Citrus greening disease in Pakistan - a review

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Abstract

The citrus greening disease, also called as Huanglongbing (HLB) disease, is the main reason for the decline of citrus trees in all citrus-growing countries. The bacterial species *Candidatus* Liberibacter is responsible for citrus greening disease and quickly spread through citrus psyllid, resulting in serious economic losses. Due to symptomatic variations, it is difficult to diagnose this disease among citrus cultivars. The disease spreads through grafting. With the help of genetic engineering, the genes are incorporated in citrus cultivars to manage the disease. The *NPR1* genes showed the resistance against this disease. The expression of *NPR1* in phloem increased the disease resistance in transgenic cultivars. It is essential to plan definite strategies to control citrus decline due to citrus greening disease in Pakistan.

Keywords: Citrus greening, Management, Psyllid, Transgenic cultivars.

Introduction

Citrus fruit is mainly grown in Pakistan and the world. Pakistan ranks 13 in the world for citrus production (FAO, 2018), and citrus is the top fruit in term of production, *i.e.* 2.36 million tons produced from an area of 206.6 thousand hectares (BOSP, 2014). The citrus fruit production is 40% in Pakistan (Arif *et al.,* 2005). Citrus Greening is the most destructive insect vector disease of citrus fruit and is currently uncurable. This disease is caused by the bacteria *Candidatus* Liberibacter species, which is phloem limited and unculturable. These bacteria reside in the phloem tissues of host plant and hinders the translocation of nutrients (Tipu *et al*., 2020). Nearly all commercial species and varieties of citrus are somewhat vulnerable to HLB. The disease has a range of symptoms which can be spotted anywhere on the plant, starting from roots to leaves, changing the chemical attributes, and sensory characteristics of the fruit (Paula *et al*., 2019). Citrus greening causes off-flavor, misshapen, inedible fruit, and yield loss in the citrus tree by declining of citrus trees. The mortality of citrus is rapid in young citrus trees (Gottwald, 2007). Liberibacter species vary in their temperature range. Heat-tolerant Asian form (*Liberibacter asiaticus*) tolerate temperature >30 °C and L. africanus tolerate temperature in the range of 22–25 °C. Asian citrus severely destroy citrus trees in the tropical and subtropical regions of Asia (Manjunath *et al.,* 2008). The causative organism of this disease is a gram-negative bacterial species of *Candidatus* Liberibacter, and it belongs to family Rhizobiaceae. The symptoms of the disease are related to citrus decline, yield loss, stunted trees and infected branches drying as disease development. Efforts are being made by using conventional and biotechnological tools to develop resistance varieties of citrus , management strategy such as reduce the sources of inoculums , control vector and grow disease-free planting materials to control citrus greening disease (Hall and Gottwald, 2011).

History

For over a hundred year Citrus greening disease has been in China and India (Husain and Nath, 1927; Chen, 1943). The disease citrus greening was first reported in the Western hemisphere about 12 years ago (Abdullah *et al.,* 2009). In other reports, HLB is thought to be first originated from China in 1919 (Fahdi *et al*., 2018). After that, greening disease has been described in India, South-East Asia, and South Africa this Citrus greening disease affected large citrus-producing areas and in the first half of the 20th century caused severe damage to the production of citrus in many countries. Citrus greening was discovered in Brazil in 2004 (Carmo *et al.,* 2005; Abdullah *et al.,* 2009) and it appeared in Florida in 2005 (Spreen *et al.,* 2014). The bacterium L. asiaticus *is* found in Florida and L. americanus is found in Brazil. In the 10 years since it appeared in Florida, the citrus industry lost over 50% in Florida and at an alarming rate decreasing the production of citrus (Stover *et al.,* 2015). In the future, the presence of this disease in citrus will harm the drive for planting new citrus trees. Citrus greening disease is the most severe disaster intimidating the citrus industry today.

