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Decoding Huanglongbing: Understanding the Origins, Impacts, and Remedies for Citrus Greening

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Citrus cultivation is a vital component of the agricultural sector, offering significant nutritional and economic benefits. However, citrus plants face numerous disorders, including Huanglongbing (HLB), also known as citrus greening disease. HLB is a devastating disease caused by the bacterial species *Candidatus Liberibacter asiaticus* (Las), primarily transmitted by the citrus psyllid and grafting. Investigating the distribution and molecular characterization of HLB in various citrus varieties and psyllid populations is crucial. Molecular techniques and iodine starch staining have been used to detect HLB symptoms. Several studies have been designed to determine the incidence and severity of HLB symptoms in different citrus varieties and locations and estimate the psyllid population density in different citrus varieties. Furthermore, these studies established a correlation between the population of psyllids and the incidence and severity of HLB in different locations and these findings suggest a potential link between psyllid infestation and the prevalence of HLB. This study highlights the prevalence of HLB in various locations and varieties, providing insights into the distribution, severity, and correlation between psyllid populations and HLB incidence. The reliability of PCR testing for Las infection confirms its effectiveness. Such findings suggest implementing integrated pest management strategies, including monitoring and controlling psyllid populations, removing infected plants, and using certified disease-free planting materials to prevent HLB spread.
Keywords: Citrus, HLB, Greening, *Candidatus Liberibacter asiaticus* (Las), Transmission and Managements.

INTRODUCTION

Citrus is considered a significant fruit crop on a global scale, cultivated in over 125 countries within the latitudinal of 35°-36°C and under suitable climatic conditions, with temperatures ranging from 4ºC-50ºC (Naz *et al.*, 2014). The botanical classification of citrus places it within the *Rutaceae* family, a taxonomic group consisting of approximately 158 genera and 1900 distinct species (Bhan *et al.*, 2016). Citrus species are mainly diploid, with a chromosome number of 2n = 2x = 18 (Vijayakumari *et al.*, 2022). The first cultivation of citrus fruit on the planet can be traced back to approximately 30 million years ago. The history of citrus cultivation is believed to date back to Nippur, an ancient city in Mesopotamia, dating back to 4000 B.C. The domestication of citrus dates back to ancient times in Southeast Asia, and its cultivation has subsequently spread to diverse regions worldwide. The taxonomic classification of citrus comprises a diverse array of species, like Mandarins, Oranges, etc. The fruit often referred to as oranges is sometimes referred to as "sweet orange," which is a prevalent and extensively cultivated variety of citrus. Citrus ranks 13th in Pakistan's

