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## Responses of different genotypes of citrus to *huanglongbing* (Citrus Greening) under field condition

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### Abstract

Citrus fruits belonging to the family Rutaceae, occupies a place of considerable importance in the fruit crop due to high economic returns. The citrus greening disease was observed in citrus germplasm orchard of All India Coordinated Research Project on Citrus, Department of Horticulture Mahatma Phule Krishi Vidyapeeth, Rahuri. Therefore attempts were made to study the detection by transmission studies, reaction of citrus varieties against the greening pathogen. In the present study, nineteen species/varieties of citrus were screened for their reaction against the citrus greening disease under field conditions. In field plant symptoms Leaf chlorotic mottling were the most diagnostic type of symptoms and was followed by vein yellowing. Fully matured leaves showed the most reliable symptoms of leaf mottling viz., yellowing along the midrib and leaves veins which spread in the adjacent tissues to produce a mottled effect. Such symptoms were observed in the citrus species of *C. grandis*, Tangerin ling ming, unidentified hybrid (Rough lemon *Chettalli* x *Cleopatra mandarin*) and *C. limon*. In case of varietal reaction of different citrus varieties or species to citrus greening diseases, significantly highest percent disease incidence (100 %) was observed on leaves of *C. sinensis*, *C. reticulata*, tangelo, *C. grandis* and *C. jambhiri*. Whereas, the *C. paradisi* (90 %), *C. limonia* (86.67 %) and *Citrus reshni* (63.33 %) showed highly susceptible reaction.

**Keywords:** Citrus greening, Citrus varieties, Field plant symptoms, Field condition, Varietal reaction

### Introduction

Citrus is believed to have originated from the region within Northeast India, South China, Indonesia and Peninsular Malaysia. It is an extremely important crop on a world basis and the total world production of citrus was estimated at over 73 million metric tons (FAO, 2003). Citrus greening disease or huanglongbing is one of the serious citrus diseases in the world. It is a major limiting factor for citrus production in parts of Asia and Africa. In areas where the disease is endemic, citrus trees live for 5-8 years and never produce usable fruits (Roistacher, 1996). Citrus greening pathogen are transmitted by the insect vector, citruspsylla. 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).

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*, *Clausenafortunella*, *Microcitrus*, *Murraya*, *Poncirus*, *Severinia*, *Swinglea*, *Todalis* and *Triphosia* (Halbert and Manjunath 2004). *Murraya paniculata* 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 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.

Considering the potential of pathogen to cause severe losses, it was felt necessary to investigate on the citrus greening in different citrus species to study the symptom pattern, transmission studies, and reaction of citrus varieties against greening disease.

## Materials and Methods

### Varietal reaction

The citrus species maintained in the orchard of Department of Horticulture, M.P.K.V., Rahuri were evaluated against the pathogen *Candidatus liberibacterasiaticus* causing citrus greening. Nineteen citrus species were evaluated against the pathogen under natural field conditions viz.,

1. *Citrus sinensis*
2. *Citrus reticulata*
3. *Citrus reshni*
4. Tangelo (*C. sinensis* X *C. paradisi*)
5. *Citrus aurantifolia*
6. *Citrus limonia*
7. *Citrus limon*
8. *Citrus paradisi*

9. *Citrus grandis*
10. *Citrus jambhiri*
11. *Citrus aurantium*
12. *Citrus mandurensis*
13. *Tangerin ling Ming*
14. *Mexivica Brazil*
15. *Poncirus trifoliata*
16. *Troyer citrange Australia*
17. *Aeglemarmelos*
18. *Citrus medica*
19. *Severiniabuxifolia*

For this, ten leaves were scored on each twig for the greening disease based on 0-5 scale. The observations for greening were recorded on 5 twigs of each replication by adopting standard methodology.

**Table 1:** Scoring scale for the citrus greening diseases

Scale	Particulars	Reaction
0	No infection	Highly resistant (HR) or Immune (I)
1	Less than 10% incidence	Resistant (R)
2	10.01 to 20% leaf area infected	Moderately resistant (MR)
3	20.01 to 40% leaf area infected	Moderately susceptible (MS)
4	40.01 to 60% leaf area infected	Susceptible (S)
5	61% and above leaf area infected	Highly susceptible (HS)

The per cent disease index was calculated according to the standard formula

$$\text{Percent chlorosis} = \frac{\text{Total number of infected leaves}}{\text{Total No. of leaves observed}} \times 100$$

On the basis of kind of symptom and percent chlorosis, different citrus species were ranked as resistant to highly susceptible.

## Results and Discussion

### Field plant symptoms

Generally citrus plants affected by citrus greening in the field are recognized by yellowing of foliage, upright leaves and the branches affected are prone to dieback. Symptoms are initially seen as yellowing of foliage of only one limb per branch or a sector of plant canopy.

