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ASIAN CITRUS PSYLLID (*DIAPHORINA CITRI* KUWAYAMA) REARING FOR TRANSMISSION OF *CANDIDATUS* LIBERIBACTER ASIATICUS IN CITRUS FOR THE MANAGEMENT STRATEGIES OF HUANGLONGBING

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ABSTRACT

Huanglongbing (HLB) is causing destruction in citrus all over the world. Control of this disease is mendatory to save the citrus industry. Asian citrus psyllid (ACP), the natural vector of HLB causing bacterium is present in abundance in citrus orchards of Punjab, pakistan. Rearing of ACP was conducted in this study for the management strategies of HLB. Citrus orchards of Faisalabad district of Punjab, Pakistan were surveyed for HLB diagnosis and acquisition of ACP from HLB positive citrus trees for ACP rearing in controlled conditions of growth room. ACP was successfully reared at 26±2 ^{O}C temperature and photoperiod of 13:11 (Light : Dark) and *Candidatus* Liberibacter asiaticus was detected by conventional PCR in the plants used for ACP rearing. PCR results confirmed the presence of *Candidatus* Liberibacter asiaticus with discrete bands having amplicon size of 1160bp, 800bp and 700bp for primer pairs OI1/OI2c, OI2/23S1 and A2/J5, respectively.

Keywords: Amplicon; blotchy mottle; citrus greening; Gram negative; symptoms

INTRODUCTION

Huanglongbing (HLB), also called citrus greening, is a fatal illness of citrus. About 100 million citrus trees have been destroyed in African and Asian countries due to HLB (Gottwald *et al*., 2007; Nageswara-Roa *et al*., 2013). More than 8000 persons associated with citrus industry lost their jobs in Florida (Chin *et al.,* 2014; Aslam *et al.,* 2017). The level of starch increases upto six times in HLB affected leaves than healthy ones (Taba *et al*., 2006; Etxeberria *et al*., 2007). Symptoms of HLB on leaves are similar to zinc deficiency (Timmer *et al*., 2003). Blotchy mottle, vein yellowing, leaf curling and prominent vein corking symptoms can be seen on different genotypes of citrus. All symptomatic leaves ultimately defoliate after few weeks to month (Zhang *et al*., 2012).

HLB is transmitted by jumping lice called citrus psyllid. There are two types of psyllid vectors confirmed for huanglongbing. One of the two psyllid vectors is *Diaphorina citri* Kuwayama (Hemiptera: Sternorryncha: Lividae) and the other is *Trioza erytreae* (del Guercio) (Hemiptera: Sternorrynca: Triozidae) (Aubert, 1987). *Candidatus* Liberibacter, a Gram negative, non culturable, phloem resident bacterium is the causal pathogen of HLB (Lopes *et al*., 2007). It has cell wall of 25 nm thickness. Three species of this bacterium have found to be the cause of HLB. One is *Ca.* L. asiaticus found in Asia and Americas, second is *Ca.* L. africanus found in Africa (Da Graca, 1991; Koizumi, 1995) and third is *Candidatus* Liberibacter americanus found only in America. *Candidatus* Liberibacter asiaticus can tolerate heat and express symptoms in cool as well as warm (22 to 35°C) ambience while *Candidatus* Liberibacter africanus is sensitive for heat and do not show symptoms above 25°C (Bove, 2006; Li *et al*., 2009). *Diaphorina citri* also called Asian Citrus Psyllid (ACP) can transmit Liberibacter asiaticus (Las) and Liberibacter americanus (Lam) in citrus and citrus relatives while, *Trioza erytreae* is the carrier and transmitter of *Candidatus* Liberibacter africanus (Laf) only (Bove, 2006; Lin *et al*., 2015). Haemolymph of ACP and other organs, like midgut, salivary glands, ovaries, malpighian tubules and muscle tissues contain great amount of Las (Ammar *et al*., 2011).

Adult psyllids are 2.7 to 3.3 mm long. The color of wings is brown with three different colors of abdomen. The ratio of

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male and female psyllids is probably equal. Adults can be seen on citrus leaves or branches sitting at the angle of 45^{O} (Wenninger *et al*., 2009). Records of average life of adult males and females are found to be variable ranging from 32 to 51 days at 24°C to 30°C for males and 90 days at 27°C for virgin females on suitable host plants (Richardson and Hall, 2013). In the presence of new leaves, females lay eggs regularly in their life. A large number of eggs may be seen on a single new flush. Generally 500-800 eggs are laid in two months reaching at maximum number of 1900 eggs. Freshly laid eggs are light yellow and oval. At maturity, egg color turns orange having two red spots for eyes. ACP has five stages of nymph or instars (Husain and Nath, 1927). Temperatures between 16 and 41.6 °C are good for oviposition but 29.6 °C is ideal (Hall *et al*., 2012). It has been known that Las carrying female psyllids are not responsible for the dissemination of Las through their eggs because eggs and first and second instars were found to be free of Las (Hung *et al*., 2004). The Las transmission investigation between male and female *D. citri* reveal that male insects transmit Las sexually to females only. A 7 days latent period is required for detection of Las in recipient female. It was observed that Las can spread within *D.citri* population horizontally without presence of infected plants (Mann *et al*., 2011).