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agricultural production, covering an area of 0.193 million hectares and yielding a total of 2.396 million tons (Mubeen *et al.*, 2015a; Fateh, *et al.*, 2017; Naqvi *et al.*, 2017; Naqvi *et al.*, 2022). Pakistan is a significant contributor to the global production of Kinnow mandarins and oranges, with a share of 95% in the Kinnow production worldwide (Naz *et al.*, 2014). In Pakistan, the yield of citrus per acre is lower than in most citrus-growing countries (Mubeen *et al.*, 2015b; Mahmood *et al.*, 2006). Citrus greening Huanglongbing (HLB) has been linked to three uncultured "*Candidatus liberibacter* species" discovered in the phloem: "*Candidatus Liberibacter asiaticus*" (Las), "*Candidatus Liberibacter africanus*," and "*Candidatus Liberibacter americanus*" (Sajid *et al.*, 2021). The most dangerous and widespread kind of "*Ca. Liberibacter*" is called "Las." HLB first became known in Southeast Asia more than 100 years ago, and it ultimately was found in Florida in the USA in 2005. The disease has quickly spread across the world's commercial citrus-producing regions (Kumagai *et al.*, 2013). Symptoms of the citrus greening disease can occur throughout the entire tree and these include yellowing of leaves with a blotchy mottling pattern that is not consistent across the leaf, as well as small, upright leaves with thickened midribs and veins. As the disease progresses, leaves drop, shoots become stunted, and branches gradually die. Other signs of citrus greening include out-of-season flowering and fruiting, small and lopsided fruit with dark, undersized seeds, and excessive fruit drop. Initially, the citrus greening disease was believed to be linked to mineral deficiency and waterlogging, as its symptoms included yellow shoots. However, in China, experiments conducted through grafting techniques confirmed that a pathogen causes the disease. Similarly, African greening was also shown to be graft-transmissible by McClean and Oberholzer, (1965). The citrus greening disease is primarily spread through grafting and insect vectors, with the two species of citrus psyllids, *Diaphorina citri* and Trioza erytreae, known to transmit the disease. *Diaphorina citri*, also called the Asian citrus psyllid, is responsible for spreading the disease in both Asia and the Americas. It's important to note that the plant part and amount of tissue used for grafting can have a significant impact on the spread of the disease. August and mid-March to April were the two times when the population of citrus psylla reached its peak (Iftikhar *et al.*, 2017). Additionally, the disease is not seed-transmissible, as infected plants tend to have a high number of aborted seeds. The climate and type of leaves influence the acquisition of the pathogen by the vector. During winter, feeding on old leaves is observed to be ideal for pathogen acquisition, while in spring, a young flush is preferred. The psyllids are more attracted to diseased plants due to the yellow-green color of the symptomatic leaves, which increases the likelihood of pathogen acquisition and transmission by the insect. Assessing losses caused by greening is a challenging task. Only certain parts of a tree may be affected, resulting in minor losses. However, in other cases, the entire tree may be infected, leading to a complete loss of the crop. While there are no detailed studies on the losses caused by greening, the literature substantiates the severity of the disease. In the Philippines, greening affected an estimated 7 million trees in 1962 and was responsible for a reduction of over 60% in the area planted to citrus between 1961 and 1970 (Catling, 1970). In 1971, it caused the death of more than 1 million trees in a single province. According to Paudyal (2015), the presence of HLB caused a 23% reduction in orange production between 2006 and 2010, which had a significant impact on Florida's economy. The total output, the total value added, and labor income all decreased, resulting in a loss of $3.9 billion. Symptom-based diagnosis is not practical and the popularity of the PCR technique has grown because of its ability to quickly and sensitively detect even trace amounts of pathogen DNA in both infected plant samples and insect vectors (Sajid *et al.*, 2022). PCR utilizes a fluorescent reporter that binds to the amplified product and emits fluorescence to indicate its presence. The intensity of the fluorescence is directly proportional to the quantity of the product that is formed. In areas where the disease is newly introduced, quarantine and eradication programs may be implemented to prevent its spread (Mei *et al.*, 2014). In established citrus-producing areas, integrated pest management strategies may be used to control the vector populations and limit the spread of the disease. These strategies may include the use of insecticides, biological control agents, and cultural practices such as the removal of infected trees and the use of disease-free planting material. The development of a protocol for treating citrus bud wood to make it free of HLB is crucial to prevent the spread of the disease through budding and grafting. Antibiotic treatment and temperature treatment are two effective methods that can be used for treating bud wood. In addition, identifying and utilizing citrus varieties that are tolerant to HLB can also be an effective management strategy. However, it is important to note that the development of resistant varieties is a long-term solution and requires significant investment in research and breeding programs. There is a mandarin cultivar, LB8-9, also known as Sugar Belle, which is gaining popularity due to its tolerance to a bacterial disease called “Huanglongbing” (Deng *et al.*, 2019).

***Citrus Greening*:** Srivastava *et al.*, 2009 stated that citrus is susceptible to various biotic and abiotic disorders, which can significantly impact its growth and yield. HLB, in particular, is a major threat to the global citrus industry. The disease has been stated to be associated with three species of parasitic gram-negative bacterial microorganisms, known as "*Candidatus Liberibacter* spp." ACPACP spreads these microorganisms spreads these microorganisms. The disease can be deadly for citrus plants and has become prevalent in the primary citrus-producing areas of Brazil and the USA. The symptoms of citrus greening can be recognized through the discoloration of leaves that display an irregular, blotchy, mottling pattern. Also, the leaves of affected citrus trees exhibit a thin and upright growth habit, with thicker veins and midribs. As the state of the plant worsens, there is a noticeable shedding of leaves, stunted growth of shoots, and a gradual withering of the branches. Additional symptoms include blooming and fruiting at odd times of the year, trim, unusually shaped fruits with tiny dark seeds, and considerable amounts of fruit falling off at inappropriate times. The Asian citrus psyllid feeds on the sap of citrus trees and, while doing so, can transmit the CLas bacterium to the tree. Once infected, the bacterium spreads throughout the tree, infecting the leaves, stems, and fruit. Wang *et al.* (2019), proposed that HLB and ACP are causing severe damage to fruit yield in different locations that produce fruit such as Caribbean countries, and the USA, with their rapid spread. HLB has been confirmed to be present in more than 50 of the 140 countries involved in citrus cultivation. The Mediterranean region and Australia are presently lacking HLB disease, however, they are confronted with a considerable threat of ACP infestation and subsequent dissemination of HLB. The potential introduction of Trioza erytreae into Spain poses a significant threat, as it could serve as a vector for disseminating HLB throughout the Mediterranean area. Consequently, HLB has emerged as a worldwide concern. Merfa *et al.* (2019), made a statement that in recent decades, the CLas, which includes ACP-vectored plant diseases, has become an adaptive and economically destructive danger to a wide range of significant plant hosts. A noteworthy instance is CLas, which is linked to the severe plant disease recognized as HLB in various citrus-producing areas worldwide. CLas is classified as α-proteobacterium and is predominantly transmitted among citrus species through the agency of the ACP *Diaphorina citri*. The initial detection of HLB occurred in Florida (USA) in 2005, after the introduction of ACP to the region in 1998. In three years, HLB spread to all citrus-cultivating areas in Florida, resulting in noteworthy financial setbacks for growers and substantial costs for taxpayers at both the state and national levels.