Leaf chlorotic mottling are the most diagnostic type of symptoms and is followed by vein yellowing. Fully matured leaves show the most reliable symptoms of leaf mottling viz.,

yellowing along the midrib and leaves veins which spread in the adjacent tissues to produce a mottled effect. Such symptoms were observed in the citrus species of *C. grandis*, Tangerin ling ming, unidentified hybrid (Rough lemon *Chettalli* x *Cleopatra mandarin*) and *C. limon*. Infected trees produce new twigs which bear small upright leaves and interveinal yellowing symptoms resembling that of zinc deficiency, some of them having dark green spots as observed on plants of *C. reticulata*, *C. sinensis* and *C. aurantifolia* (Plates 1 and 2). *C. reshni* had symptoms of both mottling and interveinal yellowing (Plate 3). Affected shoots gives plants a bushy appearance and as such leaves mature, they turn green and veins form a darker green network. Internodes become shorter, trees become stunted, bear multiple off season flowers, most of which fall off and produce small irregularly shaped or misshaped lopsided fruits with a thick and pale peel. They do not colour properly, remain green on the shaded side and drop prematurely. Chronically infected trees are sparsely foliated with extensive twig dieback and do not fruit or are unproductive.



A) *C. grandis*



B) Tangerin ling ming



C) Unidentified hybrid

D) *C. limon*

Plate 1: Leaf symptoms of mottling on different citrus species



A) *C. reticulata*

B) *C. sinensis*



C) *C. aurantifolia*

Plate 2: leaf symptoms of interveinal yellowing on different citrus species



Plate 3: Leaf symptoms of mottling and interveinal yellowing on *C. Reshni*

Field observations demonstrate that, usually, symptoms are unevenly distributed within infected trees, which represents one of the major difficulties for detection of the disease (Schneider, 1968, Martinez *et al.*, 1971, Da Graça, 1991,

Bové, 2006)<sup>[20, 12, 6, 3]</sup>. Schwarz and Green (1970) observed all major species of citrus leaves to exhibit mottling, yellow vein symptoms and out of season flowering. Ding *et al.* (2005) reported the presence of vein yellowing and blotch mottling of leaf blades. Shokrollah *et al.* (2011) found characteristic symptoms of greening to be asymmetrical blotchy mottle of leaves, underdeveloped and misshaped fruits with aborted seed. Elizabeth *et al.* (2005) observed characteristic symptoms to be mottling and leaf chlorosis resembling zinc deficiency.

**Varietal reaction**

The incidence of citrus greening caused by *Candidatus Liberibacter asiaticus* was recorded on nineteen citrus species under field conditions. Of these, four citrus species showed resistant reaction (Table 2). Significantly highest percent disease incidence (100 %) was observed on leaves of *C. sinensis*, *C. reticulata*, tangelo, *C. grandis* and *C. jambhiri*. The *C. paradisi* (90 %), *C. limonia* (86.67 %) and *Citrus reshni* (63.33 %) showed highly susceptible reaction. The citrus varieties showing susceptible reaction included (Table 2.)

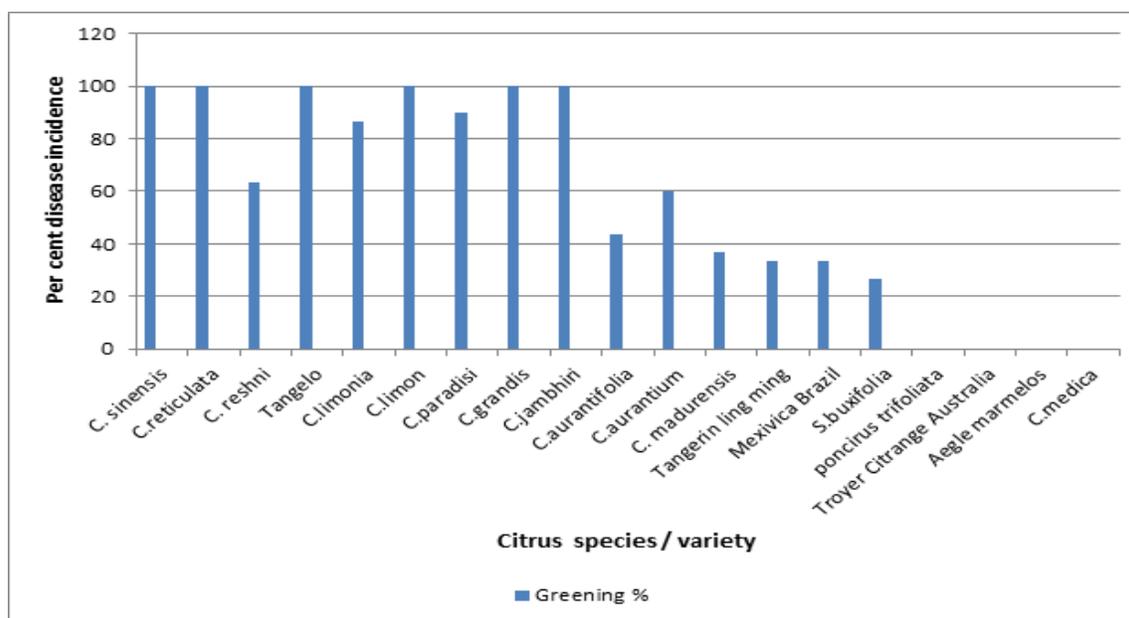
**Table 2:** Reaction of different citrus species varieties to greening under field conditions