Significance

In Pakistan, Citrus greening caused huge losses and causes various types of symptoms. HLB has been declared the most economically significant pathogens of citrus. Firstly, the citrus greening disease was known by various names: in China, yellow shoot (huanglongbing HLB); in Taiwan, likubin (decline); in India, dieback; and in Philippines, leaf mottle. This became apparent that all these were related diseases, but the name citrus "greening" was widely adopted (Paudyal *et al*., 2015). The citrus greening affects the quality and yield of citrus in Pakistan in different conditions different symptoms appear in different parts of plants which affect the quality of citrus and decrease productivity.

Disease diagnosis of the disease is difficult, specifically in the early stages. Greening affected trees are stunted and display twig dieback and severe drop of fruits. Citrus greening disease in some cases shows color invasion in which green color at the peduncular on the fruit sooner than the stylar end, as shown in normal case (McCollum and Baldwin, 2017). Hundred percent trees of rough lemon and 25% trees of sweet orange rootstock infected. Citrus greening disease destroyed about 60 million citrus trees in Africa and Asia (Shokrollah *et al.,* 2011). It is compulsory to control the citrus greening disease by different management techniques to secure the economy of Pakistan.

Pathogen

Candidatus Liberibacter spp.

Taxonomy

Classification	
Kingdom	Bacterium
Phylum	Proteobacteria
Class	Alphaproteobacteria
Order	Rhizobiales
Family	Rhizobiaceae
Genus	*Candidatus* Liberibacter

Biology of Pathogen

Candidatus Liberibacter disease-causing pathogen of citrus greening disease is the most notorious pathogen in more than 40 countries of the world (Iftikhar *et al.,* 2009). *Candidatus* Liberibacter americanus has the ribosomal 16S/23S intergenic region gene sequence and 16S rRNA gene sequence. The citrus greening pathogen was gram-negative (Cornelissen *et al.,* 2003). HLB pathogen was not grown on artificial media, instead of conventional methods such as growth, morphology, enzymatic activity its taxonomical classification was based on the 16S rRNA gene sequence. Taxonomy of Pathogen alpha subdivision of proteobacteria, genus *Candidatus* Liberibacter, family Rhizobiaceae (Abdullah *et al.,* 2009). Presently citrus greening disease caused by three species of *Candidatus* Liberibacter are which are L. asiaticus, L. africanus, and L. americanus (Haapalainen, 2014).

Strains

Citrus greening pathogen mostly occurs in two forms Asian form and African form. These two forms are based on temperature sensitivity. Asian form (*L. asiaticus*) of pathogen shows more severe symptoms than African (*L. africanus*), which shows symptoms under cooler temperature and American form remove disease symptoms from infected plants under warm condition but show symptoms under cooler conditions (Lopes *et al.*, 2007). Liberibacter Genus was described as a greening pathogen, and on PCR studies consider a member of the alpha subdivision of proteobacteria. Based on sequence homology, these 2 forms *L. asiaticus* and *L. africanus* renowned as separate species. In Brazil, the newly discovered species in American form (*L. americanus*) shows symptoms under cooler conditions whereas the Asian form shows severe symptoms in warmer conditions (Chung and Brlansky, 2009).

Pathogen Virulence

Virulence and interaction mechanism of pathogen necessary for effective control of the citrus greening disease. Liberibacter species are three in which *Candidatus* L. asiaticus (Las) is the most destructive and prevalent (Tipu *et al.*, 2020) because it is difficult to grow on media (Tabachnick *et al.*, 2015), so its virulence mechanism understandsbased onthe genomic study. Asian form *Candidatus* L. asiaticus is the most virulent and extensively disseminated worldwide.

Host range

In the citrus greening the host ranges mainly two types of hosts, one host which favors the pathogen to multiply, vector to feeds, and multiplies. Another host is called the alternate host in which the diseased psyllids are maintained (Adkar-Purushothama *et al*., 2009).