Electrical penetration graphing (EPG) studies to determine the feeding behaviour and transmission of Liberibacter by *D. citri,* reveal that ACP acquire *Candidatus* Liberibacter asiaticus 54% from young leaves, whereas only 10% from older leaves. Nymphs acquire and transmit Las more efficiently as compare to adults (McClean and Kinsey, 1964; Tjallingii, 1988). The acquisition efficiency and feeding behaviour of *D. citri* is changed by stage of citrus leaf development. Young leaves are preferred by all age group of insects. There are higher chances of acquisition of bacteria when *D. citri* complete most of its life span (from nymph to adult) on diseased branch as compare to those that ingest sap from diseased branch when become adult (Lopes *et al*., 2009). Trees infected with HLB bacterium look more attractive to adult ACP before sucking their sap. After feeding on infected trees, ACP is attracted towards healthy trees causing the dissemination of HLB bacterium. Adults need 1-25 days after acquiring Las before transmitting it to healthy trees but the adult infected with Las at nymph stage can transmit Las immediately after becoming adult (Mann *et al*., 2012; Hall *et al*., 2012).

D.citri is a local inhabitant of Asia. It was familiar in Brazil for many dacades. During 1990s it has grown in the countries of northern South America and the Caribbean. It was discovered in south Florida and Gaudeloupe in 1998 and since then it is spreading quickly in the Caribbean basin (Halbert and Nunez, 2004). Population dynamic studies of ACP in the Punjab province of Pakistan, reveal ACP population to be highest in spring (Ahmad *et al*., 2004; Razi *et al.,* 2014). Untill 2004, no report of ACP presence heared in Oman. On Mexican lime ACP was found to be present at Barka in 2005. Since then, ACP presence was recorded in most of the citrus groves of Oman especially three areas including Barka, Al-Rustaq and Masirat Al Rawajeh. HLB has been detected in the Saudi Arabia (Al-Zadjali *et al*., 2008). Australia is still free from HLB pathogen and ACP but there are strong chances of spread of HLB because of its diagnosis in the neighbours of Australia including New Guinea, Indonesia and Timor Leste (Finlay *et al*., 2009). The ACP was discovered in Brazil in 1940s, then in Florida in late 1990 and now it has been detected in a lot of citrus growing states of America, Mexico, Belize, Costa Rica and California (Grafton *et al*., 2013). More than eighty genotypes of citrus have been studied for the colonization of ACP, only *Poncirus* and some of its hybrids have been found least favourite for ACP (Westbrook *et al*., 2011; Albrecht and Bowman, 2012; Richardson and Hall, 2013).

In Pakistan, suspected presence of HLB has been reported in many publications on the basis of visual symptoms (Abbas *et al*., 2005). In Punjab, HLB was first time diagnosed in Faisalabad district in 2011 at the University of Agriculture Faisalabad, Pakistan by polymerase chain reaction for the management of this disease (Yaqub *et al.,* 2017). Monitoring of ACP is considered as an essential part of management program aimed at minimizing the occurrence and propagation of HLB. In Pakistan, HLB has been ignored unintentionally. Due to ignorance, uprooting of citrus trees has been a major practice in Pakistan without knowing the actual cause of decline. In these situations, a detailed study of HLB and its natural vector was truly needed for HLB management strategies.

The main objectives of current study were ACP rearing in controlled conditions of growthroom for HLB bacterium transmission in citrus plants and molecular detection of *Candidatus* Liberibacter asiaticus in those ACP infested plants so that the inoculated plant material could be used for HLB management practices.

MATERIALS AND METHODS

Survey of citrus orchards for HLB diagnosis and ACP collection

Different citrus orchards of district Faisalabad were surveyed for the presence of ACP and HLB diagnosis. A sweet orange orchard with a huge population of ACP nymphs and adults with numerous symptoms of HLB on leaves was selected to achieve the target (Figure 1). Symptomatic leaves for HLB diagnosis were collected from that sweet orange field and subjected to molecular studies for the detection of HLB bacterium *Candidatus* Liberibacter (Figure 2). Positivity of the field sweet orange plants and ACP infested plants in the growth room was confirmed by standard PCR using 16S ribosomal RNA gene primer pair OI1/OI2c (Jagoueix *et al*., 1996), *rplKAJL-rpoBC* operon primer pair A2/J5 (Hocquellet *et al*.,1999; Chohan *et al*., 2007) for *Candidatus* Liberibacter asiaticus and 16S/23S rDNA intergenic region (Jagoueix *et al*., 1997). Sequences of primers are given in table.

Plant's DNA extraction and conventional PCR: The DNA was isolated from leaf midribs and petioles of sweet orange plants as described by Yaqub *et al*. (2017) using cetyl trimethyl ammonium bromide method (2% CTAB, 1% Lauroyl sarcosine, 100 m*M* Tris HCl, 1.4 m*M* NaCl and 20 m*M* EDTA). A total volume of 25 µL was used in the PCR reaction mix. Amplification was carried out in a peqSTAR 96 universal gradient thermocycler with the following thermal profile: one cycle for initial denaturation at 94°C for 2 min; 35 cycles at 94°C for 30 sec, 58°C for 1 min and 72°C for 1 min; one cycle for final extension at 72°C for 10 min. The PCR

products were analyzed by gel electrophoresis using 1% agarose in 0.5X TBE buffer.

