*Brief communication*

# Candidate gene markers for *Candidatus* Liberibacter asiaticus for detecting citrus greening disease

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Citrus Huanglongbing (HLB) also known as citrus greening is one of the most devastating diseases of citrus worldwide. The disease is caused by *Candidatus* Liberibacter bacterium, vectored by the psyllid *Diaphorina citri* Kuwayama and *Trioza erytreae* Del Guercio. Citrus plants infected by the HLB bacterium may not show visible symptoms sometimes for years following infection. The aim of this study was to develop effective gene-specific primer pairs for polymerase chain reaction based method for quick screening of HLB disease. Thirty-two different gene-specific primer pairs, across the *Ca.* Liberibacter asiaticus genome, were successfully developed. The possibility of these primer pairs for cross-genome amplification across '*Ca.* Liberibacter africanus' and '*Ca.* Liberibacter americanus' were tested. The applicability of these primer pairs for detection and differentiation of *Ca* Liberibacter spp. is discussed.

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Citrus greening (Huanglongbing, HLB) is the most destructive disease of citrus, debilitating the productive capacity of citrus trees wherever it is present worldwide (Halbert and Manjunath 2004; Bové 2006). HLB has been shown to be present internationally, from its first description in the early 1900s in China to its appearance in Florida, USA, as recently as 2005 (Bové 2006). It is estimated that nearly 100 million trees in 40 countries are affected by this disease especially in Asian and Southeast Asian countries like India, China, Indonesia, Philippines, as well as the Arabian Peninsula, and Africa (Bové 1986; Halbert and Manjunath 2004; Gottwald *et al*. 2007) and more recently in Brazil and USA. The fast progression of the disease, in the orchards where the bacterial inoculum or the psyllids have not been efficiently managed, may lead to the infected tree decline in 3 to 13 years (Gottwald *et al*. 1989, 2007). In March 2004, HLB was recognized for the first time in the Americas in São Paulo State, Brazil, that resulted in removal of nearly 3 million HLB-affected sweet orange trees (NRC 2010). Florida being the second largest citrus producer in the world, its $9.3 billion annual economic benefit of the citrus industry would be lost or significantly diminished due to severe spread of HLB disease statewide (NRC 2010).

The etiologic causal agent of HLB is a fastidious, phloem-limited, gram-negative bacterium (Garnier *et al*. 1984), restricted to phloem sieve tubes of its natural host, citrus. Despite several attempts, the HLB-causing bacterium has not been obtained in pure culture (Das 2004; Li *et al*. 2006). Based on the nucleotide sequence of its 16S ribosomal RNA gene (rDNA), the HLB pathogen has been characterized as a new genus belonging to the alpha subdivision of the proteobacteria, with the *Candidatus* status '*Candidatus* Liberibacter' (Jagoueix *et al*. 1994). Three *Candidatus* spp. of the pathogen are currently known with most widespread being the Asian species '*Ca.* Liberibacter asiaticus' found in all HLB-affected countries except Africa. The African species *Ca.* Liberibacter africanus and the American species *Ca.* L. americanus are restricted to Africa and Brazil,

**Keywords.** Citrus greening; *Candidatus* Liberibacter; cross-amplification; DNA markers; polymerase chain reaction



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**Table 1.** Nucleotide sequence of forward and reverse primers used for the detection of *Candidatus* Liberibacter species

