. (1984), rootstocks can affect the expression of symptoms of HLB; 5 of 23 rough lemon rootstock selections in India induced tolerance in sweet orange scion in greenhouse. Graca and Korsten (2004) also reported that 100% of rough lemon trees were infected compared to only 25% of blood red trees on sweet orange rootstock. Sweet orange is not commonly used as a rootstock for other species of citrus as it has been detected to be susceptible to HLB.

Molecular techniques such as PCR have been used successfully to detect and differentiate different types of Candidatus Liberibacter (Bove et al., 1993; Halbert and Manjunath, 2004; Jagoueix et al., 1996; Tian et al., 1996). Based on sequence information, PCR primers have been developed to amplify ribosomal DNA for detection of HLB. Recently specific primers such as OI1 and OI2s have been used widely for detection of the HLB pathogen (Teixeira et al., 2008).

There is no information pertaining to the effects of different citrus rootstocks and interstocks against HLB disease in field or greenhouse conditions for *C. reticulata* scion. An interstock is a piece of stem of two grafted union between the rootstock and scion. Interstocks can be used to avoid incompatibility between the rootstock and scion, to take advantage of dwarfing rootstock and to improve rooting (Chanana and Gill, 2008). It may also affect the reduction of disease severity. This study was conducted to determine the best combination of citrus rootstocks and interstocks for control of HLB and also to categorize the level of HLB disease severity on different citrus combination.

### 2. Materials and methods

#### 2.1. Source of plant materials and HLB transmission by side grafting

Experiments were conducted in a research field of the University of Putra Malaysia (UPM). Because Hajiyand et al. (2009) categorized *C. grandis* and *C. hystrix* as resistant species and *C. aurantium* and *C. aurantifolia* as tolerant species against HLB disease, seeds of these citrus species were collected from the Department of Agriculture in Kuala Terengganu, Malaysia. Because we planned to use these species as rootstocks and interstocks, we planted seeds in January 2008 and grew seedlings in 5 L plastic pots with a mixture of soil, peat and sand (2:3:1 v/v). Twelve combinations of citrus rootstocks and interstocks were chosen for this study as shown in Table 1.

Interstocks were grafted onto the rootstocks using wedge grafting three months after germination. The plants were grafted at approximately 30 cm height from the soil pot level and then immediately wrapped with plastic bag for 2 weeks. Successfully grafted plants were maintained in a screened house. *C. reticulata* was used as scion for all combinations in September 2008 using wedge grafting method. The inoculum sources of HLB disease were identified in the UPM research orchard and confirmed by PCR. Infected soft wood cuttings were taken from the inoculum sources as scions and all cuttings were 10–12 cm long. All seedlings were inoculated through side grafting on scions and covered with plastic bags for two weeks. A total of 108 successful grafted seedlings were arranged in a randomized complete block design with three replications. Leaf samples were collected from the citrus combination six months after inoculation and transferred to laboratory for PCR testing.

#### 2.2. Disease severity measurement

Disease severity was measured based on symptoms of HLB on the scion growth (Table 2), according to the rating scale proposed by Kranz (1988) and Bowen (2004). Based on the leaf symptoms, the scale included:

0 = No symptom (Tolerant).
1 = Mild (blotchy mottling symptoms observed from 1 to 30% on seedlings canopy).
2 = Moderate (yellowing symptoms observed from 31 to 50% on seedlings canopy).
3 = Severe (blotchy mottling, midrib yellowing and twig dieback symptoms observed more than 50% on seedling canopy).

Disease severity = \[ \frac{\Sigma (a \times b)}{NZ} \times 100\% \]

where \( \Sigma (a \times b) \) = sum of the symptomatic plant and their corresponding rating, \( N \) = total number of sampled plant and \( Z \) = highest rating.

#### 2.3. Plant growth parameter measurements

Six months after inoculation. The grafted plant combinations were washed thoroughly and rinsed in tap water, followed by distilled water and then oven dried at 65 °C for 4–5 days. Above ground dry matter and root dry matter of the different combinations were then measured. Plant heights and stem diameters of grafted plants were also measured (Table 3). Data were collected, analyzed and means were separated using Duncan’s multiple range test (DMRT) at \( P \leq 0.05 \).