Figure 1.HLB-infected tree with symptoms of blotchy mottling and symptomatic Leaves

***Distribution*:** Alvarez *et al.* (2021) ajenen, conducted a study to discover an efficient and inexpensive approach for detecting HLB symptoms in citrus plants through the utilization of satellite images of Sentinel-2. A supervised classification approach was employed to differentiate between unaffected Persian lime trees and those suffering from HLB in the citrus-growing area of Tabasco, Mexico. The outcomes indicate that the spectral response of trees affected by HLB was greater than that of unaffected trees in the near-infrared red, and green spectral bands. Gao *et al.* (2022), stated that the disease, commonly referred to as HLB has spread throughout areas that cultivate citrus fruits, including Guangxi, Guangdong, and Fujian. Fourie *et al.* (2021) stated that HLB has yet to be detected in the southern African region. HLB is caused by a bacterium called CLas, which is spread via the ACP insect vector. Yaqub *et al.* (2017), conducted a study in Faisalabad, Pakistan during 2010-11 to identify the occurrence of HLB disease in citrus orchards. These findings verified that CLas was present in the samples. Zafarullah *et al.* (2021), conducted a comparative investigation to evaluate the effectiveness of traditional PCR and qPCR techniques, utilizing a universal 16S rDNA marker. Results suggested that utilization of a sensitive and robust qPCR assay can facilitate the screening and quantification of CLas, thereby confirming the occurrence of HLB infection in Pakistan. Ajene *et al.* (2020), conducted a study to identify the Liberibacter species that have an impact on citrus, the vectors linked to them in Eastern Africa, and their ecological distribution. Results envisaged that the potential geographical distribution in Eastern Africa encompasses significant citrus-producing areas within Ethiopia, Kenya, and Tanzania, encompassing regions where the disease has yet to be documented. Diksha, *et al.* (2023), took a study to investigate the association between CLas and various citrus cultivars from diverse locations in Western and North-Eastern India by examining the genetic diversity and interrelationships among isolates through the characterization of different genetic markers. The results of the phylogenetic analysis conducted on CLas isolates utilizing rplA-rplJ gene sequences revealed the presence of two distinct clusters. Sajid *et al.* (2021), conducted a study in the Sargodha district of Pakistan, focusing on two citrus cultivars, Kinnow and Musambi. They carried molecular techniques to confirm the presence of the greening pathogen through specific primer pairs, providing a standardized approach for future research and aiding in quick disease index. Khan *et al.* (2019), conducted a study in primary regions for citrus cultivation in KPK, Pakistan to assess HLB incidence. Tipu *et al.* (2020), aimed to determine the pathogen responsible for citrus greening in Bangladesh. The presence of the pathogen was confirmed through the amplification of specific amplicon size, and sequence analysis demonstrated a 99% identity match with *CLas* in the GenBank database, confirming its prevalence as the causal organism of citrus greening in Bangladesh. Costa *et al.*

(2021), conducted a study in the Paranavaí micro-region of Paraná, Brazil, to determine the prevalence and economic consequences of HLB. Data provided by the Paraná Agribusiness Defense Agency enabled the estimation of production losses caused by the disease. The outcomes revealed an annual HLB incidence below 2.5% in the region but with a significant increase from 0.16% to 0.96% during the considered period. Orchards with fewer than 10,000 plants exhibited higher disease incidence. The estimated economic impact of HLB was substantial, reaching US$11.8 million and US$39.2 million under different scenarios, highlighting the urgent need for HLB prevention and management.

Vector and Transmission: Das *et al.* (2022), conducted a study to identify the presence of *CLas* in *D. citri* vectors that occur naturally and to examine the genetic interrelationships and discusses the differences observed among the populations of ACP and CLas within the citrus regions situated in India. The results demonstrate that the tree topology of *D. citri* mtCOI sequences, which were collected from different locations in Indian states, exhibited a clear separation into four distinct clades. Leong *et al.* (2020), conducted a study to analyze the transmission of HLB disease, which is transmitted by *D. citri*, in connection to its spatial arrangement and flight behavior in reaction to flush cycles in a flourishing area. The results indicate that the Rebel brown-yellow traps were more effective than others in capturing adult *D. citri*. Zhang *et al.* (2022), conducted a study to explore acquiring diseases and subsequent distribution of HLB, caused mainly by the citrus psyllid, which is the greatest damaging citrus infection in the globe. These findings offer regulators a theoretical direction for determining the long-term durability of applied HLB intervention techniques. Ramsey *et al.* (2022), put up an argue that modifications in the ACP immune system and microbes during development play a crucial role in the regulation of vector ability. Das *et al.*, (2022), conducted a study to identify the presence of CLas and evaluate the genetic correlation and variations among populations of the ACP in citrus-producing areas of India. The results demonstrated that the mtCOI sequences of ACP showed an apparent division into four distinct clades, which suggests the presence of genetic diversity among different populations of ACP. Yaqub *et al.*, (2019), stated that the citrus industry is facing a significant global challenge known as HLB, which is particularly damaging to citrus orchards located in Punjab, Pakistan. The controlled rearing of Asian citrus psyllid is conducted to facilitate the development of management strategies aimed at combating HLB. A survey was conducted on citrus orchards located in the Faisalabad district to diagnose HLB. ACP was obtained from HLB-positive citrus trees and subsequently reared in a growth room under controlled conditions of a temperature of 26°C and a period of sunlight of 13 hours. The findings from the PCR analysis indicated the existence of CLas, as evidenced by distinct bands.