| Serial No. | Primer name | GenBank Entry | Gene target | Primer sequence (5'–3') | Amplicon size |
|---|---|---|---|---|---|
| 1 | HLB135<br>HLB136 | EF164805.1 | tRNA methyl RNA protein chain elongation factor EF-Tu (tufB) | F: TACTAATACGACTCACTATAGGGCTTGTTGTGCAGGGCAAGATGTGT<br>R: CCAGATGCAACGCCAACTTCACTT | 1 kb |
| 2 | HLB141<br>HLB142 | EF164805.1 | tRNA methyl RNA protein chain elongation factor EF-Tu (tufB) | F: AATCCTTGTGTCGAGGGTTCAAATCCC<br>R: TACTAATACGACTCACTATAGGGACTATATACC | 2.5 kb |
| 3 | HLB157<br>HLB158 | JF811345.1 | Outer membrane protein | F: CACCGTAGAAGGGCATATTGAT<br>R: CATGCGATTACCTATACGAAAACC | 1.3 kb |
| 4 | HLB159<br>HLB160 | FJ489642.1 | Outer membrane protein | F: CACCATGATAGTTCCTATTACTG<br>R: AAACTGCTGAATTTTATCACCCTC | 900 bp |
| 5 | HLB163<br>HLB164 | AB473570.1 | RNA polymerase beta subunit | F: GCGTTCATGTAGAAGTTGTG<br>R: CCTACAGGTGGCTGACTCAT | 500 bp |
| 6 | HLB175<br>HLB176 | CP001677.4 | ATP-binding protein; Prophase-related gene cluster-1 | F: CCTTAATACGACTCACTATAGGCGCGTATGCAATACGAGCGGCA<br>R: CGAGACCCGGGAAATACACAAAATACAGCTAAAAATCACTG | 1.5 kb |
| 7 | HLB177<br>HLB179 | CP001677.4 | Prophase-related gene cluster-1, elongation factor EF-Tu | F: CTGATATTGTGAACCATGGGAACATCCTCAC<br>R: GATAGACCAAACAGATCAGCGACAACACCGACGGC | 1.5 kb |
| 8 | HLB178<br>HLB179 | CP001677.4 | Prophase-related gene cluster-1, elongation factor EF-Tu | F: GTGAGGATGTTCCCATGGTTCACAATATCAG<br>R: GATAGACCAAACAGATCAGCGACAACACCGACGGC | 825 bp |
| 9 | CL420 | CLIBASIA_03420 | Integral membrane protein MviN | F: GAGCTTAATTAAATGAAGATTATTCGAAACTTTCTTACGGTATG<br>R: TTATTTTCGAATCATTTGTTGCAATGGGGATAGAAAATC | 1.5 kb |
| 10 | CL30 | CLIBASIA_03030 | Zinc uptake ABC transporter, permease protein | F: GAGCTTAATTAAATGTATAATGAATTCTTCATTCGTGCCCTGTTTTC<br>R: TTATTTTTTATAGAAAAAGCTTAATATAAATAAGAATAAAG | 723 bp |
| 11 | CL025 | CLIBASIA_03025 | Putative high-affinity zinc uptake system ATP-binding | F: GAGCTTAATTAAATGTCTAACGCCCTATCTATTACGCCCTTG<br>R: TTAGTGATCATGTTTGTGATTATGAATAGCTAATATTTCTG | 780 bp |
| 12 | CL20 | CLIBASIA_03020 | Zinc uptake ABC transporter | F: GAGCTTAATTAAATGAAAAATTTTTTAATAATACTTATATTTC<br>R: CTAAGAACAGTTCTTAGCTATTGAATTAGACATAC | 885 bp |
| 13 | CL555 | CLIBASIA_01555 | Hemolysin protein | F: GAGCTTAATTAAATGATGAGCGATTTTAAAATAAATTCATC<br>R: TTAATTTTTAAAATGAACAAGACGTCGAATACGCACACGAC | 963 bp |
| 14 | CL965 | CLIBASIA_02965 | ABC transporter, membrane-spanning protein | F: GAGCTTAATTAAATGCTCTTGTTATCCGGTTTTAGTATAATC<br>R: TCATTCGTACTTTCTCTGATATTTTTTCATGATATACATAG | 1.5 kb |
| 15 | CL096 | CLIBASIA_02960 | Phosphate ABC transporter, permease protein PstA | F: GAGCTTAATTAAATGAAATCGTTTTCTATCAATAAAAAGAAC<br>R: TTACCAACGCTTTTTAAAGCGATTCCTCAACCATAACATAG | 1.3 kp |
| 16 | CL250 | CLIBASIA_02250 | Extracellular solute-binding protein | F: GAGCTTAATTAAATGTTTAAACGTACAATATATACATATCTTTTATTG<br>R: CTATCGCTTGTATTTGGTCAATATCTCGTTGGCAGTAATA | 600 bp |
| 17 | CL335 | CLIBASIA_01355 | Type I secretion membrane fusion protein, HlyD | F: GAGCTTAATTAAATGCATCATTTTATGTGGAAAAATTTAAAAAG<br>R: TTACCTTTCAAATATTTTAGGCCAGTTATCGGTAATG | 1.3 kp |
| 18 | CL55 | CLIBASIA_03055 | Component of type IV pilus | F: GAGCTTAATTAAATGTTCGGAAGACGTGATAAGAAATTGATGGGCTCAG<br>R: CTACAATGCACGACTCTGTTTCTCCAGAGATTGAAGAACTTC | 1.4 kb |
| 19 | CL80 | CLIBASIA_03080 | Peptidase A24A prepilin type IV | F: GAGCTTAATTAAATGAAAGAGAGTAAAATGGTCTTTTCTGCTG<br>R: TTACGCAGAAAGGCCCATTAAAGCAACTTTAAACAAGTATG | 530 bp |