#### 2.4. DNA extraction from citrus tissues

Leaf samples were collected for evaluation from the grafted citrus plants which had been inoculated using the side grafting

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**Table 1**

List of rootstock and interstock combinations for grafting.

<table>
<thead>
<tr>
<th>Combinations</th>
<th>Rootstocks</th>
<th>Interstock</th>
<th>Scion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comb.1</td>
<td><em>C. grandis</em> cv. limau bali</td>
<td><em>C. hystrix</em></td>
<td><em>C. reticulata</em> (Blanco) cv. limau madu</td>
</tr>
<tr>
<td>Comb.2</td>
<td><em>C. grandis</em> (L) cv. limau bali</td>
<td><em>C. aurantium</em></td>
<td><em>C. reticulata</em> (Blanco) cv. limau madu</td>
</tr>
<tr>
<td>Comb.3</td>
<td><em>C. grandis</em> (L) cv. limau bali</td>
<td><em>C. aurantifolia</em></td>
<td><em>C. reticulata</em> (Blanco) cv. limau madu</td>
</tr>
<tr>
<td>Comb.4</td>
<td><em>C. hystrix</em> Chakr. cv. limau purut</td>
<td><em>C. grandis</em></td>
<td><em>C. reticulata</em> (Blanco) cv. limau madu</td>
</tr>
<tr>
<td>Comb.5</td>
<td><em>C. hystrix</em> Chakr. cv. limau purut</td>
<td><em>C. aurantifolia</em></td>
<td><em>C. reticulata</em> (Blanco) cv. limau madu</td>
</tr>
<tr>
<td>Comb.6</td>
<td><em>C. hystrix</em> Chakr. cv. limau purut</td>
<td><em>C. aurantium</em></td>
<td><em>C. reticulata</em> (Blanco) cv. limau madu</td>
</tr>
<tr>
<td>Comb.7</td>
<td><em>C. aurantium</em> (L) cv. limau samur</td>
<td><em>C. grandis</em></td>
<td><em>C. reticulata</em> (Blanco) cv. limau madu</td>
</tr>
<tr>
<td>Comb.8</td>
<td><em>C. aurantium</em> (L) cv. limau samur</td>
<td><em>C. aurantifolia</em></td>
<td><em>C. reticulata</em> (Blanco) cv. limau madu</td>
</tr>
<tr>
<td>Comb.9</td>
<td><em>C. aurantium</em> (L) cv. limau samur</td>
<td><em>C. hystrix</em></td>
<td><em>C. reticulata</em> (Blanco) cv. limau madu</td>
</tr>
<tr>
<td>Comb.10</td>
<td><em>C. aurantium</em> (S) cv. limau nipsis</td>
<td><em>C. grandis</em></td>
<td><em>C. reticulata</em> (Blanco) cv. limau madu</td>
</tr>
<tr>
<td>Comb.11</td>
<td><em>C. aurantium</em> (S) cv. limau nipsis</td>
<td><em>C. aurantium</em></td>
<td><em>C. reticulata</em> (Blanco) cv. limau madu</td>
</tr>
<tr>
<td>Comb.12</td>
<td><em>C. aurantium</em> (S) cv. limau nipsis</td>
<td><em>C. hystrix</em></td>
<td><em>C. reticulata</em> (Blanco) cv. limau madu</td>
</tr>
</tbody>
</table>

* A total of 108 successful grafted seedlings were arranged in a randomized complete block design with three replications.

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method. DNA extracts from citrus tissues were prepared following the method described by Hung et al. (2004). DNA was extracted from HLB infected tissue using cetyltrimethyl ammonium bromide (CTAB). The pellets were washed with 70% ethanol, dried and resuspended in 100 μL TE buffer.

