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Graft Transmission and Study the Symptom Pattern of Citrus Greening Pathogen on Indicator Plant

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A B S T R A C T

Citrus fruits belonging to the family Rutaceae, occupies a place of considerable importance in the fruit crop due to high economic returns. Citrus greening is one of the most severe disease of citrus. Citrus greening disease was presumed to originate in china during the 1890 (Graca 1991). It is evident that the citrus disease has emerged as a potential series threat to citrus production in all the citrus growing districts of Maharashtra. It has been proved that in Maharashtra the greening disease is the major causes a decline in citrus production. Therefore attempts were made to study the detection by transmission studies the greening pathogen. In transmission studies, sweet orange indicator plant exhibited leaf mottling and interveinal yellowing symptoms from Mosambi bud source plant and leaf mottling symptoms from Kamla bud source plant. The acid lime exhibited interveinal yellowing and vein yellowing from Mosambi bud source plant, while leaf chlorosis from kamla bud source plant. Tangelo indicator plants exhibited leaf mottling and vein yellowing symptoms from bud source of Mosambi and leaf chlorosis and leaf mottling symptoms from bud source Kamla.

Introduction

Citrus greening is the destructive G-proteobactria disease of citrus found in Asia, Africa, Peninsular (Garnier and Bove, 1996) being and important disease of family Rutaceae. It affects production of citrus in several parts of India including Maharashtra, which is major state of citrus production. This disease is generally transmitted by citrus psylla, *Diaphoria citri kuwayama.* The psylla cab be observed in all South-East Asia, India, Mauritius, Brazil (Halbert and Manjunath, 2004). In areas where the disease is endemic, citrus trees live for 5-8 years and never produce usable fruits (Roistacher, 1996). Citrus greening pathogen is transmitted by the insect vector, citrus psylla. They can also be transmitted by grafting, by dodder and possibly by seed. Even though the pathogens are bacteria, the disease does not spread by causal contamination of personnel and tools or by wind and rain. Citrus greening is caused by phloem limiting bacteria in the genus *Candidatus Liberibacter*. Three species are described, including *Candidatus Liberibacter asiaticus*, *Candidatus Liberibacter africanus* and *Candidatus Liberibacter americanus* (Texeira *et al.*, 2005).

In Maharashtra citrus greening is considered very important disease. About 54.26 thousand

ha a citrus cultivation area produce 659.15 thousand million tones (Agricultural statistics at a glance 2016). This repot was also show that most of the local citrus verities or species were infected. In citrus greening infects most of the citrus species, hybrids, cultivars and some citrus relatives. It severely affects most sweet oranges, mandarins and mandarin hybrids, as well as some citrus relatives such as Atalantia, Balsamocitrus, Calodendrum, Clausena fortunella, Microcitrus, Murraya, Poncirus, Severinia, Swinglea, Todalis and Triphosia (Halbert and Manjunath, 2004). *Murraya panniculata* is a host for the greening pathogen in Brazil but not in Taiwan.

The disease symptoms are observed in four forms i) Mottling ii) Interveinal yellowing or Zinc deficiency like symptoms iii) leaf yellowing and green island and iv) blochy mottle. Several orchard plants of the citrus germplasm at the Department of Horticulture, Central Campus, M.P.K.V., Rahuri (Maharashtra) exhibited symptoms similar to citrus greening. Greening disease is known to affect several varieties belonging to the citrus group and reduce its productive life in subsequent seasons. Therefore this study is intended to assess the citrus greening in different citrus species to study the symptom pattern, transmission studies, reaction of citrus varieties against greening disease and biochemical changes associated in the host.

Materials and Methods

Collection of samples

Diseased bud sticks of sweet orange var. kamla and mosambi were collected from different citrus species from citrus germplasm orchard of the Mahatma Phule Krishi Vidyapeeth, Rahuri (Maharashtra). For this the secateurs of budstick were disinfected with 1.5% sodium hypochlorite solution when moving from one tree to another. Disease samples were labeled appropriately and placed in plastic bag and kept in refrigerator at 5-6 ^{0} C until use. These diseased samples were brought to the net house in paper bags and transmission tests were made by budding method on the indicator plants *viz.*, sweet orange (*C. sinensis*), acid lime (*C. aurantifolia*), and tangelo (*C. reticulate x C. paradisi*) within 24 hours of collection.

Seedling Preparation

The seedlings were raised by collecting seeds from healthy fruits, treated with 0.1% Carbendazim and sown in pot culture conditions in sterilized polythene bags. The sterilized potting mixture of soil: farm yard manure:sand was used in 3:1:1 proportion for raising the seedlings.