Asian citrus psyllid rearing

For HLB management practices in citrus germplasm, it was necessary to keep the germplasm free of any other graft transmissible disease except huanglongbing. For this purpose, ACP was reared in the growth room situated in a laboratory at the Center of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture Faisalabad (UAF), Pakistan, according to a modified protocol from Nava *et al*. (2007). Screenhouse raised healthy plants of sweet orange (*Citrus sinensis*) and kinnow (*Citrus reticulata* Blanco) were acclimatized in this growth room for ACP rearing. Pruning of those plants was done before starting the rearing experiment for the growth of new flushes and equal height of all plants.

After the detection of HLB pathogen in sweet orange field tree samples, ACP was collected from those trees and released on sweet orange and kinnow plants for colonization in growth room (Figure 3) at 26±2 ^{O}C temperature and photoperiod of 13:11 (Light : Dark). ACP was released on those plants fortnightly upto one year. Macro and micro nutrients were provided to the plants in the growth room raised for ACP rearing for better growth and sufficient attractive food for adult and nymph ACP.

RESULTS AND DISCUSSION

Asian citrus psyllid production in growth room

Pruning of sweet orange and kinnow plants in the growth room for ACP rearing induced the growth of new flushes. The same work has been reported by Hall and Albano (2014) with different genotypes of citrus in a way that the selection of a rearing plant for ACP may be greatly influenced by the flushing properties of a plant species, especially if the goal is to produce large numbers of ACP. This is because ACP is dependent on flush for reproduction. As the temperature of the growth room was optimum for the growth of plants and ACP, very soon after the release, we were able to see the colonies of nymphs on the new flushes of sweet orange and kinnow varieties. According to Liu and Tsai (2000), the optimum range of temperatures for the growth of ACP population was recorded as 25-28^{O}C. The white waxy excretions called honeydew of the nymphs are the evidence of the presence of *D. citri* (Figure 4A & B; Figure 6A). The adult ACP can also be seen in the figures on citrus leaves or branches sitting at an angle of 45^{O} as mentioned by some other researchers (Bove, 2006; Wenninger *et al*., 2009).

As far as the expression of HLB symptoms in the infested plants of sweet orange and kinnow are concerned, typical symptoms of HLB in leaves expressed in both types. New flushes on both varieties arose with twisted leaves. Very clear blotchy mottled leaves as well as vein yellowing symptoms were also expressed on the infested plants (Figure 5A & B; Figure 6B).

For ACP rearing, Murraya exotica (Skelley and Hoy, 2004; Hall *et al*., 2007) and Citrus macrophylla (Hall and Richardson, 2013) has been used. Westbrook *et al*. (2011) have studied eighty seven genotypes of citrus and its relatives in field for colonization of ACP.

Table

The primer sequences for HLB diagnosis by conventional PCR studies

Primer	Sequences	Target DNA	Orientation	Region of amplification	Comments
OI1	GCGCGTATGCAA TACGAGCGGCA	Las	Forward	16s ribosomal RNA	Primer described by Jagoueix *et al*., 1996
OI2c	GCCTCGCGACTT CGCAACCCAT	Las	Reverse	16s ribosomal RNA	Primer described by Jagoueix *et al*., 1996
A2	TATAAAGGTTGA CCTTTCGAGTTT	Las	Forward	rplKAJL-rpoBC(β operon)	Primer described by Hocquellet *et al*.,1999
J5	ACAAAAGCAGAA ATAGCACGAACA A	Las	Reverse	rplKAJL-rpoBC(β operon)	Primer described by Hocquellet *et al*.,1999
OI2	5'-ATGGGTTGCGA AGTCGCGAGGC-3'	Las	Forward	16S/23S rDNA intergenic region	Primer described by Jagoueix *et al*., 1997
23S1	5'-CGCCCTTCTCT CGCGCTTGA-3'	Las	Reverse	16S/23S rDNA intergenic region	Primer described by Jagoueix *et al*., 1997

Fig. 1
Sweet orange plants bearing fruit in field with a huge population of ACP adults.

Fig. 2
Sweet orange leaf samples with HLB symptoms from field trees for molecular detection of *Candidatus* Liberibacter asiaticus. **A, C & D;** Mottling and yellow vein symptoms simultaneously, **B;** Vein yellowing.

