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Recent Advances in the Biology, Taxonomy, Epidemiology and Management of Xanthomonas Axonopodis Pv. Citri (Xac)

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Abstract

Canker which is caused by Xanthomonas pv citri (Xac) is a potential threat to the successful production of citrus throughout the world. Due to sudden climatic variation, pattern of disease appearance is changing in different climatic zones. Fluctuation in climatic conditions imposes a pronounced impact on the reproduction rate and enhances aggressiveness of the bacterium. Variation in environmental situation of various citrus zones causes mutations in Xac and produces various pathotypes. So its need of the hour, not only to identify these pathotypes by using modern approaches but also to understand biology of different isolates for devising concrete tactics towards citrus canker. This paper analysis the existing state of information and understanding regarding epidemiology, biology, taxonomy, strains of Xac and latest management practices towards citrus canker.

Keywords: Pathovars; Synthetic chemicals; Asiatic citrus canker; Induced resistance; Pathotypes

Introduction

Citrus crop is threatened by a number of factors such as fungi, bacteria, nematodes, viruses, phyto-plasma, and nutritional disorders that hinder the quality and yield of the fruit. Among these, citrus cankers is one of the most devastating diseases caused by Xanthomonas axonopodis pv.citri (Xac) and therefore, this disease is of great economic importance throughout the world's citrus-growing region, including Pakistan, which has an adverse effect on plant health and fruit development. It has played a major role in causing large losses in nurseries and orchards. (Gottwald and Irey, 2007). There are three types of citrus canker disease caused by different pathovars and variants of the bacteria viz; Canker A caused by group of X. axonopodis pv. citri strains originally found in Asia. Canker B caused by group of strains of X. axonopodis pv.aurantifolli (Xaa) strains originally found in South America and third is Canker C caused by the same form as Canker B i.e Xaa originally found in Brazil (Gottwald et al., 2002). Diseased plants are characterized by the presence of noticeable inflated necrotic lesions on leaves, twigs, thorns and fruits. These lesions grow on the surface of the leaves, twigs, thorns and fruits as light yellow, elevated, spongy eruptions. The lesions are warty, round and slightly rose on the leaves at first, while later they turn brown and enlarged and raised on one or both sides of the leaves and rupture the surfaces. (Reinking, 1919). Similar lesions are observed on twigs and fruits which are elevated, corky and surrounded by an oily margin or water-soaked. Severe attack causes severe defoliation, branches dieback and premature drop of fruit. (Das, 2003)

Xac is 1.5-2.0x 0.5-0.75 µm long, aerobic, gram-negative, smooth, rod-shaped bacterium. It has a single and polar flagellum predominantly. It produces pigmented yellow mucoid colonies on glucose or any other form of sugar on agar plates. Due to the production of extracellular polysaccharide slime, the presence of sugar makes colonies very mucoid. While the yellow color of colonies is the result of the production of Xanthomonad pigment. (Braithwaiten et al., 2002). It is chemoorganotrophic with the oxidative metabolism of glucose and is obligatorily aerobic. Conducive environmental factors are required for the rapid growth and development of Xac (Kema et al., 1996). Frequent rainfall in warm weather contributes to the development of disease, especially during storms. (Gottwald et al., 2002). Temperature ranging from 20 to 30oC with even rainfall is best suited for the disease (Reddy, 1984). The disease thus occurs in seasons and/or areas with warm and humid weather conditions in severe form. In Malaysia, Derso, et al., (2007) studied the relationship between environmental parameters and occurrence of citrus canker. They found that Citrus canker was strongly associated with temperature but not with rainfall elevation and tree age at Kuala Kangsar in West Malaysia. Certain methods had been used to study taxonomy and to distinguish between X strains with various pathotypes, including physiological and biochemical studies (Alvarez et al., 1991) DNA-DNA homology, different pairs of primers, plasmid DNA, fatty acid analysis of protein profiles, rep -PCR with pairs of primers BOX and ERIC were used to distinguish X pathotypes (Cubero & Graham, 2002). The technique of rep-PCR also allows the evaluation of Xap in some parts of the world. (Cubero and Graham, 2002).