Molecular Characterization: Wulff *et al.* (2019), conducted a study to discover a new phytoplasma from the 16SrIII group that is related to HLB symptoms, emphasizing the importance of developing diagnostic tools to assess microbiomes associated with HLB. Hong *et al.* (2019), conducted a study to examine nested PCR primer combinations to identify Las. Cui *et al.* (2021), investigated the use of next-generation sequencing analysis to find two distinct prophages in the Pakistani CLas strain. Ghosh *et al.* (2021), conducted a study to detect the presence of CLas, commonly known as citrus greening, in all four primary citrus cultivation regions. Bhutan. Singh *et al.* (2021), show that HLB disease represents a significant biotic restriction to citrus cultivation on a larger scale, including in India. Nauman *et al.*(2021), claim that although HLB disease exhibits characteristic symptoms such as mosaic or mottling patterns on leaves, stunted plant growth, deformed shape, premature fruit drop, and yellowing of reticulate venation, the detection of the bacteria and downstream studies requires high-quality DNA, which is challenging to extract from citrus leaves due to the plant's various species, age groups, thick waxy cuticles, and high production of compounds like phenolics and polysaccharides. Shafiq *et al.* (2018), took a study molecularly characterize the mtco1 gene in ACP, and evaluate the incidence of CLas in different citrus cultivars in Pakistan. Zafarullah *et al.* (2016), have conducted a study to identify and characterize the HLB pathogen in commercially grown citrus varieties, specifically Kinnow and sweet oranges. The prevalence of *Candidatus* liberibacter asiaticus exhibited seasonal variation, with a decrease during the spring season characterized by conditions of temperature and humidity that are considered unfavorable for the bacterium. CLas is known to produce symptoms under relative humidity below 40% and high-temperature conditions, with temperatures up to 35°C.

Management: Boina *et al.* (2014), suggested that the sustainable solution to this disease through chemical control of Asian citrus psyllid is the need of time (Table 1). Represent the Insecticides, chemical group, IRAC classification and their mode of action used in citrus for controlling the ACP (Asian citrus psyllid). Hussain *et al.* (2022), stated that HLB is a notable disease that affects the Kinnow Mandarin, with significant economic effects. Several management strategies have been used to manage this disease. The effective decrease in this disease is dependent upon the effective administration of micronutrients, which serve a crucial function in enhancing the resilience of citrus plants to the disease. The characteristic indicators of citrus greening include the presence of discolored leaves on citrus plants that were affected by the disease. The fruits that were infected exhibited stunted growth and were unable to achieve the characteristic orange hue of mature fruit. The iodo-starch examinations indicated that the hue of leaves that tested positive for HLB was a shade of darker shade of gray or black when observed on the cut surface. Subsequently, a combination of Zinc sulfate and

Table 1. Insecticides, chemical group, IRAC classification, and their mode of action used in citrus for controlling the ACP (Asian citrus psyllid).

Insecticide	Chemical Group	IRAC classification[b]	Mode(s) of Action
Aldicarb, fenobucarb, methomyl and oxamyl	Organophosphate	1A	Aldicarb, fenobucarb, methomyl and oxamyl
Endosulfan	Cyclodiene organochlorines	2A	Antagonist of γ-aminobutyric-acid-gated chloride channels
α-Cyhalothrin, cypermethrin, fenpropathrin, λ-cyhalothrin and ζ-cypermethrin	Synthetic pyrethroid	3A	Sodium channel modulation
Acetamiprid, clothianidin, dinotefuran, imidacloprid and thiamethoxam	Neonicotinoids and sulfoximines	4A	Agonist of nicotinic acetylcholine (nACh) receptors
Sulfoxaflor		4C	
Flupyradifurone	Butenolides	4D	Agonist of nACh receptors
Spinosad and spinetoram	Spinosyns	5	nACh receptor allosteric modulators and antagonists of GABA-gated chloride channels
Abamectin	Avermectin	6	Chloride channel activation
7C Pyriproxyfen	Insect growth regulators	7C	Juvenile hormone mimic
Diflubenzuron, flufenoxuron, lufenuron, novaluron and teflubenzuron		15 type 0	Chitin biosynthesis inhibition (benzoylphenyl ureas)
Buprofezin		16 type 0	
Pymetrozine	Selective homopteran feeding blockers	9B	Paralysis of cibarium or mouthparts used for ingesting plant sap
Pyridaben, fenpyroximate	METI insecticides	21A	Mitochondrial complex I electron transport inhibition
Spirodiclofen and spirotetramat	Tetronic and tetramic acid derivatives	23	Acetyl CoA carboxylase inhibition
Chlorantraniliprole, cyantraniliprole	Anthranilic diamides	28	Ryanodine receptor modulation
Azadirachtin, sucrose octanoate, Silwet L-77, Kinetic, petroleum spray oil, horticultural spray oil, nC24 horticultural mineral oil and oil	Compounds of unknown or uncertain mode of action	Unknown	Chitin synthesis inhibition, feeding and oviposition deterrence, suffocation and alterations in cuticle composition