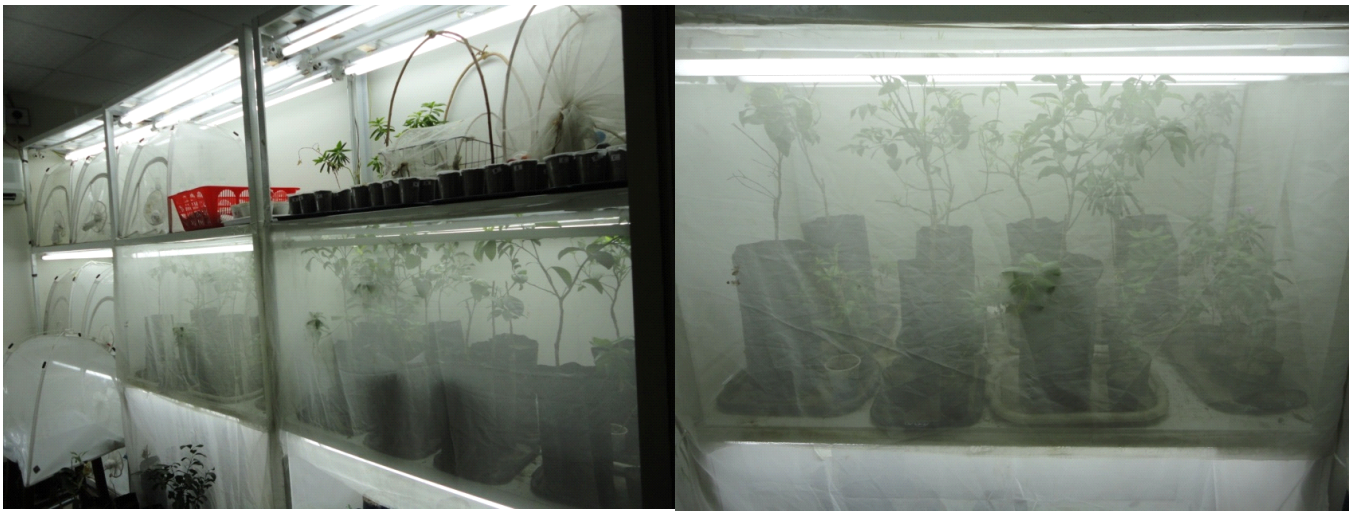


Fig. 3.
Sweet orange and kinnow plants in growth room. Racks in the growth room of CABB, UAF, Pakistan containing plants for the release and rearing of ACP. A: Sweet orange and kinnow plants. B: Kinnow plant for ACP rearing.

Fig. 4
Colonies of ACP on Sweet orange and Kinnow plants in growthroom conditions. Pictures showing colonies of ACP. **A**: ACP adults sitting on leaf and branch at an angle of 45 degree highlighted in red circle and arrow while nymplh colonies along with exudates in red diagonal. **B:** ACP nymphs and adult highlighted in red circles and red arrow showing exudate of nymphs and adult sitting at an angle of 45 degree.

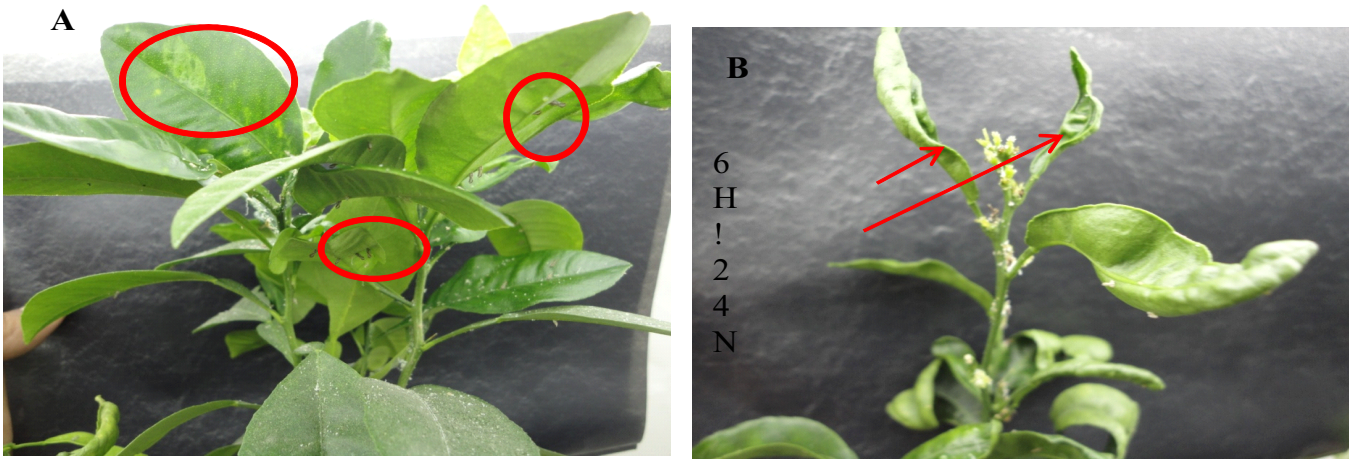


Fig. 5

HLB symptom expression after infestation by ACP: **A,** Appearance of blotchy mottle and vein yellowing symptoms in sweet orange. **B,** leaf curling sumptoms wth yellow veins in kinnow plants after acquisition of HLB bacterium from natural vector in growthroom conditions