Citrus canker is managed by various control strategies like use of chemicals, plant extracts or antagonists. However, the chemicals are the cheapest and easiest way to manage pre and post-harvest losses but it may lead to the environmental pollution and effect whole ecosystem (Campos et al., 2005). It can result in the resistance of bacterium against diseases. In order to save our surroundings as well as human beings from the toxic effects of these chemicals, there is a need to find out another way to save our crops from these pathogens (Tahir et al., 2016). Most recent work has been published on the effects of endophytes and plant byproducts, which are helpful to minimize the effects of plant pathogens. Many researchers have reported the use of several plant byproducts, possessing antimicrobial properties, against several pathogenic bacteria and fungi (Kilani, 2006). Green plants have been proved effective chemotherapeutants and can be used as an important source of natural pesticides, alternate to those of synthetic chemicals (Mahajan and Das, 2003).

Biology of Xanthomonas axonopodis pv citri

Xac belongs to the gamma class of the proteobacteria, a complex and economically important group of bacterial phyto-pathogens (Vauterian et al 1995). The bacterium is 1.5 –2.0 to 0.5–0.75 micro meter, rod-shaped, gram negative, and has a polar flagellum. Laboratory media colonies are yellow due to pigment development of Xanthomonadin. Due to the production of exopolysaccharides slime, colonies become very mucoid when glucose or other sugars are added to the culture medium (Goto, 1992). The bacterium produces lesions on leaves, roots, and fruit. Once the lesions have free water, the bacteria ooze out and can spread to new plants. Wind-driven rain is the primary dispersal agent, and wind speeds of > 18 mph helps the bacteria to penetrate through pores, cuts and bugs. When tissues mature, they are immune to infection. Almost all infections of the leaf and stem occur within the first 6 weeks of growth, unless there is an infestation of the leaf miner. The most critical period for fruit disease is when the diameter of orange fruit ranges from 0.25-1.5 inches. When stomata open, the fruit is particularly susceptible to bacterial penetration. Fruit remains vulnerable to disease in oranges and tangerines for the first 60 to 90 days and in grapefruit for 120 days. After time, infection can lead to the formation of small and unobtrusive pustules. Severe tropical storms, hurricanes, and tornadoes can take the pathogen over long distances, up to miles, under heavy winds. Disease is also spread by the movement of diseased plant material such as bud wood, rootstock, seedlings and budded trees. Farm implements and workers can also act as a vector of the disease (http://edis.ifas.ufl.edu).