2.5. PCR conditions, primers and gel electrophoresis

Conventional PCR were 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, 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, 0.75 units of Taq DNA polymerase and 200 ng DNA extracts from citrus tissues were prepared following the method. DNA extracts from citrus tissues were prepared following the method described by Hung et al. (2004). DNA was extracted from HLB infected tissue using cetyltrimethyl ammonium bromide (CTAB). The pellets were washed with 70% ethanol, dried and resuspended in 100 μL TE buffer.

A moderate level of HLB severity was recorded for both Comb.2 (C. grandis used as the rootstock and C. aurantifolia as the interstock) and Comb.3 (C. grandis used as the rootstock and C. aurantifolia as the interstock for C. reticulata). The disease severities for Comb.2 were calculated as 0% two months and 11.11% four and six months after inoculation. In contrast, severities were 11.11% two months and 22.22% four and six months after inoculation for Comb.3.

Disease symptoms were not observed on Comb.4 (C. hystrix as the rootstock and C. grandis as the interstock). In contrast, disease severity was measured 11.11% two months and 22.22% four and six months after inoculations on Comb.5 (C. aurantium rootstock with C. hystrix interstock). A moderate level of disease severity was observed on Comb.6 (C. hystrix as the rootstock and C. aurantifolia as the interstock) with 22.22% two months and 33.33% four and six months after inoculation.

A mild level of disease severity was recorded for Comb.7 (C. aurantium as the rootstock and C. grandis as the interstock) and also in Comb.9 (C. aurantium as the rootstock and C. hystrix as the interstock) with 11.11% two months and 22.22% on four and six months after inoculation for both Comb.7 and Comb.9. In contrast, severe HLB was observed on Comb.8 (C. aurantium as the rootstock and C. aurantifolia as the interstock and C. reticulata as the scion) with 22.22%, 44.44% and 55.55% two, four and six months after inoculation, respectively.

A mild level of disease severity was recorded for Comb.10 (C. aurantifolia as rootstock with C. grandis as interstock) and the disease was measured 0%, 11.11% and 22.22% two, four, and six months after inoculation, respectively. In contrast, severe HLB symptoms were observed on Comb.11 (C. aurantifolia as the rootstock and C. aurantifolia as the interstock), as disease severity was measured 22.22%, 55.55% and 65% two, four and six months after inoculation, respectively.

Table 2

<table>
<thead>
<tr>
<th>Combinations</th>
<th>Rootstocks</th>
<th>Interstock</th>
<th>Scion</th>
<th>Disease severity (%)</th>
<th>Severity group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2nd month</td>
<td>4th month</td>
</tr>
<tr>
<td>Comb.1</td>
<td>C. grandis</td>
<td>C. hystrix</td>
<td>C. reticulata</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Comb.2</td>
<td>C. grandis</td>
<td>C. aurantifolia</td>
<td>C. reticulata</td>
<td>0.00</td>
<td>11.11</td>
</tr>
<tr>
<td>Comb.3</td>
<td>C. grandis</td>
<td>C. aurantium</td>
<td>C. reticulata</td>
<td>11.11</td>
<td>22.22</td>
</tr>
<tr>
<td>Comb.4</td>
<td>C. hystrix</td>
<td>C. grandis</td>
<td>C. reticulata</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Comb.5</td>
<td>C. hystrix</td>
<td>C. aurantifolia</td>
<td>C. reticulata</td>
<td>11.11</td>
<td>22.22</td>
</tr>
<tr>
<td>Comb.6</td>
<td>C. hystrix</td>
<td>C. aurantium</td>
<td>C. reticulata</td>
<td>22.22</td>
<td>33.33</td>
</tr>
<tr>
<td>Comb.7</td>
<td>C. aurantium</td>
<td>C. grandis</td>
<td>C. reticulata</td>
<td>11.11</td>
<td>22.22</td>
</tr>
<tr>
<td>Comb.8</td>
<td>C. aurantium</td>
<td>C. hystrix</td>
<td>C. reticulata</td>
<td>22.22</td>
<td>44.44</td>
</tr>
<tr>
<td>Comb.9</td>
<td>C. aurantium</td>
<td>C. hystrix</td>
<td>C. reticulata</td>
<td>11.11</td>
<td>22.22</td>
</tr>
<tr>
<td>Comb.10</td>
<td>C. aurantifolia</td>
<td>C. grandis</td>
<td>C. reticulata</td>
<td>0.00</td>
<td>11.11</td>
</tr>
<tr>
<td>Comb.11</td>
<td>C. aurantium</td>
<td>C. aurantifolia</td>
<td>C. reticulata</td>
<td>22.22</td>
<td>55.55</td>
</tr>
<tr>
<td>Comb.12</td>
<td>C. aurantifolia</td>
<td>C. hystrix</td>
<td>C. reticulata</td>
<td>11.11</td>
<td>33.33</td>
</tr>
</tbody>
</table>