Manganese sulfate was administered to specifically chosen diseased plants at varying concentrations. Then, various vegetative, physiological in nature, and biochemical characteristics of citrus fruit that were suffering from citrus greening, including fruit diameter, Juice percentages, total soluble solids, weight, total solids insoluble in water, titratable acidity ascorbic acids were statistically analyzed. Zhang *et al.* (2011), stated no established cure for citrus HLB has been around for over a century. To control HLB spread, a propagation test system was used to screen therapeutic compounds on both infected periwinkle and citrus plants with CLas. The application of SP to periwinkle cuttings that were infected resulted in a regeneration rate of 70%, surpassing the regeneration rates achieved by alternative treatments, which were less than 50. Li *et al.* (2019), took a study to determine the lowest dosage of oxytetracycline (OTC) required to effectively control citrus HLB disease using a new method developed to evaluate the effect of OTC treatment on CLas titers in infected plants. Munir *et al.* (2022), conducted a study to establish a valuable method of managing HLB by modifying the citrus endophytic bacteria with the use of an indigenous endophyte, thereby ensuring sustainability. Umair *et al.*, (2023), conducted a study to investigate phylogenic silver nanoparticles' (AgNPs') potential to successfully recover the condition of citrus plants afflicted with HLB disease in a biocompatible manner.

***Conclusions and Future Aspects*:** HLB is a severe threat to the citrus industry in Pakistan, as it affects all commercial varieties and regions. The disease is caused by Las, which is transmitted by psyllids. The spatial distribution and molecular characterization of HLB revealed that the Musambi variety and Northwest location were the most susceptible to HLB. The psyllid transmission of HLB is a complex and dynamic process that involves multiple interactions among biotic and abiotic factors. Understanding these interactions can help to design better strategies to reduce the psyllid population and prevent Las transmission. Opportunities for future improvements in the management of HLB disease may involve the development of resistant citrus cultivars to mitigate susceptibility to the disease, as well as the study of biological control strategies, such as the development of beneficial microorganisms or predators, to specifically target the bacteria responsible for the disease or its vectors.

Improving the potential for early detection and diagnosis via advanced methods and tools may prove advantageous.

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REFERENCES

Ajene, I.J., F.M., Khamis, B. van Asch, G. Pietersen, N. Seid, I. Rwomushana, F.L. Ombura,G. Momanyi, P. Finyange, B.A. Rasowo and C.M. Tanga. 2020. Distribution of Candidatus Liberibacter species in Eastern Africa, and the first report of Candidatus Liberibacter asiaticus in Kenya. Scientific Reports 10:3919. https://doi.org/10.1038/s41598-020-60712-0

Alvarez, M.E.Y., C.F. Ortiz-García and J.A. Rincón-Ramírez. 2021. Detection of huanglongbing of citruses (Citrus sp.) through Sentinel-2satellite images in Huimanguillo, Tabasco, Mexico. Agro Productividad. https://doi.org/10.32854/agrop.v14i12.2053

Bhan, C., A.K. Srivastava, H. Prasad, S.K Bairwa and S. Chawla. 2016. A study on reduction in fruit drop to improve yield and quality of kinnow (Citrus reticulata blanco) fruit. Journal of Progressive Agriculture 7:97-101. https://doi.org/10.18805/ijare.a-5964

Boina, D.R., and J.R. Bloomquist. 2015. Chemical control of the Asian citrus psyllid and of huanglongbing disease in citrus. Pest Management Science 71:808-823. https://doi.org/10.1002/ps.3957

Catling, H.D., 1970. Distribution of the psyllid vectors of citrus greening disease, with notes on the biology and bionomics of Diaphorina citri. FAO Plant Protection Bulletin 18:8-15. https://swfrec.ifas.ufl.edu/hlb/database/pdf/00000839.pdf

Costa, G.V.D., C.S.V.J. Neves, R.B. Bassanezi, R.P. Leite Junior and T.S. Telles . 2021. Economic impact of Huanglongbing on orange production. Revista Brasileira de Fruticultura 43. https://doi.org/10.1590/0100-29452021472