Approximately all the citrus varieties are susceptible to citrus greening disease. Severe disease symptoms vary in many citrus cultivars like grapefruit, mandarin, sweet oranges, and tangelo but in sour orange, rough lemon, and lemon few symptoms are shown (Ghosh *et al.*, 2018). Grapefruit is tolerating a variety of citrus than sweet orange, there is no resistant variety of citrus, but tolerant varieties are present. The citrus cultivars like pummelo and kumquat were considered resistant at first but with time develop disease and show the symptoms of mottling due to the evolution of citrus greening pathogen (Tsai and Liu, 2000; Bove *et al.*, 2006). Another potential and alternative host of citrus greening pathogen and Asian citrus psyllid is *Murraya paniculate* (Killini *et al.,* 2016).

Disease signs and symptoms

Citrus greening disease based on symptoms diagnosing is very difficult as citrus varieties are often affected by many other problems. Symptoms of citrus trees depend on their age, time, and stage of infection (Ghosh *et al.*, 2018). The main symptom is 'yellow shoots' in which leaves are dark green, pale green, partially green, partially yellow, with many different shades of yellow and blending with no sharp boundaries between the diverse shades of color (Chung and Brlansky, 2009). On the single branch or shoot, leaf yellowing appears. The characteristic symptoms of the citrus greening disease are mottling and leave chlorosis which resembles zinc deficiency symptoms (Abdullah *et al.*, 2009).

Dieback and defoliation in the citrus plant due to citrus greening disease (Hu *et al.*, 2011). In Asia citrus life is 5–8 years on average where the disease spread in epidemic form in the hotter region. Citrus fruits are under-developed and poorly colored (Abdullah *et al.*, 2009).

Transmission of pathogen

Firstly, it was considered that citrus greening disease is associated with mineral deficiency and water-logging due to yellowing of stem symptoms (Hall *et al.*, 2013; Ghosh *et al.*, 2018). Transmission of *Candidatus* Liberibacter spp. through grafting of diseased plant parts. Citrus greening disease is not transmitted through seeds (Manjunath *et al.*, 2008). This disease is not transmitted through seed because disease citrus plants have aborted seeds in high number. Citrus greening commonly spread through grafting and insect vectors. Two vector species transmit the pathogen such as citrus psyllid, *Diaphorina citri,* and *Trioza erytreae* (Fig. 1). The disease vector resistant to heat and tolerate high temperature but does not tolerate high rainfall and humidity conditions. Climatic conditions and leaves types play a major role in carrying the pathogen by the vector (Hall *et al.*, 2013).

Epidemiology

The citrus greening disease is caused by the Liberibacter spp. which shows a different reaction to temperature. In Asia, *Ca.* L. asiaticus (*las*) is more heat-tolerant and at a temperature above 30 °C they can survive. In lower-lying hotter areas usually found *Ca.* L. asiaticus. African form (L. africanus) cause symptoms of citrus greening are suppressed by long exposure to temperatures above 30 °C. Therefore *Ca*. L. africanus is usually found in citrus at an elevation above 700 m. *Ca*. L. africanus thermo-labile and survive at temperatures in the range of 22–25 °C (Manjunath *et al.*, 2008). *Ca.* L. americanus in America newly discovered in Brazil appears to closely resemble in symptoms expression and severity with the *Ca.* L. asiaticus, but the results of tests in Brazil show that the African type is heat intolerant.

Management

To control or management of citrus greening disease use different strategies. To prevent the dispersal of pathogens legislative control is used. There are established following legislative control measures:

- Regulatory plants: Citrus species like *Rutaceae, Fortunella* spp., *Poncirus trifoliate,* and *Citrus* genus living plants.
- Regulatory of Pathogen and insect: citrus greening pathogen bacteria and vector Psyllid.
- Mother stock prevention: To prevent citrus greening mother stock isolate to prevent the citrus plant from insect vector which carry the pathogen.

The citrus greening disease is managed by quarantine measures, certification programs, clean stock to produce disease-free plants and prevent the movement of infected nursery stock. The vectors of citrus greening disease must be controlled. Chemicals spray is used during winter months to reduce the psyllid vector population when psyllid is not active and only over-wintering adults are present (Bassanezi *et al.*, 2013).