* A total of 108 successful grafted seedlings were arranged in a randomized complete block design with three replications.

* Severeities 1–30% – mild group, 31–50% – moderate group, > 51% severe group and no symptom – tolerant group.

Table 3

<table>
<thead>
<tr>
<th>Combinations</th>
<th>Rootstocks</th>
<th>Interstocks</th>
<th>Scion</th>
<th>Above ground dry matter (g)</th>
<th>Root dry matter (g)</th>
<th>Plant height (cm)</th>
<th>Stem diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comb.1</td>
<td>C. grandis</td>
<td>C. hystric</td>
<td>C. reticulata</td>
<td>23.57 b</td>
<td>1.37 a</td>
<td>83.67 a</td>
<td>8.60 a</td>
</tr>
<tr>
<td>Comb.2</td>
<td>C. grandis</td>
<td>C. aurantifolia</td>
<td>C. reticulata</td>
<td>21.73 bcd</td>
<td>0.80 b</td>
<td>74.67 bc</td>
<td>4.50 c</td>
</tr>
<tr>
<td>Comb.3</td>
<td>C. grandis</td>
<td>C. aurantium</td>
<td>C. reticulata</td>
<td>20.00 de</td>
<td>0.73 bc</td>
<td>65.00 def</td>
<td>3.93 cd</td>
</tr>
<tr>
<td>Comb.4</td>
<td>C. hystrix</td>
<td>C. grandis</td>
<td>C. reticulata</td>
<td>25.60 a</td>
<td>0.82 b</td>
<td>78.33 ab</td>
<td>8.00 a</td>
</tr>
<tr>
<td>Comb.5</td>
<td>C. hystrix</td>
<td>C. aurantifolia</td>
<td>C. reticulata</td>
<td>20.20 de</td>
<td>0.70 bc</td>
<td>66.33 de</td>
<td>3.57 cd</td>
</tr>
<tr>
<td>Comb.6</td>
<td>C. hystrix</td>
<td>C. aurantium</td>
<td>C. reticulata</td>
<td>20.00 de</td>
<td>0.71 bc</td>
<td>56.67 f</td>
<td>3.60 cd</td>
</tr>
<tr>
<td>Comb.7</td>
<td>C. aurantium</td>
<td>C. grandis</td>
<td>C. reticulata</td>
<td>21.50 cd</td>
<td>0.82 b</td>
<td>70.00 cf</td>
<td>4.47 c</td>
</tr>
<tr>
<td>Comb.8</td>
<td>C. aurantium</td>
<td>C. hystrix</td>
<td>C. reticulata</td>
<td>17.90 f</td>
<td>0.53 c</td>
<td>46.67 g</td>
<td>3.00 d</td>
</tr>
<tr>
<td>Comb.9</td>
<td>C. aurantium</td>
<td>C. hystrix</td>
<td>C. reticulata</td>
<td>21.13 d</td>
<td>0.63 bc</td>
<td>65.00 def</td>
<td>3.83 cd</td>
</tr>
<tr>
<td>Comb.10</td>
<td>C. aurantifolia</td>
<td>C. grandis</td>
<td>C. reticulata</td>
<td>23.17 bc</td>
<td>0.82 b</td>
<td>80.00 ab</td>
<td>5.93 b</td>
</tr>
<tr>
<td>Comb.11</td>
<td>C. aurantifolia</td>
<td>C. aurantium</td>
<td>C. reticulata</td>
<td>17.33 f</td>
<td>0.50 c</td>
<td>35.33 h</td>
<td>2.97 d</td>
</tr>
<tr>
<td>Comb.12</td>
<td>C. aurantifolia</td>
<td>C. hystrix</td>
<td>C. reticulata</td>
<td>18.87 ef</td>
<td>0.62 bc</td>
<td>60.00 ef</td>
<td>3.50 cd</td>
</tr>
</tbody>
</table>