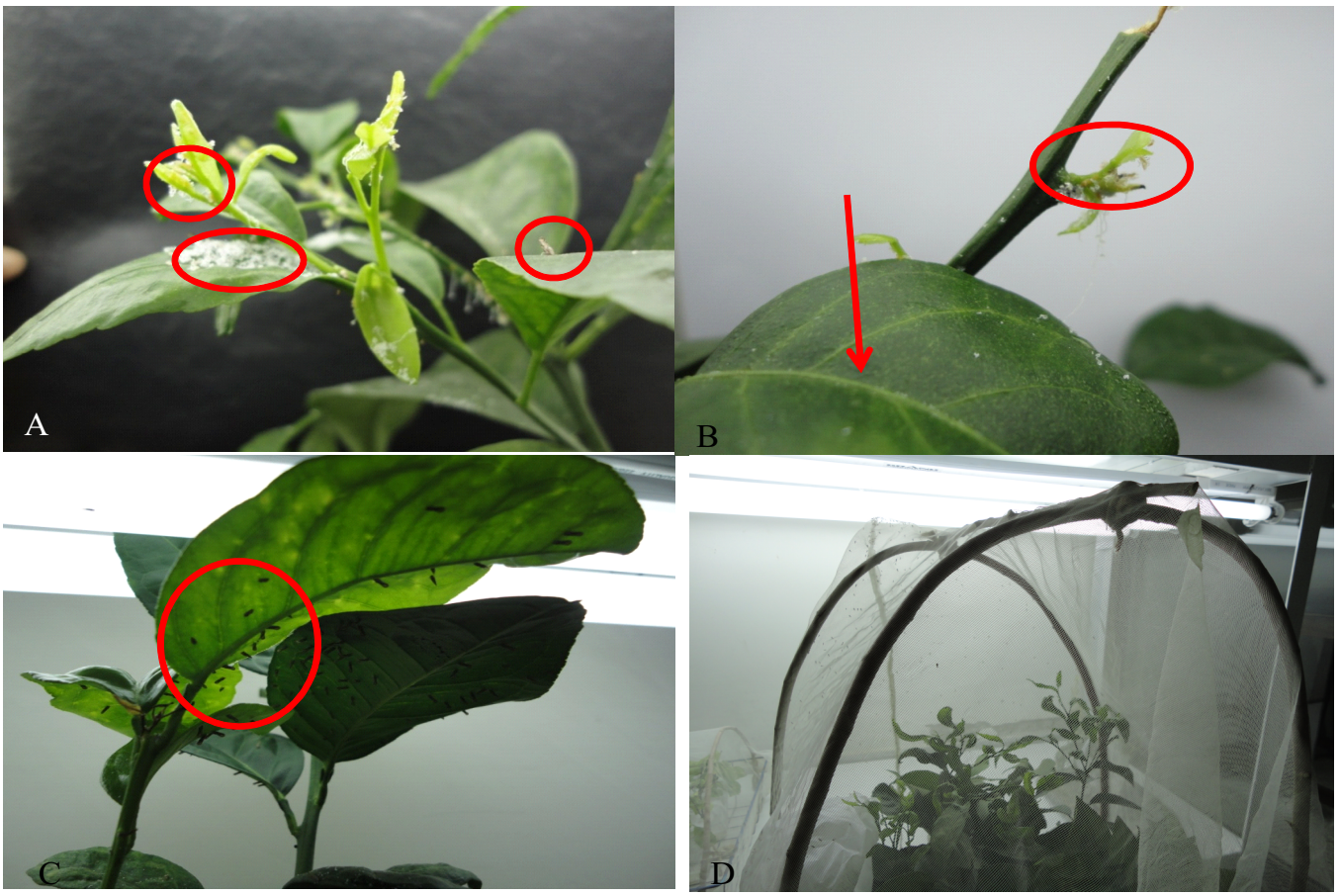


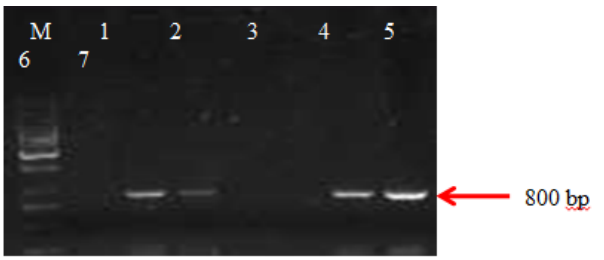
Fig. 6

Successful rearing of ACP resulting in establishment of ACP colonies and expression of HLB symptoms on the source plants.

Successful rearing of ACP in growth room,CABB,UAF, Pakistan. A: ACP nymph and its exudate on leaves at left side and adult on right side. B: Vein yellowing symptom on sweet orange leaf with ACP nymps on new flushes. C:A large no. of adult ACP on sweet orange leaves. D: ACP adults and nympls in cage having sweet orange and kinnow plants for ACP rearing.

Huanglongbing diagnosis in ACP infested plants

The sweet orange and kinnow plants were used for the colonization and infestation by ACP in controlled conditions of growth room. As the insect vector of HLB pathogen was captured from HLB positive plants and released on the growth room plants for one year, those plants were kept under observation for HLB symptom appearance. Blotchy mottle and vein yellowing symptoms along with twisted small sized leaves were found to be present in almost all of the infested pants (Figure 5A & B; Figure 6B). After one year of infestation, they were tested for the presence of HLB pathogen by conventional PCR. The primer pairs OI1/OI2c and A2/J5 were used for the amplification of pathogen's 16S ribosomal RNA gene and *rplKAJL-rpoBC* beta operon respectively. A product of 1160 nucleotides (Figure 8) and ~700 nucleotides (Figure 9) was observed for OI1/ OI2c and A2/J5 primer pairs respectively on agarose gel as also reported by other researchers (Ruanguang and Akarapisan, 2006; Aslam *et al*., 2017). For the amplification of 16S/23S rDNA intergenic region, primer pair OI2/23S1 was used, resulting in an amplicon of about 800 bp (Figure 7) indicating the presence of *Candidatus* Liberibacter asiaticus in the sweet orange and kinnow plants infested by ACP as reported by the founder of the primer OI2/23S1 (Jaguoeix *et al*., 1997) and other researchers (Subandiyah *et al*., 2000; Ding *et al*., 2009).