Three approaches are used to manage disease in the areas where the citrus greening disease is not already established: Identify the early symptoms of the disease by regular survey and removed the diseased plants. Used the disease-free plant material for replanting and control vector by survey and pesticide application (Alvarez *et al.*, 2016). The bacterium associated with citrus greening disease by the detection of PCR or real-time PCR. Vector psyllids are testing by real-time PCR for the occurrence of the bacterium related to citrus greening disease to provide an early warning of the presence of the disease in the area where the citrus greening disease is not established. (Manjunath *et al.*, 2008).

Genetic engineering of citrus greening

Resistant germplasm utilization to control or low spread of citrus greening is complex due to the commercially short availability of rootstock which protects from disease. It's only a potential disease management strategy to identify and incorporate resistant traits from tolerant Citrus spp. and citrus relatives (Westbrook *et al.*, 2011). The difficult and time-consuming methods are conventional breeding for citrus cultivars improvement. For citrus cultivar genetic improvement, the fastest method is Genetic engineering (Gmitter *et al.*, 2009). Genetic engineering of citrus cultivars by using or incorporation of genes that defend from citrus greening disease and activate plant systemic acquired resistance (SAR) (Zhang *et al.*, 2010). SAR provides the expression of specific resistance genes, resistance against the pathogen, and plant defense (Kuc, 1982). SAR produces the pathogenesis-related (PR) proteins and protects broad spectrum microorganisms (Ward *et al.*, 2007). Salicylic acid (SA) induced SAR defense response. Plants are more susceptible to pathogen infection if the salicylic acid pathway nonactive in plants (Forouhar *et al.*, 2005). For instance, the transgenic plant of Arabidopsis when over-expressing gene nahG which encoding the SA hydroxylase enzyme is unable to accumulate SA because SA hydroxylase degradation it into catechol. Therefore, such plants are susceptible to *Pernospora paraitica* and *Pseudomonas syringa* microorganisms infection.

Arabidopsis mutants are most susceptible to pathogen infection in which Salicylic acid (SA) pathways induction-deficient are unable to accumulate SA (Nawrath and Metraux 2007). To regulate the signal transduction pathway which leads to systematic acquired resistance (SAR) the main pathway is the Pathogenesis Related *NPR1* gene. The transcription factor that controls the PR gene expression regulates by the *NPR1* gene which acts as a regulator and also mediates the SA-induced expression of SAR and PR genes (Kinkema *et al.*, 2007). Citrus plants show improved resistance to many pathogens and provide resistance against Citrus greening disease by the over expressing *NPR1* gene (Xu *et al.*, 2015). For citrus greening disease resistance, incorporation of At*NPR1* in phloem tissue of citrus cultivars and over expression of AtNAR1 gene in transgenic citrus cultivar in phloem tissues via the exploitation of phloem specific Arabidopsis constitutive CaMV35S promoter or sucrose-proton symporter 2(AtSUC2) promoters. Transgenic citrus plants show effective citrus greening resistance by overexpressing the At*NPR1* gene.

Mechanism of resistance gene

A serious phloem limited bacterial disease citrus greening is usually fatal and mostly commercial citrus cultivars lack resistance to citrus greening disease. For sustained disease resistance to citrus greening, over expression of AtNAR1 gene in transgenic citrus cultivar in phloem tissues with phloem specific *Arabidopsis* SUC2 (AtSUC2) promoter orconstitutive CaMV 35S promoter (Fig. 2). Over expression of At*NPR1*genein normal trees that enhanced resistance to citrus greening disease. Phloem specific expression of *NPR1* shows expression in phloem and increases disease resistance in transgenic citrus cultivars which remain disease free even after 36 months of planting in the disease field and show less severity of citrus greening disease. Expression of *NPR1* gene also activates plant defense signaling pathways by activating some native genes of the citrus plant. For the development of all plant, T-DNA derived consumer friendly GM tree *NPR1* gene serve as the main component.