**Table 1** (continued)

| Serial No. | Primer name | GenBank Entry | Gene target | Primer sequence (5'–3') | Amplicon size |
|---|---|---|---|---|---|
| 20 | CL165 | CLIBASIA_05165 | Hypothetical protein | F: GACCTTAATTAATGGATCAAAAGCAACAAGCATTCCATG<br>R: TTATTGTGCAGTGTTCTTTTTCTGTTTTGTCTG | 480 bp |
| 21 | CL195 | CLIBASIA_05195 | Hypothetical protein | F: GAGCTTAATTAATGAACGTGCTAGCCATATTAAATACCGTTTG<br>R: TTATCCTCCTAGCCAAAGGATCTTTTCCGC | 615 bp |
| 22 | CL200 | CLIBASIA_05200 | Hypothetical protein | F: GAGCTTAATTAATGGCAAAAGATTTCGCTTCTTTATTAATTG<br>R: CTACCACGAACGGATATCATTCTGCTGTAAT | 564 bp |
| 23 | CL605 | CLIBASIA_05605 | Hypothetical protein | F: GAGCTTAATTTATGAGTTTCTTAGATTCGAGTGTAAAATTCGGTTC<br>R: TCATCGTACATGCACCCCTGATAAAAGACTCG | 360 bp |
| 24 | CL620 | CLIBASIA_05620 | Hypothetical protein | F: GAGCTTAATTAAATGTACGCTCACAAATACACAAAAGAAAGAATTG<br>R: TTAGTCATGTTTAGTTAACTTCGGAAGCTCAAG | 500 bp |
| 25 | CL635 | CLIBASIA_05635 | Hypothetical protein | F: GAGCTTAATTAATGGGACAATTAAAGCAATATTACCTCGAAGAG<br>R: TCAAATCTGACTGGCGTAATAATCTTCTTTATG | 420 bp |
| 26 | CL665 | CLIBASIA_05665 | Hypothetical protein | F: GAGCTTAATTAATGGGAAGAAAAGTTTTAACACCTGAAGAAAGG<br>R: TTAACCTCTCTTATAAATTAGTTCTTCTATGG | 417 bp |
| 27 | CL875 | CLIBASIA_02875 | Putative flagellar motor switch protein | F: GAGCTTAATTAAATGATCAAAAAAGTCTCAAAAATTTCTTAC<br>R: TTACTGAGCGTTTAAAATTTCTATTACCCGCACTCCAAAG | 430 bp |
| 28 | CL910 | CLIBASIA_02910 | Flagellar MS-ring protein | F: GAGCTTAATTAAATGGCTATTTTAGATCAATTATTGCAGTTTTTC<br>R: CTAGCTAATATGTTGAGTATATCGATCTTCTATTTC | 1.7 kp |
| 29 | HLB180<br>HLB181 | CP001677.4 | Transcriptional regulator | F: GCCGTCGGTGTTGTCGCTGATCTGTTTGGTCTATC<br>R: AACGGTTAGTTCACGCTAGAGGATATACTAAAACGG | 500 bp |
| 30 | CL345 | CLIBASIA_01345 | Serralysin | F: GAGCTTAATTAAATGCATAATATAAAACCGGTTTATACAACTG<br>R: TCAGGAAAAATCATGATTTATATCATGCATAGAATATAAATC | 2.0 kb |
| 31 | OMP1 | CLIBASIA_00995 | Outer membrane protein | F: TGCAGGCTCGGTACCACAAACTAA<br>R: TGCATCATAGCCACCTGTCACTGT | 475 bp |
| 32 | OMP2 | CLIBASIA_02425 | Outer membrane protein | F: ACTCGCCTCTTTCTGTTCTGCTC<br>R: AATCCTCCGACTTCAACACCGACA | 474 bp |