16S&23S IR primers showing 800bp amplicon specific for Las. M=1Kb ladder, 1= NTC, 2=Positive control, 3 to7 = HLB positive samples

Fig. 7
Amplification of16S & 23S intergenic region showing 800bp amplicon specific for *Candidatus* Liberibacter asiaticus

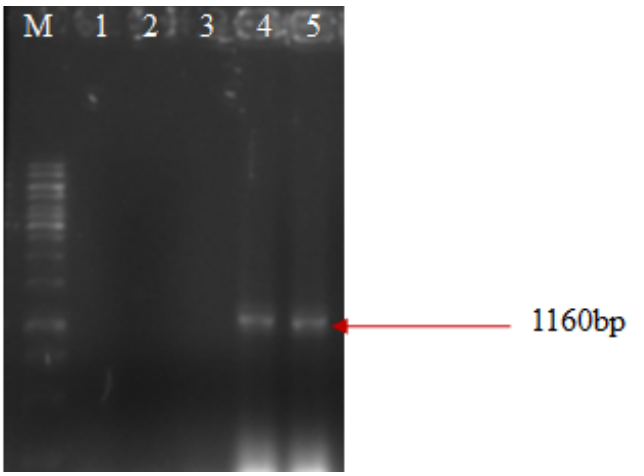


Fig. 8
Candidatus Liberibacter asiaticus detection in sweet orange plants infested by ACP. Amplicons of 1160 bp obtained in plants 4&5 by using16S rDNA primer OI1/OI2C. M1 = 1Kb DNA ladder, lane 1= no template control, 2&3= healthy controls, 4&5 HLB infected samples

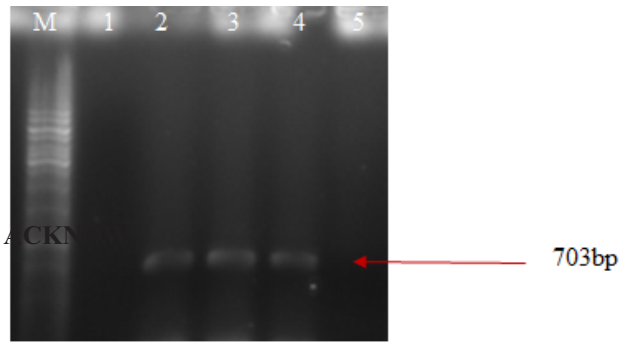


Fig. 9
Candidatus Liberibacter asiaticus detection in sweet orange plants infested by ACP. Amplicons of 703 bp obtained in plants 2-4 by using *Las* specific primer A2/J5. M=1Kb ladder, 1=healthy control, 2= positive control,3, 4 &5= inoculated plants

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Authors' contributions

MSY, IAK and RA designed the study and wrote the manuscript with input from all authors. MDG and MAA analyzed the data. SA and KT gave their suggestions for improvement in the manuscript. All authors read and approved the final manuscript.

REFERENCES

Aslam, R., I. A. Khan, K. U. Rahman, M. Asghar, 2017. Detection of '*Candidatus* Liberibacter asiaticus' the causal organism of huanglongbing, in mandarin group of citrus. Int. J. Agri. Biol., 19: 255–258.

Abbas, M., M. M. Khan, S. M. Mughal and I. A. Khan, 2005. Prospects of classical cross protection technique against Citrus tristeza closterovirus in Pakistan. Hortic. Sci., (Prague) 32(2): 74-83.

Ahmed , S., N. Ahmad and R. R. khan, 2004. Studies on population dynamics and chemical control of citrus psylla, *Diaphorina citri.* Int. J. Agri. Biol., 6(6): 970-973.

Albrecht, U. and K. D. Bowman, 2012. Transcriptional response of susceptible and tolerant citrus to infection with *Candidatus* Liberibacter asiaticus. Plant Sci., 185-186:118-130.

Al-Zadjali, T. S., R. Ibrahim and A. K. Ai-Rawahi, 2008. First record of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) from the sultanate of Oman. Insecta Mundi, 0039: 1-3.

Ammar, E. D., G. Robert, J. Shatters and D. G. Hall, 2011. Localization of *Candidatus* Liberibacter asiaticus, associated with citrus huanglongbing disease, in its psyllid vector using fluorescence in situ hybridization. Phytopathol., doi: 10.1111/j.1439-0434.2011.01836.x

Aubert, B., 1987. Trioza erytreae Del Guercio and Diaphorina citri Kuwayama (Homoptera: Psylloidea), the two vectors of citrus greening disease: biological aspects and possible control strategies. Fruits, 42: 149-162.