Seedlings of one year age having pencil size thickness of stem portion from different citrus species *viz;* sweet orange (*C. sinensis*), acid lime (*C. aurantifolia*) and tangelo (*C. reticulata X C. paradisi*) were raised from seed, for conducting the transmission test by inoculation with the 'T' budding or shield budding method on these indicator plants.

Bud transmission

The seedling prepare for experimental purpose when attained one year age and pencil size thickness of stem portion with the help sharp knife previously disinfected with 1.5 % sodium hypoclorite was used to make a slice into the stem about 8-10 cms above soil using a continuous slicing motion taking care not to injure the xylem portion of seedling. The bud of mosambi and Kamla citrus species was cut free of the budstick with one smooth slicing stroke of knife (previously disinfected with 1.5% Naclo) and were inserted into the incision made on the seedling of sweet orange, acid lime and tangelo. The "buds"

were secured to the stem by wrapping with 200 gauge polyethylene grafting tape taking care to stretch the tape while wrapping the bud. The seedlings were irrigated after completing of inoculation and maintained under temperature ranging from 25-30^{0}C and humidity 70-80 percent (Plate 1,2). Negative controls were incorporated in each inoculation test (Roistacher, 1991).

Transmission and symptomatology studies

The wrapping tapes were removed 21 days after inoculation, by cutting the tape with a previously disinfected knife or razor blade and the inoculum examined for survival. The seedlings are also cut back or topped to 20-25 cms from soil level. The time in weeks from inoculation to appearance of first symptoms under optimum growth and temperature conditions was noted. Observation on date of inoculation, source of inoculums, indicator plant used was also taken. Field symptoms were recorded from different citrus species of age 19 years from the orchards maintained at the All India Coordinated Research Project on Citrus, Department of Horticulture, MPKV, Rahuri (Maharashtra).

Results and Discussion

The present studies were carried out in respect of transmission and symptomatology studies of citrus greening, reaction of citrus varieties against greening pathogen under in vivo conditions. The results presented here are as under.

Transmission studies

Transmission were made by using the 'T' budding or shield budding method on indicator plants *viz.,* sweet orange (*C. sinensis*), acid lime (*C. aurantifolia*) and tangelo (*C. reticulate x C. paradisi*) by collecting the bud sticks from diseased plants of the sweet orange varieties Mosambi and Kamla. The indicator plant symptom represent characteristic symptom produced by the greening disease and used for diagnosis or confirmatory for the presence of *Candidatus Liberibacter asiaticus* pathogen in the citrus tree plants, since the bacterium is known to be an unculturable pathogen**.**

Indicator plants symptoms

The symptoms exhibited on the indicator plants *viz*., sweet orange, acid lime and tangelo are presented in Table 1. The sweet orange indicator plants exhibited the characteristic symptoms of greening from the sweet orange varieties Mosambi and Kamla buds in 101 and 105 days respectively (Plate 3). The symptoms from Mosambi source plant include leaf mottling and interveinal yellowing while symptoms from Kamla source plant were leaf mottling on Sweet orange. The acid lime indicator plants exhibited typical symptoms of greening disease from bud source of Mosambi and Kamla plants in 106 and 107 days respectively (Plate 4). The symptoms from Mosambi bud source included interveinal yellowing and vein yellowing, while Kamla source plant symptoms included leaf chlorosis on acid lime. The indicator plants of tangelo showed symptoms of disease from Mosambi and Kamla bud source plants in 110 and 115 days respectively (Plate 5). The Mosambi bud source symptoms included leaf mottling and vein yellowing, while the Kamla bud source could produce leaf chlorosis and leaf mottling on tangelo.

Citrus greening is one of the most devastating diseases of affecting citrus cultivars worldwide and in India, leading to citrus decline. It is caused by an unculturable bacterium designated as *Candidatus Liberibacter asiaticus* (Garnier *et al.,* 2000). The disease is transmitted in the field by

tissue grafting and its detection is made by indexing on indicator plants like sweet orange and acid lime, and is the practical method for confirmation for its presence in field trees (Nariani, 1985). It is also detected by the florescent marker and by Polymerase Chain Reaction techniques. The plants can be affected at any stage in the field because of its transmission by the psylla insect vector, *Diaphorina citri*. Various citrus species and varieties are severely affected by the disease and symptoms range from yellowing of the foliage, leaf mottling and micronutrient deficiencies (Jagdish Chandra and Singh, 1997). The present investigations were therefore attempted on transmission studies, symptomatology, varietal reaction to pathogen.