respectively (Teixeira *et al*. 2005). *Ca.* Liberibacter asiaticus', a heat-tolerant species, and '*Ca.* Liberibacter africanus', a heat-sensitive species, are vectored by both *Diaphorina citri* Kuwayama and *Trioza erytreae* Del Guercio, while '*Ca.* Liberibacter americanus', a heat-tolerant species, is vectored only by *D. citri* Kuwayama (McClean and Oberholzer 1965; Capoor *et al*. 1967; Bové 2006).

The typical symptoms of HLB disease are blotchy mottling of leaves (NRC 2010) often resembling zinc deficiency symptoms which leads to the typical appearance of yellowing of shoots in the tree. The infected trees show the open growth, stunning twig dieback appearance and severe fruit drop (da Graca and Korsten 2004). Additionally, symptoms of mottling and chlorosis of leaves, and small misshapen fruits with aborted seeds are characteristics of HLB-infected trees. The causal agent of HLB in Florida is *Ca.* Liberibacter asiaticus while both *Ca.* Liberibacter asiaticus and *Ca.* Liberibacter americanus isolates of HLB disease have been detected in Brazil (Bové 2006; NRC 2010). In some instances, more than one species of *Ca.* Liberibacter have been observed in plants as mixed infections (Bové 2006). Primers based on 16S RNA gene have been developed to differentiate between species of *Ca*. Liberibacter. However, availability of additional marker primer pairs to other gene loci would help ascertain the infection by *Ca*. Liberibacter spp. In this study, efforts have been made to develop various candidate gene makers specific to *Ca.* Liberibacter asiaticus for early detection of HLB disease. The effectiveness of the primer pairs developed were also tested for cross-species amplification, if any, against the other two HLB-causing species *Ca.* Liberibacter africanus and *Ca.* Liberibacter americanus.

Total nucleic acids from HLB-infected citrus was extracted from 100 mg of tissue (leaf and/or inner bark tissue from healthy as well as HLB-infected plants, Tatineni *et al*. 2008) and also from HLB-infected psyllids. Total nucleic acid samples of *Ca.* Liberibacter africanus were kindly provided by Prof G Pietersen (University of Pietoria, South Africa) and total nucleic acid samples from *Ca*. Liberibacter asiaticus as well as *Ca.* Liberibacter americanus from Brazil were kindly provided by Mr Luis Matos (Dominican Institute of Agriculture and Forestry Research, Dominican Republic).