Bove, J. M., 2006. Huanglongbing: a destructive, newly emerging, century old disease of citrus. J. plant pathol., 88 (1): 7-37.

Chin, E., D. O. Mishchuk, J. Bruce, M. Cilia, G. Coaker, C. Davis, H. Jin, W. Ma, G. Sellar, C. LeVesque, K. Godfrey and C. M. Slupsky, 2014. An interdisciplinary approach to combat HLB. CRB- funded research project report. Citrograph Magazine, P 28-34.

Chohan, S. N., R. Qamar, I. Sadiq, M. Azam, P. Holford and A. Beattie, 2007. Molecular evidence for the presence of huanglongbing in Pakistan. Aust. Plant Dis. Not., 2: 37-38.

Da Graca, J. V., 1991. Citrus greening disease. Ann. Rev. Phytopathol., 29:109–36.

Ding, F., X. Deng, N. Hong, Y. Zhong, G. Wang and G. Yi, 2009. Phylogenetic analysis of the citrus Huanglongbing (HLB) bacterium based on the sequences of 16S rDNA and 16S/23S rDNA intergenic regions among isolates in China. Eur. J. Plant Pathol., 124: 495-503.

Etxeberria, E., P. Gonzalez, D. Achor, and G. Albrigo, 2009. Anatomical distribution of abnormally high levels of starch in HLB-infected Valencia orange trees. Physio. and Mol. Plant Pathol., 74: 76–83.

Finlay, K. J., A. L. Yen, J. P. Aurambout, G. A. C. Beattie, P. Barkley and J. E. Luck, 2009. Consequences for Australian biodiversity with establishment of the Asiatic Citrus Psyllid, *Diaphorina citri*, under present and future climates. Biodiversity, 10(2&3): 25-32.

Gottwald, T. R., J. V. da Graca and R. B. Bassanezi, 2007. Citrus Huanglongbing: The pathogen and its impact. Online. Plant Health Progress doi: 10.1094/PHP- 2007-906-01- RV.

Grafton-Cardwell, E. E., L. L. Stelinski and P. A. Stansly, 2013. Biology and management of asian citrus psyllid, vector of the huanglongbing pathogen. Ann. Rev. Entomol., 58: 413-432.

Halbert, S. E., and C. A. Nunez, 2004. Distribution of the asian citrus psyllid, *Diaphorina citri* Kuwayama (Rhynchota: Psyllidae) in the Caribbean basin. Florida Entomol., 87(3): 401-402.

Hall, D. G., and J. P. Albano, 2014. An evaluation of different plant species for rearing asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera Psyllidae). J. Citrus Pathol., 1: 139-141.

Hall, D. G., and M. L. Richardson, 2013. Toxicity of insecticidal soaps to the Asian citrus psyllid (*Diaphorina citri*) and two of its natural enemies. J. Applied Entomol., 137: 347-354.

Hall, D. G., M. L. Richardson, E. Ammar and S. E. Halbert, 2012. Asian citrus psyllid, *Diaphorina citri*, vector of citrus huanglongbing disease. Entomologia Experimentalis et Applicata.146: 207–223.

Hall, D. G., S. L. Lapointe, and E. J. Wenninger, 2007. Effects of a particle film on biology and behaviour of *Diaphorina citri* (Hemiptera: Psyllidae) and its infestations in citrus. J. Econ. Entomol., 100: 847-854.

Hocquellet, A., P. Toorawa, J. M. Bove and M. Garnier, 1999. Detection and identification of two *candidatus* liberobacter species associated citrus huanglongbing by PCR amplification of ribosomal protein genes of the beta operon. Mol. Cell. Probes., 13: 373-379.

Hung, T. H., S. C. Hung, C. N. Chen, M. H. Hsu and H. J. Su, 2004. Detection by PCR of *Candidatus* Liberibacter asiaticus, the bacterium using citrus huanglongbing in vector psyllids: application to the study of vector-pathogen relationships. Plant Pathol., 53: 96-102.

Hussain, M. A., and D. Nath, 1927. The citrus psylla (*Diaphorina citri* Kuwayama) [Psyllidae:Homoptera]. Memories of the Department of Agriculture in India, Entomological Series 10: ID 27.

Jagoueix, S., J. M. Bove and M. Garnier, 1996. PCR detection of two *Candidatus* Liberibacter species associated with greening disease of citrus. Mol. Cell. Probes, 10: 43-50.

Jagoueix, S., J. M. Bove and M. Garnier, 1997. Comparison of the 16S/23S ribosomal intergenic regions of *Candidatus* Liberibacter asiaticus and *Candidatus* Liberibacter africanus, the two species associated with citrus huanglongbing (greening) disease. Int. J. Systematic Bacteriol., 20 (1): 224-227.

Koizumi, M., 1995. Virus or virus-like diseases of citrus in tropical and subtropical zones. JIRCAS International symposium Series, 3: 61-66.

Li, W., L. Levy and J. S. Hartung, 2009. Quantitative distribution of '*Candidatus* Liberibacter asiaticus' in citrus plants with citrus huanglongbing. Phytopathol., 99: 139-144.