Status of Citrus greening disease in Pakistan

Worldwide as well as in Pakistan citrus production is maximum. In Punjab and Khyber Pakhtunkhwa, the citrus greening disease or HLB is severe. During the surveys, observed the incidence of disease in different citrus cultivars like 25–40% in sweet orange, 22% in Kinnow, 15% in grapefruit, 10% in sweet lime, and 2% in lemon. At different localities vary the disease incidence and disease severity (Razi *et al.*, 2014). Diagnosis the abnormality by micrometry of thin sections stained with phenolic thionin and observed the organism called Bacteria like organisms (Razi *et al.*, 2014). It is investigating that greening disease causes a serious threat to the citrus industry in Pakistan. On citrus greening disease only, limited work is available in Pakistan. The first research in the late 1980s in which surveyed and collected infested and diseased samples of bacterium *D. citri* associate with citrus greening disease was detected in midribs of leaves and sieve tubes (Grimaldi and Catara, 1989). Observation of samples collected from the province under an electron microscope showed that pathogens associate with greening present in phloem cells.

Future scenario

In the future, early detection improves control of vector, and new management strategies combined with economic threshold models to profits and maximum rotation should lead to increasing planting viability and productivity and a more viable, profitable, and stable industry. In the long term, the answer to an endemic citrus greening situation is of course disease resistance. Unfortunately, no resistance source has yet been identified either for conventional breeding or transgenic improvement system. However, it is hoped that efforts in host sequencing, pathogen, and bioinformatics will point to resistance genes or vulnerabilities of pathogen and therefore disease resistance mechanisms paths for the future. At present, the citrus industry produces many citrus cultivars which are moderately and highly susceptible to HLB. Therefore, when sources of resistance are identified then incorporate into citrus cultivars by using transgenic technology to increase productivity and market demands.

Conclusion

In the world as well as in Pakistan citrus Greening a destructive disease and cause citrus decline. Pakistan as well as in neighboring countries prevalence threats of citrus greening disease. Citrus greening disease can be overcome or increase in citrus production by growing nurseries free from pathogen with professional techniques. The citrus greening problem can be efficiently solved by using certified citrus nurseries and investigate the pathogen and host relation. By using certified bud wood programs might be the best way to establish Disease-free citrus orchards. Integrated pest management program used to control citrus greening disease.

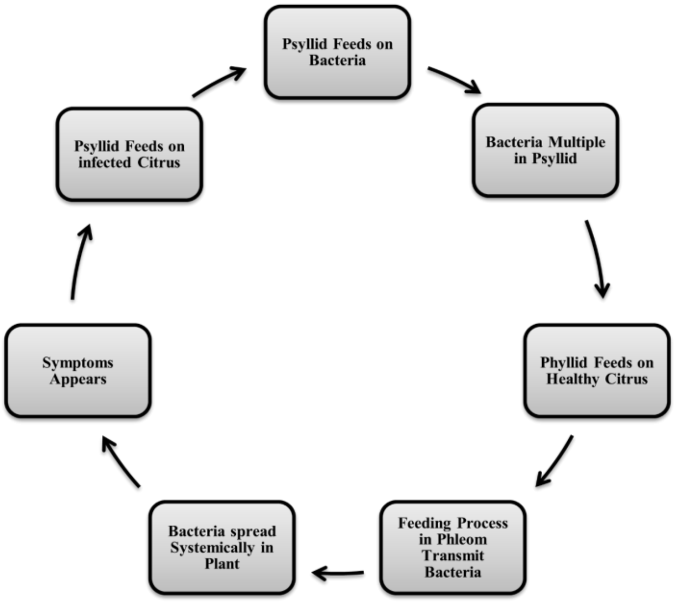

Fig 1: Life cycle of Huanglongbing (HLB)