Conventional PCR with SpeedSTAR HS DNA polymerase (Takara Bio, WI) was used to examine the presence of the HLB bacterium in citrus as well as psyllid samples. The PCR reaction (20 µL volume) consisted of 1 µL of template DNA, 0.2 µM each oligonucleotide (gene-specific forward and reverse primers; table 1), 0.25 mM dNTPs, 1× buffer (FBII; Takara Bio, USA), and 0.125 µL (5 U/µL) of SpeedSTAR HS DNA polymerase. Amplification was carried out using the following protocol (primer pairs 1–19, table 1): 94°C for 2 min; followed by 10 cycles at 94°C for 10 s, 54°C for 10 s, and 72°C for 90 s; followed by 25 cycles at 94°C for 10 s, 58°C for 10 s, and 72°C for 90 s; followed by final extension at 72°C for 5 min. For primer pairs 20–32 (table 1), the PCR cycle was modified slightly with the extension being carried out for 60 s instead of 90 s. PCR reaction (15 µL) was analysed through 1.5% agarose gel in 1× Tris-acetate-EDTA buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.5) and PCR amplicons were visualized using ethidium bromide. The PCR products were eluted from the gel and purified using GeneClean® Kit (MP Biomedicals, USA) following the manufacturer's instructions and sent to core sequencing facility, Interdisciplinary Center for Biotechnology Research (ICBR), University of Florida, Gainesville, FL, for gene sequence confirmation. For confirmation of *Ca*. Liberibacter asiaticus, *Ca.* Liberibacter africanus as well as *Ca.* Liberibacter americanus bacterium, the total nucleic acids samples were also screened against HLB gene-specific 16S rDNA primer pairs developed by Teixeira *et al*. (2005). The HLB-free, healthy citrus samples were used as a negative control in PCR amplifications. All the experiments were performed in triplicates and the results obtained were reconfirmed.

Availability of increasing number of nucleotide (genome) sequences in the public domain provides a fast and efficient approach to develop gene-specific PCR molecular markers (Chen *et al*. 2008; Soneji *et al*. 2010). Based on *Ca.* Liberibacter asiaticus whole genome sequence information (Duan *et al*. 2009; GenBank accession number NC_012985; NZ_ABQW01000001 to NZ_ABQW01000034), 50 gene-specific primer-pairs for HLB pathogen were developed using the online free primer development software, PrimerQuest$^{SM}$-Integrated DNA Technologies, USA. Of the 50 primer pairs, 32 primer pairs (table 1) amplified specific amplicons from the total nucleic acids isolated from *Ca.* Liberibacter asiaticus–infected citrus plants as well as infected pysllids (figure 1). No PCR products were amplified from the healthy citrus plants as well as psyllids. The GenBank accession number, primer name, loci studied, and the expected PCR product size are mentioned in table 1. The study also successfully standardized a common PCR thermocyler program for all the primer pairs developed. The PCR amplicon sizes observed on the agarose gel electrophoresis were compatible with the *Ca.* Liberibacter asiaticus–infected citrus plants as well as infected pysllids. The PCR amplification of all the primer pairs were also screened against the *Ca.* Liberibacter asiaticus infected samples obtained from Brazil and the amplicon sizes were found to be compatible with *Ca.* Liberibacter asiaticus infected samples from Florida (figure 2). The amplified gene fragments from agarose gel were gel-eluted (for both the American as well as Brazilian *Ca*. Liberibacter samples), gene-cleaned (GeneClean® Kit; MP Biomedicals, USA), sequenced and the sequence information were reconfirmed with respect to their GenBank accession numbers (table 1). The nucleotide