Impact of environmental factors on the growth, development and spread of Xac

Dissemination of Xac occurs through combination of wind and rain. The spread is increased due to the larval feeding of Asian citrus leaf miner (CLM) (Bock et a1. 2005; Gottwald et a1., 2007). There is highest concentration of Xac inoculum, when the viable lesions become wet. The concentration of inoculum gets lowered in rainwater when an hour is passed after stop of rain and wind. However, multiplication of bacteria is highest between storms to provide a large concentration of initial inoculum for new wind event. There is the sufficient growth of Xac between temperature of 20 to 30oC and this temperature therefore supports large populations in tropical and subtropical environments throughout the year. In addition to favorable temperatures, rain events occurring regular, brief and in combination to strong winds is highly beneficial for Xac spread. (Bock et a1., 2005). Low velocity winds lead to higher plant injury resulting in more severe diseases, both in frequency and incidence. Hurricanes and tornadoes are able to disperse large amounts of inoculum. (Bock et a1. 2010; Gottwald and Irey, 2007). Symptoms may develop around 7 days after infection under favorable conditions of inoculum, temperature and humidity. When there are low inoculum and temperature levels, symptoms may take 2 months or more to manifest (Schubert et a1., 2001). Typically, when temperature reaches 40oC, symptoms are produced on Tahiti lime, provided the ample length of the leaf, but not on sweet orange. There is the severe attack of CC on sweet orange and Tahiti lime during long wetness of the leaves (Christiano et a1. 2009; Dalla Pria et a1. 2006). In newly expanding tender leaves, stem and immature green fruit tissues, citrus canker produces local eruptive lesions. Leaf and fruit lesions are often surrounded by water-soaked margins which are surrounded by a chlorotic halo, but chlorotic haloes are not present in stem lesions. There is an increase in diameter of citrus canker lesions with age which later turn brown, having corky appearance. Finally, the heavy infestation may result in complete defoliation (Schubert et a1. 2001). Young lesions have a higher concentration of inoculum as compared to older lesions. (Bock et a1, 2005). Host epidermis breaks within 1–3 weeks after infection, depending on type of host, temperature and initial inoculum. This results in release of bacteria to the surface where they can disperse to start a new infection cycle. (Brunings and Gabriel, 2003). Higher concentrations of Xac inoculum is present in bacterial exudates which advance the bacteria to new exposed young tissues at the same plant or new plants (Timmer et a1., 1991). Xac enters the host tissue through cuts and wounds and through natural openings like stomata and hydathodes (Lee, 1921). Mainly, Xac persists in the edges of the lesions seasonally. It survives only 1-3 days on surfaces like clothes, agriculture implements and not more than 2 months in soil as a result of saprophyte rivalry. (Schubert et al., 2001). During mild winters of tropical environments, Xac cells survive in the boundaries of older lesions (Pruvost et a1., 2002).

Taxonomy of Xanthomonas axonopodis pv.citri

Citrus canker (CC) also known as the Asian citrus canker (ACC), was first found in US during an epidemic condition in several states of southeastern US in early 1900s (Stevens, 1914). Specimens were collected by Hasse from different states like, Florida, Mississippi and Louisiana in 1914 and bacterium was successfully isolated (Hasse, 1915). Hasse identified the bacterium as Pseudomonas citri after experiments on characterization and pathogenicity. (Hasse, 1915). Since then, the bacterium has been identified as Phytomonas and then Xanthomonas citri and placed in different genera. (Dowson, 1939). Strains of this species were kept as A strains which shows that these have associations with ACC. Two more strains of Xanthomonads which cause CC were discovered in the 1970s and were put in group C strains that cause canker in Key lime and group B strains with a broader range of host strains. (Rosetti, 1977). Until 1978, the bacterium was called as X. citri. Then it was given name as X. campestris pv. citri. (Young et al., 1978). Afterwards, Gabriel et a1. (1989) transferred the bacterium to X. citri but the strain B and C were designed as Xcc. Young et al., (1978) argued that there was a need of additional research to position CC strains in X. Afterwards, the bacterium strain was moved to X. compestris pv aurantifolii (Young et al., 1991). Using DNA–DNA hybridization (DDH) method, strains A were referred as Xac and B, C strains as Xaa by Vauterin et al., (1995). S1 nuclease techniques were used by Schaad et al., (2005) to place the Xac strains in X. smithii and the Xaa strains in X. fuscans. Thereafter, the recommendations were revised by Schaad et al. (2006) to put these bacterial strains in Xc in year 2007. Recently, major alterations to taxonomy of Xanthomonads were suggested by Constantin et al. (2016). They suggested that, a range of pathovars can be added to X by the use of a polyphasic approaches (Constantin et al., 2016). For the moment, both Xac and Xfa are the current nomenclature names of citrus canker causing bacteria.