Transmission studies

The transmission studies were carried out using indicator plants under in vivo conditions. The pathogen in different citrus varieties were transmitted through 'T' budding from varieties of Kamla and Mosambi of sweet orange varieties which are highly susceptible to the citrus greening disease (Gupta and Baranwal, 2017). They were inoculated on sweet orange, acid lime and tangelo indicator seedlings to assess the presence of *Candidatus Liberibacter asiaticus* in the field plants of sweet orange when used as bud source. These indicator plants expressed diagnostic symptoms of citrus greening from different sources of inoculums of citrus varieties.

Plate 1 : Citrus indicator seedlings inoculated and raised in insect proof condition

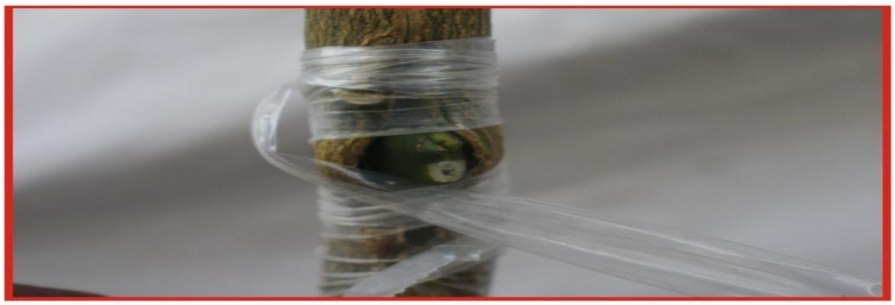

Plate 2 : Bud inoculum of greening source plant inoculated and affixed with grafting tape on Indicator seedling

Healthy sweet orange plant

From source mosambi

From source kamla

Plate 3: Indicator plants of sweet orange showing symptoms of citrus greening

Healthy acid lime plant

From source mosambi

From source kamla

Plate 4 : Indicator plants of acid lime showing symptoms of citrus greening

Healthy tangelo plant

From source mosambi

From source kamla

Plate 5 : Indicator plants of tangelo showing symptoms of citrus greening

Table.1 Reaction of different indicator plants to greening disease

Indicator plants	Source plant	Symptoms	Incubation period (Days)
Sweet orange	Mosambi	Leaf mottling, Interveinal yellowing	101
Sweet orange	Kamla	Mottling	105
Acid lime	Mosambi	Interveinal yellowing, vein yellowing	106
Acid lime	Kamla	Leaf chlorosis/yellowing	107
Tangelo	Mosambi	Mottling, vein yellowing	110
Tangelo	Kamla	Leaf chlorosis, Mottling	115

The present investigations are in agreement with the findings of Chen (1943) who transmitted the disease experimentally by grafting which result into establishment of the causal agent as pathogen. Capoor (1966) and Choudhari *et al.*,(1980) have observed greening to be transmitted by grafting in healthy seedlings of sweet orange, Rangpur lime, acid lime, and grape fruit cv Marsh seedless when raised in green house.

The results are similar to findings of Nariani *et al.,* (1967) who found variation in transmission of greening agent when buds from sweet orange were used as inoculum. Greening agent was transmitted by bud or wedge grafting. Varied greening transmission percentage was observed by Lopes *et al.,* (2011) for *Candidatus Liberibacter asiaticus* when budsticks from field greening affected trees used as inoculum sources. Evers *et al.,* (1992) reported that citrus greening was graft transmitted to healthy, green house grown indicator seedlings of *C. sinensis*, *C. aurantifolia* and other species.

Symptomatology

Indicator plants

The indicator plants of sweet orange exhibited leaf mottling and interveinal yellowing symptoms exhibited within 101-105 days. The interveinal yellowing, vein yellowing and leaf chlorosis were the indicator plant symptoms observed within 106-107 days in acid lime. The tangelo indicator plants showed leaf mottling, vein yellowing and leaf chlorosis symptoms from different inoculum sources in 110-115 days.

Lopes and Frare (2008) observed that several inoculum sources produced leaf symptoms and symptoms similar to those of iron, manganese and zinc deficiencies, but blotchy mottle leaves were found characteristic symptoms of sweet orange on indicator plants. Jantasorn *et al.*, (2007) reported that different samples of citrus produced symptoms, and newly emerging leaves were yellow, showed blotchy mottle for the presence of disease.

The indexing results of present investigations revealed similar symptoms found on various indicator plants

When a indicator plant sweet orange, acid lime and tangelo inoculated with pathogen *Candidatus Liberibacter asiaticus* produced symptoms like leaf mottling, interveinal yellowing, vein yellowing and leaf chlorosis within 101 to 115 days after bud inoculation of mosambi and kamla.

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