



## RESEARCH PAPER

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## Economical and quick indexing of citrus greening disease in Sargodha, Pakistan

Ashara Sajid<sup>\*1</sup>, Muhammad Usman Ghazanfar<sup>1</sup>, Zahoor Hussain<sup>2</sup>, Salman Ahmed<sup>1</sup>, Yasir Iftikhar<sup>1</sup>

<sup>1</sup>Department of Plant Pathology, College of Agriculture, University of Sargodha, Pakistan

<sup>2</sup>Department of Horticulture, College of Agriculture, University of Sargodha, Pakistan

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### Abstract

Citrus greening disease (CGD), a century old prokaryotic disease is one of the most destructive and widely distributed diseases in the citrus growing areas of the world. This disease has become a serious threat to citrus industry. Zinc spray gives a clue for the disease as persistence of symptoms even after the foliar application. The early detection of CGD is one of the key steps to manage the disease in time. Therefore, during a survey in 2018-2019, the study was carried out for routine, economical and quick detection of CGD in infected samples collected on the basis of symptomology. The samples were collected from the citrus orchards infected with greening disease. Iodo-starch test was performed to observe the accumulation of starch in the symptomatic leaves. Moreover, the infected bud-wood were grafted on healthy plants showed the mottling symptoms after 6-8 months. Dark grey edges of the infected leaves indicated the accumulation of starch. Among the five varieties as indicator, Sweet orange, kinnow and Feutralls' early showed the mottling symptoms on indicator citrus plants. This confirmed the transmissibility of CGD and iodo-starch test was found reliable along with symptomology and vegetative propagation for early and quick detection of CGD. The iodo-starch test was effective as backed up by vegetative transmission.

\*Corresponding Author: Ashara Sajid ✉ [phytdoctor123@gmail.com](mailto:phytdoctor123@gmail.com)

## Introduction

There is diversified pathogen community in citrus plantation and have their role in citrus decline. Citrus decline is a complex, contributed by many biotic factors. Viruses and nematodes have a vital role in citrus decline and comprehensive screening against nematodes has been done previously (Rehmal *et al.*, 2020). Among them viruses and prokaryotes are key pathogens which cause disease resulting in the decline of citrus orchards. Citrus greening, a prokaryotic disease is graft-transmissible systemic infection. Citrus greening disease (CGD), also known as “Huanglongbing (HLB)” is well known century old disease probably originated in china where it was named yellow shoot disease (Akhtar and Ahmad 1999; Batool *et al.*, 2007; Iftikhar *et al.*, 2016). Since then, the disease has been detected through different assays and reviewed by different researchers (Batool *et al.*, 2007; Bove 2006, Graca 1991; Iftikhar *et al.*, 2016). CGD is a widely distributed and devastating disease in all citrus growing areas of the world. Three strains *Libriobacter candidatus asiaticum*, *africanus* and *americanum* are prevalent in Asia, Africa and Brazil respectively (Batool *et al.*, 2007). In Pakistan, like other crops CGD is infecting citrus at a large scale. The disease has been detected in Pakistan for the first time in 70s’ (Cochran 1976). Further, the disease was characterized by Akhtar and Ahmad (1999) on the basis of symptoms. Moreover, the pathogen was detected molecularly confirmed the presence of CGD in Pakistan (Chohan *et al.*, 2007).

As the pathogen is gram negative, uncultrable phloem limited bacterium, therefore, early and quick detection was the need of time and many scientists worked on it and few has success stories (Etxeberría *et al.*, 2007; Razi *et al.*, 2012). The pathogen was successfully transmitted through grafted (Batool *et al.*, 2007; Bove 2006). Although different studies have been carried out to monitor the disease and its detection through symptoms and PCR but early and economical detection and its reliability is the need of time in Pakistan. Therefore, the study was carried out with the aim to follow-up the efficiency of already established quick indexing protocols for the CGD detection.