Management approaches towards Xanthomonas axonopodis pv.citri (Xac)

Control measures include eradication across regions where citrus canker is not yet widespread, minimizing spread, reducing inoculum sources, and protecting vulnerable tissues from infection. (Behlau et al., 2016). For areas where citrus canker has become widespread and elimination is suitable no more, action is taken to manage disease and to prevent or reduce plant loss. An integrated citrus canker management program involves planting citrus canker-free nursery stocks, selecting resistant cultivars, deploying arboreal windbreaks, spraying with copper-based bactericides, controlling citrus leaf miner (CLM) and applying systemic acquired resistance (SAR) inducers (Behlau and Belasque., 2014). Primary control method of citrus canker is copper based bactericides. When climatic conditions favour the pathogen and young plants are rich in number then copper application is adopted during summer and spring season. Fixed or insoluble copper forms like copper hydroxide, copper oxychloride, and copper oxide are the most commonly used types. Fixed copper is less phytotoxic to plants due to the slow release of copper ions and has good residual action against Xac than non-fixed copper. If conditions are favorable to CC outbreaks, copper sprays and wind breaks must be collectively to manage the disease. Even with windbreaks, daily copper reapplications are necessary to save fruits that are continually expanding over a period of 90–120 days, depending on the citrus cultivar. (Behlau et al., 2010). Amamectin and neonicotinoid insecticides are mainly used to control the larvae in the mining industry. (Powell et al., 2007). In former studies, neonicotinoid soil formulations were extremely productive in minimizing foliar disease and defoliation caused by CC on grapefruit trees (Graham and Myers 2013). Systemic neonicotinoid insecticides such as imidacloprid, thiamethoxam, and clothianidin can be used year-round as soil drenches on non-bearing citrus trees to control CLM and the leaf miner infection associated with Xac. Soil-applied neonicotinoid insecticides are applied in newly developed orchards orchards (up to 3 years), while amamectin can be applied on trees of every age. When detecting the first galleries of CLM mined leaves, usually in the feather leaf stage, foliar-applied insecticides give shield of a shorter period as compared to soil applications and are applied during the summer and autumn to safe leaf growth. Neonicotinoids take much time to reach the canopy and these insecticides must be performed approximately 14 days before leaf blushes when the CLM larvae start feeding (Rogers et al., 2015). Biological control of CLM is less effective. The famous and studied natural enemy of CLM is the wasp Ageniaspis citricola, and though this predator may have extreme levels of parasitism, it has not been shown to be an equally effective alternative to the use of chemical sprays, particularly when CLM is highly infested. (Hoy and Jessey, 2004). Streptomycin and zinc oxide are other chemicals that have shown positive results but usually are not used due to different causes to control citrus canker. While it has been shown that copper-based bactericides are successful in controlling citrus canker. They cannot fully suppress the inoculum, so, regular applications are needed throughout the spring and summer to minimize losses. (Behlau et al., 2017).Use of copper biocides is a common method to control disease, however, Xac has plasmid-borne genes that make it resistant to copper sprays and is a limiting factor in the long term use of copper. D-Leucine and 3-indolylacetonitrile (IAN) also inhibit Xac from biofilm production and increase the sensitivity of copper sulfate ($CuSO_4$) treatment. In addition, IAN has inhibited chemotaxis and motility gene expression. (Li and Wang, 2014). Applying ASM for SAR induction has also shown effectiveness in reducing the incidence and severity of citrus canker (Graham and Myers, 2013). Control of Xac through foliar chemical applications that activate the host plant's systemic resistance does not seem to be particularly effective on its own (Graham and Leite, 2004). SA foliar therapies reduce lesion incidence (Wang and Liu, 2012).

Future Perspective:

Citrus canker is potential threat to all the cultivars of citrus throughout the world, caused by several strains of Xac and new strains also emerge due to mutation. So, there is dire need of further investigation on the strains identification.

There is a great similarity among the strains, so there is a need to identify different strains by using modern techniques.

Better understanding of aggressive strains can help us in identification of a resistant source against citrus canker.

The mode of entry and spread of pathogen should be further studied for better understanding of biology and ecology of pathogen.

As there are different environmental conditions in different citrus growing areas, so, there should be further research on strains of different environmental conditions.

Citrus canker has been controlled effectively by chemicals for a long time but due to residual effects of chemicals, there is a need of some alternative approaches to manage the disease.

Certain antagonists and plant extracts are evaluated against Xac but there is a chance that they may be effective in lab but not effective in field. So, the efficacy of biological agents should be studied.

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