Cui, X., K. Liu,S. Atta, C. Zeng, C. Zhou and X. Wang. 2021. Two unique prophages of 'Candidatus Liberibacter asiaticus' strains from Pakistan. Phytopathology®111:784-788. https://doi.org/10.1094/phyto-10-20-0454-sc

Das, A.K., C.N. Rao, A. George andS.A. Chichghare. 2022. Molecular identification and characterization of the Asian citrus psyllid vector, Diaphorina citri (Hemiptera: Psyllidae) and the transmitted Huanglongbing-associated bacterium, Candidatus Liberibacter asiaticus in India. Journal of Plant Pathology 104:1097-1110. https://doi.org/10.1007/s42161-022-01155-6

Deng, H., D. Achor,E. Exteberria, Q. YuD. Du D. Stanton, G. Liang and F,G, Gmitter Jr. 2019. Phloem regeneration is a mechanism for Huanglongbing-tolerance of "Bearss" lemon and "LB8-9" Sugar Belle® mandarin. Frontiers in Plant Science 10:277. https://doi.org/10.3389/fpls.2019.00277

Diksha, D., V.K Sidharthan, P. Singhal, N. Choudhary, S.K. Sharma, S.U. Nabi and V.K Baranwal. 2023. Multigene sequence analysis reveals occurrence of genetic variability in Candidatus Liberibacter asiaticus associated with Huanglongbing of Western and North-Eastern India. Indian Phytopathology 76:261-272. https://doi.org/10.1007/s42360-022-00592-8

Fateh, F.S., T. Mukhtar, M.R. Kazmi, N.A. Abbassi and A.M. Arif. 2017. Prevalence of citrus decline in district Sargodha. Pakistan journal of agriculture sciences 54:9-13. https://doi.org/10.21162/pakjas/17.5643

Fourie, P.H., W. Kirkman, G. Cook, C. Steyn, R. de Bruyn, R. Bester, R. Roberts, D.D.M. Bassimba,C.M. José and H.J. Maree. 2021. First report of 'Candidatus Liberibacter africanus' associated with african greening of Citrus in Angola. Plant Disease 105:486-486. https://doi.org/10.1094/pdis-06-20-1392-pdn

Gao, F., B. Wu, C. Zou, Y. Bao, D. Li, W. Yao, C.A. Powell and M. Zhang. 2022. Genetic Diversity of "Candidatus Liberibacter asiaticus" Based on Four Hypervariable Genomic Regions in China. Microbiology Spectrum10:e02622-22. https://doi.org/10.1101/2022.03.21.485244

Ghosh, D.K., A.D. Kokane, S.B. Kokane, J. Tenzin, M.G.

Gubyad, P. Wangdi, A.A. Murkute, A.K. Sharma and S. Gowda. 2021. Detection and molecular characterization of 'C andidatus liberibacter asiaticus' and citrus tristeza virus associated with citrus decline in Bhutan. Phytopathology® 111:870-881. https://doi.org/10.1094/phyto-07-20-0266-r

Hong, Y., Y. Luo, J. Yi, L. He, L. Dai and T. Yi. 2019. Screening nested-PCR primer for 'Candidatus Liberibacter asiaticus' associated with citrus Huanglongbing and application in Hunan, China. PLoS One 14:e0212020. https://doi.org/10.1371/journal.pone.0212020

Hussain, Z., Y. Iftikhar, M. Mubeen, M.Z. Saleem, M.U. Naseer, M. Luqman, R. Anwar, F. Khadija and A. Abbas. 2022. Application of micronutrients enhances the quality of kinnow mandarin infected by citrus greening disease (Huanglongbing). Sarhad journal of agriculture 38:360-371. https://doi.org/10.17582/journal.sja/2022/38.1.360.371

Iftikhar, Y., I.U. Haq, W. Raza,M.I. Ullah, S. Ali, F.N. Khoso and M. Mubeen. 2017. Seasonal Fluctuation of Asian Citrus Psylla Populations In The Citrus Orchards Infected With Huanglongbing In Sargodha, Pakistan. Pakistan Journal of Phytopathology 29:175-179. https://doi.org/10.33866/phytopathol.029.01.0328

Khan, R., S.H. Shah, I. Ahmad, A. Rafi and M. Fahim. 2019. Prevalence and diversity of Candidatus Liberibacter species in main citrus growing areas of Malakand in Northwest Pakistan. Plant Protection 3:105-116. https://doi.org/10.33804/pp.003.03.3107

Kumagai, L.B., C.S. LeVesque, C.L. Blomquist, K. Madishetty, Y. Guo, P.W. Woods, S. Rooney-Latham, J. Rascoe, T. Gallindo, D. Schnabel and M. Polek. 2013. First report of Candidatus Liberibacter asiaticus associated with citrus huanglongbing in California. Plant Disease 97:283-283. https://doi.org/10.1094/pdis-09-12-0845-pdn