## Material and methods

### *Symptomology and Iodo-starch test*

A survey was carried out in the citrus orchards of Sargodha district to collect the citrus samples from diseased plants. The infected samples were collected from kinnow mandarin because of vast cultivation in citrus orchards. Symptomology was the basic criterion to collect the samples. Host plants were marked for future investigations. Suspected to be infected samples were brought to the laboratory in sterile polythene bags. Iodo-starch test as described by Etxeberria *et al.*, (2007), was performed for quick indexing followed by confirmation through graft transmission. During the samples collection for iodo-starch test, following points were kept in mind;

- i. The spray of zinc sulphate was applied to avoid the zinc deficiency symptoms.
- ii. The symptomatic leaves from branches were not detached or wounded and collected from sun directed part of the citrus trees.

### *Transmission of CGD*

Five citrus species Viz., Kinnow, Feutrell’s Early, Musambi, lemon and lime as indicator plants were used for the vegetative propagation (Razi *et al.* 2012). Infected bud wood were collected from marked citrus host plants and grafted on the indicator plants. Indicator plants were kept in insect free area to observe for symptoms development for 6-8 months.

## Results and discussion

Initial detection through characteristic symptoms, as described by Batool *et al.*, (2007) and Polek *et al.*, (2007), was carried out. Symptoms were persisted even after 21 days of zinc foliar application. Mottling of leaves and color inversions were the most common along with lopsided fruits in most of the cases. Yellowing of leaves and seed abortion were the other symptoms (Fig. 1). Iodo-starch test showed the dark grey areas at the edges in CGD infected leaves as compared to healthy leaves (Fig. 2). Transmission trails showed the successful transfer of disease from diseased plant to healthy plant in 3 varieties out of 5. Maximum disease transmission was in musambi in which 7 out of 10 plants showed the mottling symptoms (Table 1). Mottling and crinckling symptoms were appeared after 7 weeks of grafting (Fig. 3).

**Table 1.** Transmission of citrus greening disease in different varieties.

SN	Varieties	Total Number of Plants	Number of Plants Infected	% Infection
1	Kinnow	10	6	60
2	Feutrrall's Early	10	5	50
3	Musambi	10	7	70
4	Lemon	10	0	00
5	Lime	10	0	00

**Fig. 1.** a) Mottling, b and c) Lopsided fruit and color inversion.**Fig. 2.** Iodo-starch test showed the dark grey area indicated by arrow.**Fig. 3.** Leaves of citrus plant grafted with CGD infected bud wood showing mottling.

Iodo-starch test was developed by Etxeberria *et al.*, (2007) and found its reliability in case of early detection (Saifullah *et al.*, 2015). Our findings were in accordance with previous results. our results also revealed the efficacy of the iodo-starch test as confirmation through vegetative transmission of disease. The method was easy, quick and economical as compared to serological and molecular detection.

Although serological and molecular assays are sensitive and have more accuracy but costly and cannot do large number of samples in quick time and in economical way. The iodo-starch test was also supported by graft transmission that will be helpful for initial, quick and effective identification of disease to formulate the management strategies. Grafting is a conventional but reliable method not only for disease transmission but also disease identification and host reaction. Citrus greening disease was also transmitted when infected bud wood was grafted on healthy plants of different varieties. Our results were also confirmed the previous studies (Bove *et al.*, 1996; Lopes and Frare 2008; Razi *et al.*, 2012), where CGD was successfully transmitted to different citrus cultivars. The result of Razi *et al.*, (2012) revealed the successful transmission of GCD on local citrus germplasm in Pakistan. Economical, quick and easy detection of citrus greening disease is not only the aim of scientists and progressive farmers but also helpful in formulating of management strategies.

### Conclusions

Citrus greening disease has been a devastating disease of emergence and re-emergence importance in all citrus growing areas of the world. The early quick detection in field plays an important role to formulate the management strategies. Moreover, lemon and lime were tolerant towards CGD. Serological and molecular assays are more sensitive and expensive but some chemical assays like iodo-starch test are not only economical but also reliable.

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### Authors contribution

MUG and ZH conceived the idea. AS executed the research plan and SA helped in paper write up and proof reading.

### Compliance with ethical standards

The authors declare that the review is in compliance with ethical standards of the journal.

### Conflict of interest

The authors declare no conflict of interests and give their consent for publishing the material.

### Research involving human participants and/or animals

The authors declare that the manuscript does not contain research involving Human Participants and/or Animals.

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