Lin, H., G. Pietersen, C. Han, D. A. Read, B. Lou, G. Gupta, and E. L. Civeroloa, 2015. Complete genome sequence of "*Candidatus* Liberibacter africanus," a bacterium associated with citrus huanglongbing. Genome Announc, 3(4): e00733-15. doi:10.1128

Liu, Y. H., and J. H. Tsai, 2000. Effects of temperature on biolology and life table parameters of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae). Annals of Appl. Biol., 137(3): 201-206.

Lopes, S. A., C. F. Frare, P. T. Yamamoto, A. J. Ayers and J. C. Barbosa, 2007. Ineffectiveness of pruning to control citrus huanglongbing caused by *Candidatus* liberibacter americanus. Euro. J. Plant Pathol., 119: 463-468.

Lopes, S. A., E. Bertolini, G. F. Frare, E. C. Martins, N. A. Wulff, D. C. Teixeira, N. G. Fernandes and M. Cambra, 2009. Graft transmission efficiencies and multiplication

of *Candidatus* Liberibacter americanus and *Ca.* Liberibacter asiaticus in citrus plants. Phytopathol., 99: 301-306.

Mann, R. S., J. G. Ali, S. L. Hermann, S. Tiwari, K. S. Pelz-Stelinski *et al*., 2012. Induced release of a plant-defense volatile deceptively attracts insect vectors to plants infected with a bacterial pathogen. PLoS Pathogens 8: e1002610.

Mann, R. S., K. Pelz-Stelinski, S. L. Hermann, S. Tiwari and L. L. Stelinski, 2011. Sexual transmission of a plant pathogenic bacterium, Candidatus Liberibacter asiaticus, between conspecific insect vectors during mating. PLoS ONE 6(12): e29197.

McClean, D. L. and M. G. Kinsey, 1964. A technique for electronically recording aphid feeding and salivation. Nature, 202:1358-1359.

Nageswara-Rao, M., M. Irey, S. M. Garnsey and S. Gowda, 2013. Candidate gene markers for *Candidatus* Liberibacter asiaticus for detecting citrus greening disease. J. Biosci., 38(2): 229-237.

Nava, D. E., M. L. G. Torres, M. D. L. Rodrigues, J. M. S. Bento and J. R. P. Parra, 2007. Biology of *Diaphorina citri* (Hem., Psyllidae) on different hosts and at different temperatures. J. Appl. Entomol., 131: 709–715.

Razi, M. F., M. L. Keremane, C. Ramadugu, M. Roose, I. A. Khan and R. F. Lee, 2014. Detection of Citrus huanglongbing associated '*Candidatus* Liberibacter asiaticus' in Citrus and *Diaphorina citri* in Pakistan, seasonal variability and implications on disease management. Phytopathol., 104 (3): 257-268.

Richardson, M. L. and D. G. Hall, 2013. Resistance of *Poncirus* and *Citrus* × *Poncirus* Germplasm to the Asian Citrus Psyllid. Crop Sci., 53:183–188.

Ruangwong, O. and A. Akarapisan, 2006. Detection of *Candidatus* Liberibacter asiaticus causing Citrus Huanglongbing disease. J. Agric. Technol., 2(1): 111-120.

Skelley, L. H. and M. A. Hoy, 2004. A synchronous rearing method for the Asian citrus psyllid and its parasitoids in quarantine. Bio. Control, 29: 14-23.

Subandiyah, S., T. Iwanami, S. Tsuyumu and H. Ieki, 2000. Comparison of 16SrDNA and 16S/23S intergenic region sequence among citrus greening organisms in Asia. Plant Dis., 84: 15-18.

Taba, S., K. Nasu, K. Takaesu, A. Ooshiro and Z. I. Moromizato, 2006. Detection of citrus huanglongbing using an iodo starch reaction. 53: 19-23.

Timmer, L. W., S. M. Garnsey and P. Broadbent, 2003. Disease of Tropical Fruit Crops. Diseases of citrus. In: R. C. Ploetz (ed.). p163-195. CABI Publishing, USA.

Tjallingii, W. F., 1988. Electrical recording of stylet penetration activities. Pages 95-108. In: A. K. Minks & P. Harrewijn (eds.). Aphids, their Biology, Natural Enemies and Control. v. 2B. Elsevier, Amsterdam, The Netherlands.

Wenninger, E. J., D. G. Hall and R. W. Mankin, 2009. Vibrational communication between the sexes in *Diaphorina citri* (Hemiptera:Psyllidae). Annals Entomol. Soc. Amer., 102: 547–555.

Westbrook, C. J., D. G. Hall, E. Stover and Y. P. Duan, 2011. Colonization of citrus and citrus related germplasm by *Diaphorina citri* (Hemiptera: Psyllidae). Hortscience, 46 (7): 997-1005.

Yaqub, M. S., I. A. Khan, M. Usman and I. A. Rana, 2017. Molecular detection of *Candidatus* Liberibacter asiaticus, the causal organism of huanglongbing (citrus greening) in Faisalabad, Pakistan for huanglongbing management. Pak. J. Agric. Sci., 54: 21–26.

Zhang, M., C. A. Powell, Y. Guo, M. S. Doud and Y. Duan, 2012 . A graft-based chemotherapy method for screening effective molecules and rescuing huanglongbing-affected citrus plants. Phytopathol., 102 (6): 567-574.