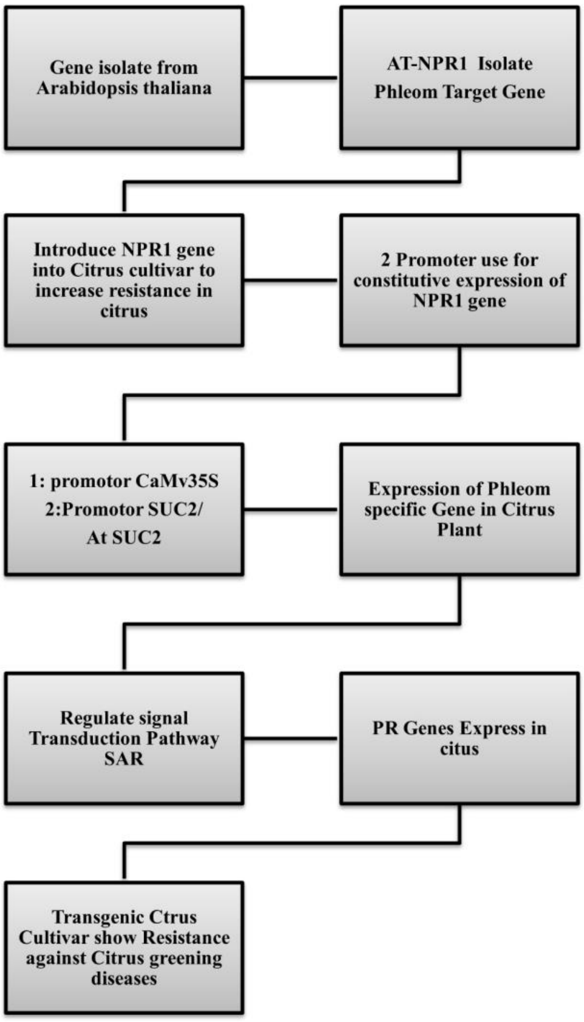

Fig. 2: Transformation gene into citrus germplasm to develop resistant cultivar.

References

Abdullah TL, Shokrollah H, Sijam K, Akmar SN, 2009. Control of Huanglongbing (HLB) disease with reference to its occurrence in Malaysia. *J. Biotechnol.,* **8**: 4007-4015.

Adkar-Purushothama CR, Quaglino F, Casati P, Ramanayaka JG, Bianco PA, 2009. Genetic diversity among '*Candidatus* Liberibacter asiaticus' isolates based on single nucleotide polymorphisms in 16S rRNA and ribosomal protein genes. *Ann. Microbiol*., **59**: 681-688.

Al Fahdi A, Al-Mamari A, Shahid MS, Maharachchikumbura SS, Carvalho CM, Elliot SL, Al-Sadi AM, 2018. Characterization of Huanglongbing disease associated with acid lime (*Citrus aurantifolia Swingle*) in Oman. *J. Plant Pathol*., **100**: 419-427.

Alvarez S, Rohrig E, Solís D, Thomas MH, 2016. Citrus greening disease (Huanglongbing) in Florida: economic impact, management and the potential for biological control. *Agric. Res*., **5**: 109-118.

Arif M, Ahmad A, Ibrahim M, Hassan S, 2005. Occurrence and distribution of virus and virus-like diseases of citrus in north-west frontier province of Pakistan. *Pak. J. Bot*., **37**: 407-421.

B. O. S. PUNJAB BOSGOT, 2014. Statistical Pocket Book of the Punjab.

Bassanezi RB, Jr JB, Montesino LH, 2013. Frequency of symptomatic trees removal in small citrus blocks on citrus huanglongbing epidemics. *Crop. Prot*., **52**: 72-77.

Bove JM, Genomique DR, Pathogene P, Recherche C De, Bordeaux I De, Bourlaux E, Cedex O, Df A, Pereira A, Araraquara P, 2006. Huanglongbing : a destructive, newly-emerging, century old disease of citrus. *J. Plant Pathol*., **88**: 7-37.