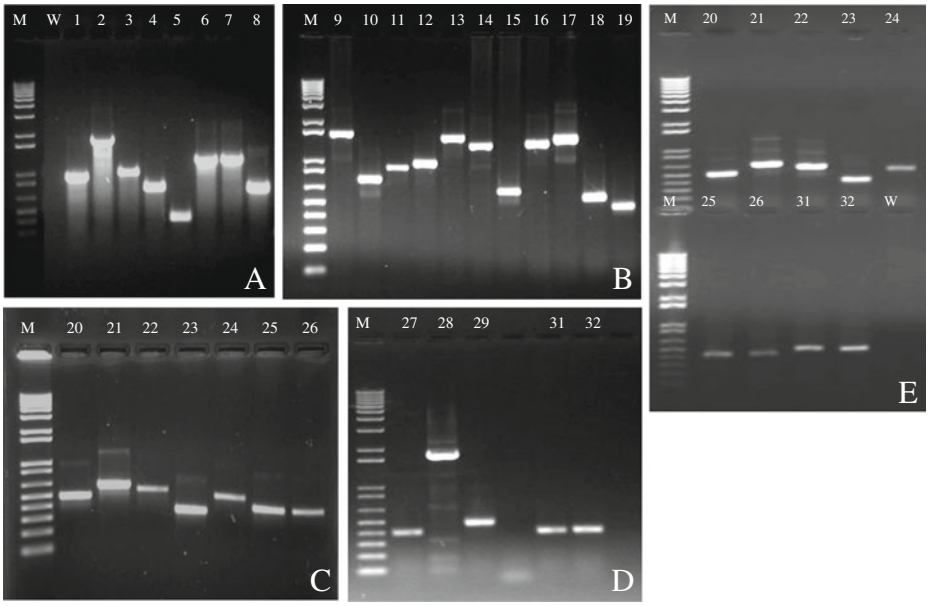


**Figure 1.** PCR amplification of *Candidatus* Liberibacter asiaticus specific sequences. (**A**, **B**, **C**, **D**) In HLB infected citrus plants. (**E**) In HLB infected psyllids. (The numbers in the lane corresponds to primer pairs in table 1); M: 1 kb DNA ladder; W: Water.

sequences were also aligned using ClustalW2 – multiple sequence alignment (*http://www.ebi.ac.uk/Tools/msa/clustalw2/*) and 98.5-100% sequence homology was observed between the American and Brazilian *Ca.* Liberibacter samples.

Total nucleic acids from citrus infected with *Ca.* Liberibacter asiaticus and *Ca.* Liberibacter americanus obtained from Brazil and the *Ca.* Liberibacter africanus from South Africa were reconfirmed by PCR experiments using primer pairs HLB75$^{(+)}$/HLB177T$^{(-)}$ (*Ca.* Liberibacter asiaticus), HLB77$^{(+)}$/HLB177T$^{(-)}$ (*Ca.* Liberibacter africanus) and HLB73$^{(+)}$/HLB166$^{(-)}$ (*Ca.* Liberibacter americanus) (table 2). The PCR amplification from these primer pairs, developed from the *Ca.* Liberibacter 16S rRNA genes, allowed us for a direct identification of all the three species.

In order to understand the cross-compatibility of the primer pairs developed in this study, across the *Ca.* Liberibacter spp., viz *Ca.* Liberibacter asiaticus, *Ca.* Liberibacter africanus, and *Ca.* Liberibacter americanus, cross-species amplification studies were carried out. Of the 32 primer pairs, only three primer pairs (1, 7, and 10; table 1; figure 3) showed cross-genome amplification in all the three *Ca.* Liberibacter spp. and three primer pairs (3, 5 and 9; table 1) showed cross-genome amplification between *Ca.* Liberibacter asiaticus and *Ca.* Liberibacter africanus and no PCR product amplicons were observed for the *Ca.*

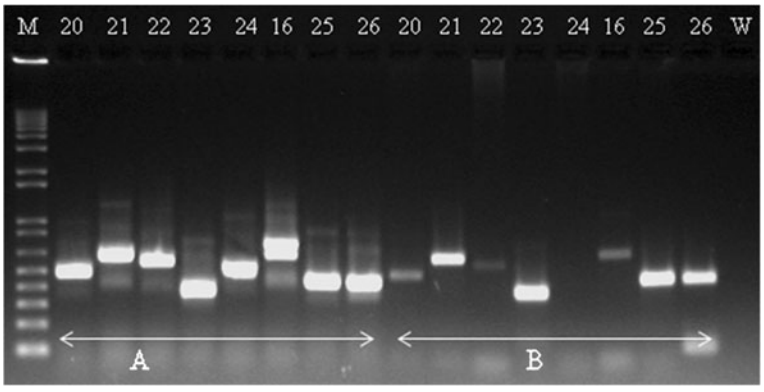


**Figure 2.** PCR amplification of *Candidatus* Liberibacter asiaticus samples. **(A)** HLB samples from Florida. **(B)** HLB samples from Brazil. The numbers in the lane corresponds to primer pairs in table 1. W: water. M: 1 kb DNA ladder.