Leong, S.S., S.C.T. Leong and G.A.C. Beattie. 2020. Incidence and spread of Huanglongbing (HLB) or citrus greening disease in relation to the distribution and fluctuation of Diaphorina citri Kuwayama (Hemiptera: Psyllidae) population in a citrus orchard in Sarawak, Malaysia. Serangga 24-42. http://journalarticle.ukm.my/16586/1/37916-134204-1-PB.pdf

Li, J., Z. Pang, S. Duan, D. Lee, V.G. Kolbasov and N. Wang. 2019. The in planta effective concentration of oxytetracycline against 'Candidatus Liberibacter asiaticus' for suppression of citrus Huanglongbing. Phytopathology 109:2046-2054. https://doi.org/10.1094/phyto-06-19-0198-r

McClean, A.P.D., and P.C.J. Oberholzer. 1965. Citrus psylla, a vector of the greening disease of sweet orange-research note. South African Journal of Agricultural Science 8:297-298. https://doi.org/10.5070/c52jb2v3kb

Mei, H., X. Deng, T. Hong and X. Luo. 2014. Early detection and grading of citrus huanglongbing using hyperspectral imaging technique. Transactions of the Chinese Society of Agricultural Engineering 30:140-147. Google Scholar.

Merfa, M.V., E. Pérez-López, E. Naranjo, M. Jain, D.W. Gabriel and L. De La Fuente. 2019. Progress and obstacles in culturing 'Candidatus Liberibacter asiaticus', the bacterium associated with Huanglongbing. Phytopathology 109:092-1101. https://doi.org/10.1094/phyto-02-19-0051-rvw

Mubeen, M., H.M. Arshad, Y. Iftikhar, I. Bilqees, S. Arooj and H.M.A. Saeed. 2015a. In-vitro efficacy of antibiotics against *Xanthomonas axonopodis* pv. citri through inhabitation zone techniques. International Journal of Agriculture and Applied Sciences 7:67-71. Google Scholar.

Mubeen, M., H.M. Arshad, Y. Iftikhar, M. Irfan Ullah and I. Bilqees. 2015b. Bio-chemical characterization of *Xanthomonas axonopodis* pv. citri: a gram-negative bacterium causing citrus canker. International Journal of Science and Nature 6:151-154. Google Scholar.

Munir, S., Y. Li,P. He, P. He, P. He, W. Cui, Y. Wu, X. Li, Q. Li, S. Zhang and Y. Xiong, Y. 2022. Defeating Huanglongbing pathogen Candidatus Liberibacter asiaticus with indigenous citrus endophyte Bacillus subtilis L1-21. Frontiers in Plant Science 12:789065. https://doi.org/10.3389/fpls.2021.789065

Naqvi, S.A.H., J. Wang, M.T. Malik, U.U.U. Umar, A. Hasnain, M.A. Sohail, M.T. Shakeel, M. Nauman, M.Z. Hassan, M. Fatima and R. Datta. 2022. Citrus canker-distribution, taxonomy, epidemiology, disease cycle, pathogen biology, detection, and management: A critical review and future research agenda. Agronomy 12:1075. https://doi.org/10.3390/agronomy12051075

Naqvi, S.A.H., S. Atta, H. Liu,A.U. Rehman and A.A. Khan. 2017. Serological and molecular based detection of graft-transmissible pathogens associated with citrus from non-core areas of Pakistan. Pakistan Journal of Agricultural Sciences 54. https://doi.org/10.21162/pakjas/17.5861

Nauman, M., U.U. Umar, S.A. Naqvi, A.U. Rehman, M.T. Malik, M. Shahid, M. Akbar and M. Umair. 2021. Impact Of Improved Dna Extraction Method From Citrus Leaves Midrib And Pcr For The Detection Of Citrus Greening (Candidatus Liberibacter). Pakistan Journal of Phytopathology 33:161-170. https://doi.org/10.33866/phytopathol.033.01.0671

Naz, S., K. Shahzadi, S. Rashid, F. Saleem, A. Zafarullah and S. Ahmad. 2014. Molecular characterization and phylogenetic relationship of different citrus varieties of Pakistan. Journal of Animal & Plant Sciences 24: Google Scholar.

Ramsey, J.S., E.D. Ammar, J.E. Mahoney, K. Rivera, R. Johnson, D.O. Igwe, T.W. Thannhauser, M.J. MacCoss, D.G Hall and M. Heck. 2022. Host plant adaptation drives changes in Diaphorina citri proteome regulation, proteoform expression, and transmission of 'Candidatus Liberibacter asiaticus', the citrus greening pathogen. Phytopathology 112:101-115. https://doi.org/10.1094/phyto-06-21-0275-r

Raza, M.U., F. Abasi, M. Shahbaz, M. Ehsan, W. Seerat, A. Akram, N.I. Raja, Z.U.R. Mashwani, H.U. Hassan and J. Proćków. 2023. Phytomediated Silver Nanoparticles (AgNPs) Embellish Antioxidant Defense System, Ameliorating HLB-Diseased 'Kinnow'Mandarin Plants. Molecules 28:2044. https://doi.org/10.3390/molecules28052044