Carmo Teixeira D do, Saillard C, Eveillard S, Danet JL, Costa PI da, Ayres AJ, Bové J, 2005. "*Candidatus* Liberibacter americanus", associated with citrus huanglongbing (greening disease) in São Paulo State, Brazil. *Int. J. Syst. Evol. Microbiol*., **55**: 1857-1862.

Chung KR, Brlansky R, 2009. Citrus diseases exotic to Florida: Huanglongbing (citrus greening). *Plant. Pathol.*, **58**: 1-4.

Cornelissen JHC, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich DE, Reich PB, Steege H Ter, Morgan HD, Heijden MGA Van Der, Pausas JG, Poorter H, 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Aust. J. Bot.*, **51**: 335-380.

Dala-Paula BM, Plotto A, Bai J, Manthey JA, Baldwin EA, Ferrarezi RS, Gloria MB, 2019. Effect of huanglongbing or greening disease on orange juice quality, a review. *Front Plant Sci*., **22**: 1976.

FAO, 2018. FAO Statistical Pocketbook 2018, Food and Agriculture Organization of the United Nations.

Forouhar F, Yang Y, Kumar D, Chen Y, Fridman E, Park SW, Chiang Y, Acton TB, Montelione GT, Pichersky E, Klessig DF, Tong L, 2005. Structural and biochemical studies identify tobacco SABP2 as a methyl salicylate esterase and implicate it in plant innate immunity. *Proc. Natl. Acad. Sci*., **102**: 1773-1778.

Ghosh DK, Motghare M, Gowda S, 2018. Citrus Greening : Overview of the Most Severe Disease of Citrus.*Adv. Agric. Res. Technol. J.*, **2**: 83-100.

Gmitter FG, Grosser JW, Castle WS, Moore GA, 2009. A comprehensive citrus genetic improvement programme. In: *Citrus Genetics and Breed Biotechnology*. CABI International. pp. 9-18.

Gottwald TR, 2007. Citrus canker and citrus Huanglongbing, two exotic bacterial diseases threatening the citrus industries of the Western Hemisphere. *Outlooks Pest. Manage*., **18**: 274-279.

Grimaldi V, Catara A, 1989. Detection of citrus tristeza and greening in Pakistan through electron microscopy. *J. Phytopathol*., **126**: 17-21.

Haapalainen M, 2014. Biology and epidemics of *Candidatus* Liberibacter species, psyllid-transmitted plant-pathogenic bacteria. *Ann. Appl. Biol*., **165**: 172-198.

Hall DG, Gottwald TR, 2011. Pest Management Practices Aimed at Curtailing Citrus Huanglongbing Disease. *Outlooks Pest. Manage*. **22**: 189-192.

Hall DG, Richardson ML, Ammar ED, Halbert SE, 2013. Asian citrus psyllid, *Diaphorina citri*, vector of citrus huanglongbing disease. *Entomol. Exp. Appl*., **146**: 207-223.

Hu WZ, Wang XF, Zhou Y, Li ZA, Tang KZ, Zhou CY, 2011. Diversity of the omp gene in *Candidatus* Liberibacter *asiaticus* in China. *J. Plant Pathol*., **93**: 211-213.

Iftikhar Y, Aslam Khan M, Rashid A, Mughal SM, Killiny N, 2016. Metabolomic comparative analysis of the phloem sap of curry leaf tree (*Bergera koenegii*), orange jasmine (*Murraya paniculata*), and Valencia sweet orange (*Citrus sinensis*) supports their differential responses to Huanglongbing. *Plant signal. & behave*., **11**: e1249080.

Kinkema M, Fan W, Dong X, 2007. Nuclear Localization of NPR1 Is Required for Activation of PR Gene Expression. *Plant Cell*., **12**: 2339-2350.

Kuc J. 1982, Induced Immunity to Plant Disease. *Biocience*, **32**: 854-860.