**Table 2.** Nucleotide sequence of forward and reverse primers used for the amplification of *Candidatus* Liberibacter 16S rDNA (Teixeira *et al*. 2005)

| Serial No. | Primer name | Primer sequence (5′—3′) | PCR product size | HLB bacterium |
|---|---|---|---|---|
| 1 | HLB73<br>HLB116 | F: AGTCGAGCGAGTACGCAAGTACT<br>R: CAACTTAATGATGGCAAATATAG | 1027 bp | *Ca.* Liberibacter americanus |
| 2 | HLB75<br>HLB177T | F: CGCGTATGCAATACGAGCGGCA<br>R: GCCTCGCGACTTCGCAACCCAT | 1027 bp | *Ca.* Liberibacter asiaticus |
| 3 | HLB77<br>HLB177T | F: GCGCGTATTTTATACGAGCGGCA<br>R: GCCTCGCGACTTCGCAACCCAT | 1027 bp | *Ca.* Liberibacter africanus |

Liberibacter americanus. High rates of transferability of molecular loci across species within a genus have been reported (Peakall *et al*. 1998; Scott *et al*. 2001; Clauss *et al*. 2002; Dirlewanger *et al*. 2002; Gaitán-Solís *et al*. 2002; Jones *et al*. 2002; Williamson *et al*. 2002; Thiel *et al*. 2003; Eujayl *et al*. 2004; Saha *et al*. 2004). It has also been observed that the molecular loci may exhibit decreasing variation as the evolutionary distance increases from the species used to develop the primer pairs (Sun and Kirkpatric 1996; White and Powell 1997; Peakall *et al*. 1998; Roa *et al*. 2000; Thiel *et al*. 2003). For some of the primer pairs, 1, 3 and 10, more than one amplicon was observed. Amplification of more than one amplicon using EST-SSR markers has been reported in previous studies and was attributed to the possible amplification of both orthologous and paralogous copies of the target region (Varshney *et al*. 2005; Sim *et al*. 2009), or homoloci from different genomes (Gupta *et al*. 2003; Saha *et al*. 2004). The cross-genome amplicons for the primer pairs, 1, 3, 5, 7, 9 and 10 (table 1), for all the three *Ca*. Liberibacter species, were gel-eluted, gene-cleaned (GeneClean® Kit; MP Biomedicals, USA), sequenced and the sequence information were blasted against the GenBank database. Except the *Ca*. Liberibacter asiaticus for which the primers were originally developed, no sequence homology was observed in *Ca.* Liberibacter africanus, and *Ca.* Liberibacter americanus for the primer pairs. Studies have also observed loss of sequence homology when markers developed from one species were screened on related species (Asp *et al*. 2007; Sim *et al*. 2009; Yu *et al*. 2011). While cross-amplification of different types of molecular loci even across genera is possible, more attention should be given to the nature of the amplified fragments before inferring synteny or orthology in genetic diversity studies (table 3). As new sequencing technologies are emerging, longer nucleotide sequence reads at much lower costs than what is available right now are anticipated, making genome sequencing the method of choice for cross-genome/genera studies (Soneji *et al*. 2010; Zeid *et al*. 2010).