Sajid, A., M.U. Ghazanfar, S. RaufZ. Hussain, S. Ahmad and Y. Iftikhar. 2021. Incidence and molecular detection of greening disease in two citrus cultivars in Sargodha, Pakistan. Sarhad journal of agriculture 37:296-301. https://doi.org/10.17582/journal.sja/2021/37.1.296.301

Sajid, A., Y. Iftikhar, M.U. Ghazanfar, M. Mubeen, Z. Hussain and E.A. Moya-Elizondo. 2022. Morpho-chemical characterization of Huanglongbing in mandarin (Citrus reticulata) and orange (Citrus sinensis) varieties from Pakistan. Chilean journal of agricultural research 82:484-492. https://doi.org/10.4067/s0718-58392022000300484

Shafiq, M., R. Fatima, S. Mushtaq, H.M. Salman, M. Talha, S. Razaq and M.S. Haider. 2018. Molecular characterization of Asian citrus psyllid (Diaphorina citri) using mitochondrial cytochrome oxidase 1 (mtCO1) gene from Punjab Pakistan. World Journal of Biology and Biotechnology 3:203-207. https://doi.org/10.33865/wjb.003.03.0170

Singh, S.J., P. Kashyap, P. Kumar, R. Kumar, A.S. Panwar and V.K. Baranwal. 2021. Molecular identification of citrus greening bacterium associated with Kinnow Mandarin in Western Uttar Pradesh, India. Indian Phytopathology 74:1135-1141. https://doi.org/10.1007/s42360-021-00396-2

Srivastava, A.K., S. Singh, A.K. Singh, A.K. Singh and E. Ngullie. 2009. Mineral nutrition of citrus: Chronological advances 11:319-417. Scientific Publishers, Jodhpur, India.https://doi.org/10.1080/01904160802592706

Tipu, M.M.H., M.M. Rahman, M.M. Islam,F.E. Elahi, R. Jahan and M.R. Islam. 2020. Citrus greening disease (HLB) on Citrus reticulata (Mandarin) caused by Candidatus Liberibacter asiaticus in Bangladesh. Physiological and Molecular Plant Pathology 112:101558. https://doi.org/10.1079/pwkb.20147801168

Vijayakumari, N., Y.B. Lahane and A. Rekha. 2022. Ploidy analysis among Citrus mutants using leaf meristematic tissue. Journal of Horticultural Sciences 17:34-40. https://doi.org/10.24154/jhs.v17i1.1186

Wang, N. 2019. The citrus huanglongbing crisis and potential solutions. Molecular plant 12:607-609. https://doi.org/10.1016/j.molp.2019.03.008

Wulff, N.A., C.G. Fassini, V.V. Marques, E.C. Martins,D.A.B. ColettiD.D.C. Teixeira, M.M. Sanches and J.M. Bové. 2019. Molecular characterization and detection of 16SrIII group phytoplasma associated with Huanglongbing symptoms. Phytopathology 109:366-374. https://doi.org/10.1094/phyto-03-18-0081-r

Yaqub, M.S., I.A. Khan and R. Aslam. 2019. Asian citrus psyllid (Diaphorina citri kuwayama) rearing for transmission of Candidatus liberibacter asiaticus in citrus for the management strategies of huanglongbing. Pakistan Entomologist 41. Google Scholar.

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. Pakistan Journal of Agricultural Sciences 54. https://doi.org/10.21162/pakjas/17.4455

Zafarullah, A., and F. Saleem. 2016. Detection and molecular characterization of 'Candidatus Liberibacter spp. causing "huanglongbing"(HLB) in indigenous citrus cultivars in Pakistan. Pakistan Journal of Botany 48:2071-2076. https://doi.org/10.21162/pakjas/17.4455

Zafarullah, A., S. Naz and F. Osman. 2021. Comparison of a Conventional PCR and Quantitative Real Time PCR (Qpcr) Assay for the Detection of Huanglongbing Disease Associated With'candidatus Liberibacter Asiaticus'in Citrus Varieties. JAPS: Journal of Animal & Plant Sciences 31: https://doi.org/10.36899/japs.2021.5.0346

Zhang, F., Z. Qiu, T. Feng, , Y. Dai and G. Fan. 2022. Modeling the Importation and Local Transmission of Huanglongbing Disease: Bifurcation and Sensitivity Analysis. International Journal of Bifurcation and Chaos 32:2250117. https://doi.org/10.1142/s0218127422501176

Zhang, M., C.A. Powell, , L. Zhou,Z. He,E. Stover and Y. Duan. 2011. Chemical compounds effective against the citrus Huanglongbing bacterium 'Candidatus Liberibacter asiaticus' in planta. Phytopathology 101:1097-1103. https://doi.org/10.1094/phyto-09-10-0262