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STUBBORN DISEASE OF CITRUS CAUSED BY *SPIROPLASMA CITRI*: A SHORT NOTE

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ABSTRACT

Citrus fruit is considered a very nutritious and delicious diet. Citrus consists of lemon, oranges, mandarin, and grapefruit which have economic value in the world. Significant losses occur in citrus due to numerous diseases. Among various diseases, citrus trees are seriously affected by a phytopathogenic mollicute *Spiroplasma citri* which causes stubborn disease. *S. citri* is transovarially transmitted by several leafhopper species. Symptoms include smaller and cupped leaves, small size, crook fruits with aborted seeds. Moreover, irregularity on the fruits such as different sizes, shapes, and typically lighter, smaller fruits than its healthy counterpart has also been observed. The affected fruits often drop before maturity. The color inversion is often seen with the stylar end remaining green and the peduncle end showing color. Various molecular and biochemical tests are conducted to identify *S. citri*. Under *in-vitro* conditions, *S. citri* grows on SP4 media where a fried egg-like shaped colony is observed. Keeping the importance of the stubborn citrus disease, the present short note briefly described the symptomology, detection, transmission, and management.

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INTRODUCTION

Citrus belongs to one of the largest groups of trees and shrubs. Citrus belongs to the genus *Citrus* L. and the family *Rutaceae*. It is believed that the southern Himalayas, China, and North East of India were the origins of citrus. Citrus trees are grown in tropical and subtropical climates. Citrus and citrus products are good sources of vitamin C, dietary fiber and minerals that are crucial for growth and development (Iftikhar et al., 2020). In addition, it contains other nutrients like carbohydrates, folate, thiamine, potassium, calcium, niacin, phosphorous, copper, vitamin B, magnesium, and several phytochemicals. As a plant food, it does not contain cholesterol, sodium, and fats. It is directly used as a fruit as well as juice. Citrus fruit is added to drinks and food to enhance the flavour of food (Mubeen et al., 2015a; Mubeen et al., 2015b). About 140 countries cultivate citrus fruit all over the world. In the world, Brazil is the biggest citrus-producing country. Other prominent citrus-producing countries are USA, Spain, Japan, Israel, Turkey, Egypt, Cuba, South Africa and Pakistan (Anwaar et al., 2020). Mandarin, sweet lime, bitter orange, grapefruit, lime, and lemon are commercially available globally. Significant losses occur in citrus crops due to biotic and abiotic factors. Citrus trees are affected by several viruses, bacteria, fungi, and virus-like diseases (Mubeen et al., 2015b). Among these diseases, citrus trees are seriously affected by a prokaryotic pathogen, *S. citri* that causes stubborn citrus disease (Gumpf and Calavan, 1981).

The stubborn disease was referred to as a disease in California in 1944 (Bové and Garnier, 2002). The arid and hot regions viz. Arizona, Mediterranean Basin, California, North Africa and the Middle East are highly affected by stubborn citrus disease caused by *S. citri*. In cold areas, it does not cause devastating effects. PCR and RFLP are used to detect Spiroplasma and phytoplasma. It is a severe and lethal pathogen that harmed the citrus industry (Yokomi et al., 2008). The insect vector, dodder, and grafting are involved in disease transmission. The trees with severe infection produced smaller and fewer fruits and had lower yields (Lee et al., 2006). The industry scale of the citrus market is about one trillion dollars, contributing most of the agricultural income (Lee et al., 2019). Therefore, the management of citrus cultivation to maintain citrus productivity and profitability is very important. This review will give a quick rundown of *S. citri* and its impact on citrus production, cultivation, and management of stubborn disease.

The causal pathogen

The prokaryotic pathogen *Spiroplasma citri* causes citrus stubborn disease. *S. citri* belongs to class *Spiroplasmacae*, family *mollicutes*, having spiral morphology. It is a cell wall-less bacterium in the class *mollicutes* that moves slowly in the phloem sieve tubes of the tree. Among mollicutes, Spiroplasma has well-defined morphology. *S. citri* has the largest genome with low G+C content. The obligation of cholesterol for growth, complete resistance to penicillin, low guanine and cytosine contents of cellular deoxyribonucleic acid (DNA), small genome size, are among the numerous cultural properties (Gumpf and Calavan, 1981).