Note: Means within a column with different letters are significantly different and means followed by the same letters are not significantly different at P = 0.05. A total of 108 successful grafted seedlings were arranged in a randomized complete block design with three replications.
respectively. Similarly, a moderate level of disease severity also recorded for Comb.12 (C. aurantifolia as rootstock with C. hystrix as interstock for C. reticulata) with 11.11% two months and 33.33% four and six months after inoculation.

3.2. Plant growth of different citrus combinations

Above ground dry matter, root dry matter, stem diameter and plant height were measured six months after inoculation (Table 3). Significant differences were observed among the citrus combinations at the 5% significance level. The above ground dry matter weights of Comb.4 (25.600 g) and Comb.1 (23.566 g) were significantly higher than those of the other combinations, and those of Comb.5 (17.333 g) and Comb.8 (17.900 g) were in the lowest group.

The root dry matter value of Comb.1 (1.366 g) was significantly higher than the other combinations and those of Comb.8 (0.533 g) and Comb.11 (0.500 g) were significantly lower than the others. Comb.7 (0.823 g), Comb.10 (0.820 g), Comb.4 (0.816 g) and Comb.2 (0.800 g) had the similar root dry matter values. Although Comb.1 (83.667 cm) had the highest plants but the height value was not significantly different from those of Comb.4 and Comb.10. The lowest height value was recorded for Comb.11 (35.333 cm). The stem diameter measures of Comb.1 (8.600 mm) and Comb.4 (8.000 mm) were significantly higher than those of the other combinations, while those of Comb.11 (2.900 mm) and Comb.8 (3.000 mm) were in the lowest group.

3.3. PCR detection of HLB in plants of various citrus combinations

DNA was extracted from the midrib tissue of leaves for each inoculated citrus combination and the template DNA was for PCR amplification with the primer pair OI1/OI2. A band of about 1160 bp was obtained from the infected citrus combinations. Second PCR amplification was also conducted with the same Primer of OI1/OI2. Results of conventional PCR showed absence or presence of C. Liberibacter asiaticus bacterial cells among the different citrus combinations in Fig. 1 and Fig. 2. Based on conventional PCR (Fig. 1), HLB was absent in Comb.1, Comb.2, Comb.3, Comb.4, Comb.5, Comb.9 and Comb.12.

In contrast, HLB was present in Comb.6, Comb.7. Comb.8, Comb.10 and Comb.11. Second PCR amplification was used to amplify 16S rDNA of HLB bacteria on all citrus combinations. Results are presented in Fig. 2. Candidatus Liberibacter asiaticus was successfully detected in all combinations, Comb.1 to Comb.12.

4. Discussion

The grafted of rootstocks and interstocks with scions is a horticultural technique used to improve crop production and enhance tolerance to pests and diseases. An interstock is a piece of stem inserted by means of two graft unions between the stock and scion. It is used to avoid any incompatibility between the stock and scion. It is used to take advantage of dwarfing rootstock to restrict tree size and improve rooting (Chanana and Gill, 2008; Colburn and Graham, 2007). Interstocks are also used to decrease the thickness of the trunk at the grafting point (Chanana and Gill, 2008).