In this study, the effectiveness of the application of PCR primer pairs specific to *Ca.* Liberibacter asiaticus, causing HLB disease in citrus, was studied. In the absence of any durable resistant citrus cultivars, the management of HLB at least in the immediate future is to eradicate the source of pathogen, the infected citrus trees and the transmitting psyllid vector (Halbert and Manjunath 2004; Pelz-Stelinski *et al.* 2010). Management of citrus greening disease requires rapid and large scale detection, followed by removing of infected trees, and this practice is hindered due to lack of quick, sensitive and large-scale detection methods. PCR-based methods currently being used are based on the 16S rRNA gene region (Jagoueix *et al.* 1997; Teixeira *et al*. 2005; Das *et al.*

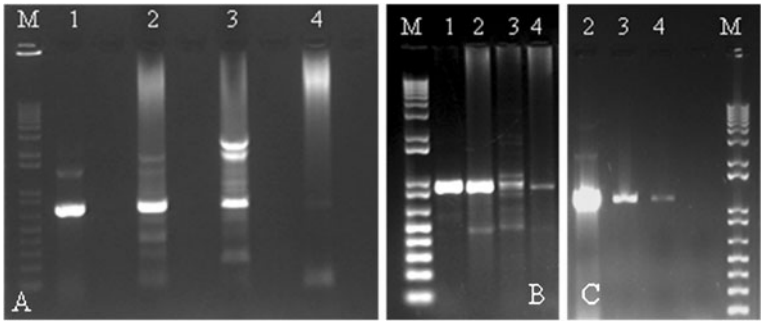


**Figure 3.** Cross-species amplification of *Candidatus* Liberibacter species. M: 1 kb DNA ladder. Gel A (primer 10 from table 1), B (primer 1 from table 1), C (primer 7 from table 1): Lane 1, *Candidatus* Liberibacter asiasticus from Florida; Lane 2, *Candidatus* Liberibacter asiasticus from Brazil; Lane 3, *Candidatus* Liberibacter africanus; Lane 4, *Candidatus* Liberibacter americanus.

**Table 3.** A few representative studies with no sequencing information presented in the cross-species/genome studies

| Serial No. | Species name | Cross-species/genome studies |
|---|---|---|
| 1 | *Aphis fabae* | Gauffre and Coeur d'acier (2006) |
| 2 | *Asparagus racemosus* | Ginwall *et al*. (2011) |
| 3 | *Blattella germanica* | Booth *et al*. (2007) |
| 4 | Citrus | Dong *et al*. (2006) |
| 5 | *Jatropha curcas* | Mittal and Dubey (2010) |
| 6 | Rice, Maize, Soybean, Wheat | Guo *et al*. (2003); Bandopadhyay *et al*. (2004) |
| 7 | *Microtus arvalis* | Walser and Heckel (2008) |
| 8 | *Liriodendron chinense* | Yao *et al*. (2008) |
| 9 | *Locusta migratoria* | Zhang *et al*. (2003) |
| 10 | *Pogona vitticeps* | Quinn *et al*. (2010) |
| 11 | *Rhizobia* | Ya-mei *et al*. (2008) |
| 12 | *Ruditapes variegate* | An *et al*. (2009) |
| 13 | *Casuarina* spp. | Yasodha *et al*. (2005) |
| 14 | *Suta* spp. | Stapley *et al*. (2005) |
| 15 | *Elaeocarpus* spp. | Jones *et al*. (2002) |

2007; Das 2009). Because these conserved regions are homologous to sequences of the host and/or citrus associated endophytes, reliable and specific detection and diagnosis of HLB is of concern (Lin *et al*. 2008). Thus, the candidate gene markers developed in this study, across the wide genome of *Ca.* Liberibacter asiaticus, will essentially benefit the citrus industry by providing simple, sensitive and rapid detection method for large-scale detection/management of HLB under field, nursery, bud-wood certification and epidemiological conditions. It will also be useful for the identification of *Ca.* Liberibacter asiaticus where more than one species of HLB pathogen is present.

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Capoor SP, Rao DG and Viswanath SM 1967 *Diaphorina citri* Kuway., a vector of the Huanglongbing disease of citrus in India. *Indian J. Agric. Sci.* **37** 572–576

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