Detection of *Spiroplasma citri*

S. citri can be detected based on culturing procedure in liquid media and can be identified under a dark field microscope (Wei et al., 2021). In low agar media, the spiroplasma form fried egg fuzzy colonies with occasional surrounding satellite colonies because of their ability to move through the agar matrix (Abd El-Fatah et al., 2016). Serological based detection methods have also been used to detect *S. citri* in the field (Umar et al., 2020). Polymerase chain reaction (PCR) is a better technique to detect *S. citri* in infected plants with 100-1000-time sensitivity (Yokomi et al., 2008). The nested and single PCR and RFLP (restriction fragment length polymorphism) have been applied for the detection of *S. citri* based on primers (spiraline) gene sequence (Fletcher et al., 2006).

Symptomatology

The symptoms on mature trees are usually less noticeable. Under field conditions, diagnosing the stubborn disease is difficult due to symptom resemblance with a nutritional deficiency (Iftikhar et al., 2020). During early infection in the plant, *S. citri* used sterol and carbohydrates that cause mottling and stunting. On the leaves, the symptoms include smaller and cupped leaves and are smaller in size. Affected trees have short internodes and produce small and fewer fruits. The tree gives the appearance of witches's broom. The infected trees habitually produce few fruits which are smaller in size and acorn-shaped (Figure 1). Even when fruits are mature, they habitually do not color at the end of the stem, and seeds are also aborted. The fruits become differing sizes, shapes, and typically lighter, smaller than their healthy counterparts, crooked and have aborted seeds. The colour inversion is often seen with the stylar end remaining green and the peduncle end showing color. The leaves are smaller, cupped, and often have a vertical appearance and become unnaturally thick, mottled, or have a chlorotic pattern (Shi et al., 2014).

Transmission

The primary vector of stubborn disease is the leafhopper. This is a plant-sucking insect that has piercing mouthparts. The insect feeds into the phloem of the plant (Roistacher, 1991). The beet leafhopper transmitted virescence agent (BLTVA) and yellow aster were two distinct phytoplasma diseases. BLTVA yellows initially cause mild chlorosis, lower leaves become purple, excessive side root proliferation and premature flowering. The graft, mechanical, and dodder transmission are also involved. This is a phloem limited pathogen and transmitted by graft propagation (using infected scion, rootstock, or transfer from an infected plant to a healthy plant (Gumpf and Calavan, 1981; Lee et al., 2006).

Figure 1: Hard appearance like the stone of fruit and aborted seeds **(A)**; Infected cupped leaves, thick, mottle or had the chlorotic pattern **(B)**; Infected trees had stunted growth **(C)** and produced small size fruits **(D)**

Management

Preventing *S. citri* from accessing and infecting young, vulnerable plants is the most efficient method to avoid stubborn citrus disease. Several cultural traditions are the most effective means of doing this. *S. citri* is spread by a variety of insect vectors, including the beet leafhopper. Planting trap plants, such as sugar beets, which the insect vectors prefers but are not susceptible to obstinate citrus disease nearby, to attract disease-carrying insects away from citrus trees, is one of the successful strategies against the beet leafhopper (Mello et al., 2010a; Mello et al., 2010b). A chemical component may eliminate the insect vector and prevent enhanced efficacy by spraying pesticides on the trap plants, eliminating the insect vector, and preventing the bacteria from reaching the citrus crop. Because older trees are less vulnerable to *S. citri* infection, it is important to avoid infection while the tree is still growing. Citrus trees under six should be destroyed since they will never be productive. In contrast, diseased trees older than six should be individually assessed and either have symptomatic portions removed or completely replaced with a healthy plant. The obstinate citrus disease may be transmitted via grafting. Therefore, make sure the mother tree is clear of *S. citri* before propagating it. To avoid introducing *S. citri* into an orchard, plants should be purchased from regions where the disease is not present. Furthermore, it is critical to keep a careful eye on weeds in new orchards to ensure that they are not vulnerable hosts of *S. citri* and eliminate any susceptible ones as soon as possible (Mello et al., 2010b).

Conclusions and future prospects

Citrus stubborn is one of the major diseases of citrus throughout the citrus growing regions of the world. The disease is transmitted by grafting therefore, make sure that grafts/buds should be free from the pathogen. Moreover, the leafhoppers that are vectors of stubborn citrus disease should be controlled eco-friendly to minimize pathogen transmission to the citrus orchards.

The pathogen's cultural, biochemical, biophysical, and genetic properties should be investigated to find a weak point so that this pathogen can be managed to avoid losses to citrus.

AUTHOR'S CONTRIBUTION

All the authors equally participated in collecting, organizing, writing and editing the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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