Lopes SA, Frare GF, Yamamoto PT, Ayres AJ, Barbosa JC, 2007. Ineffectiveness of pruning to control citrus huanglongbing caused by *Candidatus* Liberibacter americanus. *Eur. J. Plant Pathol*., **119**: 463-468.

McCollum G, Baldwin E, 2017. Huanglongbing: devastating disease of citrus. *Hort. Rev*., **44**: 315-361.

Manjunath KL, Halbert SE, Ramadugu C, Webb S, Lee RF, 2008. Detection of ‘ *Candidatus* Liberibacter asiaticus’ in *Diaphorina citri* and Its Importance in the Management of Citrus Huanglongbing in Florida. *Phytopathology.*, **98**: 387-396.

Nawrath C, Metraux J-P, 2007. Salicylic acid induction-deficient mutants of arabidopsis express PR-2 and PR-5 and accumulate high levels of camalexin after pathogen inoculation. *Plant Cell*, **11**: 1393-1404.

Paudyal KP, 2015. Technological advances in huanglongbing (HLB) or citrus greening disease management. *J. Nep. Agric. Res. Counc*., **1**: 41-50.

Razi MF, Keremane ML, Ramadugu C, Roose M, Khan IA, Lee RF, 2014. Detection of citrus huanglongbing associated *Candidatus* Liberibacter asiaticus’ in Citrus and *Diaphorina citri* in Pakistan, Seasonal Variability, and Implications for Disease Management. *Phytopathology*, **104**: 257-268.

Shokrollah H, Abdullah TL, Sijam K, Abdullah SN, 2011. Potential use of selected citrus rootstocks and interstocks against HLB disease in Malaysia. *Crop Prot.*, **30**: 521-555.

Spreen TH, Baldwin JP, Futch SH, 2014. An economic assessment of the impact of Huanglongbing on citrus tree plantings in Florida. *Hort. Sci*., **49**: 1052-1055.

Stover E, McCollum GT, Driggers R, Lee R, Shatters R, Duan YP, Ritenour M, Chaparro JX, Hall DG, 2015. Resistance and tolerance to huanglongbing in citrus. *Acta. Hort*., **1065**: 899-904.

Tabachnick WJ, 2015. Diaphorina citri (Hemiptera: Liviidae) vector competence for the citrus greening pathogen ‘*Candidatus* Liberibacter asiaticus’. *J. Econ. Entomol.*, **108**: 839-848.

Tipu MM, Rahman MM, Islam MM, Elahi FE, Jahan R, Islam MR, 2020. Citrus greening disease (HLB) on *Citrus reticulata* (Mandarin) caused by *Candidatus* Liberibacter asiaticus in Bangladesh. *Physiol. Mol. Plant Pathol*., **1**: **112**: 101558.

Tsai JH, Liu YH, 2000. Biology of *Diaphorina citri* (Homoptera: Psyllidae) on Four Host Plants. *J. Econ. Entomol*., **93**: 1721-1725.

Ward ER, Uknes SJ, Williams SC, Dincher SS, Wiederhold DL, Alexander DC, Ahl-Goy P, Metraux J-P, Ryals JA, 2007. Coordinate Gene Activity in Response to Agents That Induce Systemic Acquired Resistance. *Plant Cell*., **3**: 1085-1094.

Westbrook CJ, Hall DG, Stover E, Duan YP, Lee RF, 2011. Colonization of Citrus and Citrusrelated germplasm by Diaphorina citri (Hemiptera: Psyllidae). *Hort. Sci*., **46**: 997-1005.

Xu M, Li Y, Zheng Z, Dai Z, Tao Y, Deng X, 2015. Transcriptional analyses of mandarins seriously infected by *Candidatus* Liberibacter asiaticus. *PLoS One*, **10**: e0133652.

Zhang X, Francis MI, Dawson WO, Graham JH, Orbović V, Triplett EW, Mou Z, 2010. Over-expression of the Arabidopsis NPR1 gene in citrus increases resistance to citrus canker. *Eur. J. Plant Pathol*., **128**: 91-100.