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...
"growing" 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 styal 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**

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**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 Bransky, 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 understands based on the 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 motting 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 paniculata* (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 leaf 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 trifoliata*, 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 paratitica 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 AtNPR1 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 AtNPR1 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 or constitutive CaMV 35S promoter (Fig. 2). Over expression of AtNPR1 gene in 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


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