Rootstocks should have proper vigour and growth habits, resistant to soil borne pathogens and other pests, and they can be used in a wide range of graft compatibility and easy to propagate. Great care must be given in the selection of the best rootstock for soil type, each growing conditions, pest and disease problems. An ideal rootstock is likely to vary with soil type, growing conditions, pests and diseases. HLB can affect almost all citrus cultivars like orange, tangelo and mandarin (Knapp et al., 2004). However, based on a study conducted by Hajivand et al. (2009) C. reticulata is the most susceptible among the types of citrus grown in Malaysia. It was chosen as scion and grafted on different citrus combinations. HLB symptoms were not observed on the scion with C. grandis as rootstock and C. hystrix as interstock (Comb.1).

The present study confirmed the high rate of HLB disease severity when C. aurantium was used as rootstock with C. aurantifolia as the interstock or vice versa. In some cases, the rootstock can affect symptoms, the levels of disease severity for C. grandis and C. hystrix were higher when they were combined with C. aurantifolia or C. aurantium as rootstocks or interstocks.

This study also showed that growth performance was related to the HLB severity in various citrus combinations. Above ground dry matter, plant height, root dry matter and stem diameter were also different on the citrus combinations for citrus reticulata scion. The results were similar to those of Storey and Walker (1999), who...


considered that the permeability of the root system in citrus rootstocks could be different. In this case a high ratio of above ground dry matter, root dry matter, plant height and stem diameter were observed on the citrus combination with low levels of disease severity. For instance, low values of root dry matter, plant height and stem diameter were observed on citrus combination with high levels of disease severity. Rootstocks affect over 20 traits, primarily horticultural and physiological characters, tolerance to pests and diseases and scion compatibility (Castle et al., 1989, Jose et al., 2004). In South Africa, the percentage of greening in Valencia oranges was higher on trifoliate orange rootstock than on Empress Mandarin or Troyer Cитrante. The trifoliate rootstock might cause an extension of the growth flesh period and thus extend the feeding time for insect vector (Van Vuuren and Moll, 1985). However, no differences were found in a study on the effects of 13 rootstocks on symptoms in Ponkan mandarin (Lin, 1963).

Conventional PCR of 16S rDNA were successfully employed to produce an 1160 bp PCR product. Halbert and Manjunath (2004) used molecular approaches such as PCR and strain specific DNA probes successfully to detect and differentiate types of C. lib-erbacter in infected plants. Unfortunately, detection may not be always reliable. Sometimes, trees with classic greening symptoms test negative with PCR. The HLB pathogen was not detected in citrus combinations with low levels of disease severity, such as C. grandis rootstock with C. hiystrix interstock, C. grandis rootstock with C. aurantium interstock, C. hystrix rootstock with C. aurantium interstock, C. hystrix rootstock with C. aurantium interstock, C. hystrix rootstock with C. aurantium interstock, C. aurantium rootstock with C. hystrix interstock and C. aurantium rootstock with C. hystrix interstock for C. reticulata scion. The conventional PCR test showed positive result on plants with severe symptom of blotchy motting because they had the highest titers of the bacterium (Teixeira et al., 2008). In contrast, the HLB pathogen was detected in all citrus combinations when second PCR amplification was used.

In conclusion, citrus combinations can be categorized into tolerant (no symptom), mild, moderate and severe groups. No HLB symptoms were observed on Comb.1 (C. grandis as rootstock with C. hystrix as interstock) and Comb.4 (C. hystrix as rootstock with C. grandis as interstock) and these were in the tolerant group. The mild group included Comb.2 (C. grandis as rootstock with C. aurantiofolia as interstock), Comb.3 (C. grandis as rootstock with C. aurantium as interstock), Comb.5 (C. hystrix as rootstock with C. aurantiofolia as interstock), Comb.7 (C. aurantium as rootstock with C. grandis as interstock), Comb.9 (C. aurantium as rootstock with C. hystrix as interstock) and Comb.10 (C. aurantium as rootstock with C. grandis as interstock). The moderate group consisted of Comb.6 (C. hystrix as rootstock with C. aurantium as interstock) and Comb.12 (C. aurantiofolia as rootstock with C. aurantium as interstock). Finally, the severe group included Comb.8 (C. aurantium as rootstock with C. aurantiofolia as interstock) and Comb.11 (C. aurantium as rootstock with C. aurantium as interstock) for C. reticulata scion.