S. No	Species/Variety	Per cent disease incidence	Reaction
1	<i>C. sinensis</i>	100.00 (90.00)	Highly susceptible
2	<i>C. reticulata</i>	100.00(90.00)	Highly susceptible
3	<i>C. reshni</i>	63.33 (52.775)	Highly susceptible
4	Tangelo	100.00 (90.00)	Highly susceptible
5	<i>C. limonia</i>	86.677 (68.85)	Highly susceptible
6	<i>C. limon</i>	100.00 (90.00)	Highly susceptible
7	<i>C. paradisi</i>	90.00 (71.565)	Highly susceptible
8	<i>C. grandis</i>	100.00 (90.00)	Highly susceptible
9	<i>C. jambhiri</i>	100.00(90.00)	Highly susceptible
10	<i>C. aurantifolia</i>	43.33 (41.154)	Susceptible
11	<i>C. aurantium</i>	60.00 (50.853)	Susceptible
12	<i>C. madurensis</i>	36.66 (37.22)	Moderately susceptible
13	Tangerin ling ming	33.33 (35.21)	Moderately susceptible
14	Mexivica Brazil	33.00 (35.00)	Moderately susceptible
15	<i>S. buxifolia</i>	26.66 (30.996)	Moderately susceptible
16	<i>Poncirus trifoliata</i>	0 (0.00)	Resistant
17	Troyer Citrange Australia	0 (0.00)	Resistant
18	<i>Aeglemarmelos</i>	0 (0.00)	Resistant
19	<i>C. medica</i>	0 (0.00)	Resistant
	SE(±)	1.52	
	CD @ 5%	4.38	

Figure in parentheses are arc sin transformed values.

*C. aurantium* (60 %) and *C. aurantifolia* (43.67 %). The citrus varieties showing moderately susceptible reaction included *C. madurensis* (36.66 %), Tangerin ling ming (33.33 %),

Mexivica Brazil (33.33 %) and *Severiniabuxifolia* (26.66 %). The *Poncirus trifoliata*, Troyer Citrange Australia, *C. medica* and *A. marmelos* showed resistant reaction.

**Fig 1:** Incidence of greening disease in different citrus species/varieties

In the present study, nineteen species/varieties of citrus were screened for their reaction against the citrus greening disease under field conditions. The data on the disease reaction of greening on leaves under natural field conditions indicated that, of the nineteen species 16 of them were infected by the greening disease (Fig. 1). The per cent disease incidence among the different citrus species varied considerably. However, *Poncirus trifoliata*, Troyer Citrange Australia, *Aeglemarmelos* and *Citrus medica* exhibited resistant reaction to the disease. *C. madurensis*, Tangerin ling ming and Mexivica Brazil showed moderately susceptible reaction, while *C. aurantium* and *C. aurantifolia* were susceptible. On the contrary, the species showing highly susceptible reaction

on leaves were *Citrus sinensis*, *Citrus reticulata*, tangelo, *C. grandis* and *C. jambhiri*, *C. paradisi*, *C. limonia* and *C. reshni*. Ahmad (2011) [2] observed citrus species namely *Citrus reticulata*, *C. aurantium*, *C. grandis*, *C. madurensis* and *Murrayapaniculata* tolerant towards *Candidatus Liberibacter asiaticus*. Chaudhari *et al.* (1980) screened several citrus varieties and found *Citrus reshni*, *Citrus limonia*, *C. jambhiri*, *C. amblycarpato* show tolerant reaction. While Rangpur lime var. L-2, Rough lemon 58 III-IV and Florida Rough exhibited susceptibility with high reaction. Nariani *et al.* (1973) [14] observed mandarin and sweet orange to be very susceptible. Timmer *et al.* (2000) observed most sweet oranges, mandarins and mandarin hybrids to be severely

affected. Manicom and Van Vuuren (1990) noted sweet orange, mandarins and their hybrids with severe symptoms, while trifoliolate orange were regarded more tolerant. Similarly, earlier work and more recent observations have shown that there is a correlation between disruption of the sugar movement pathway and the appearance of chlorotic symptoms in leaves of citrus greening infected trees (Achor *et al.*, 2008, Kim *et al.*, 2009 and Takushi *et al.*, 2007) [1, 11, 21]. The lack of sugar export leads to accumulation of starch granules within chloroplasts, often to the point of chloroplast disruption, which results in chlorotic leaves. Sometimes, the chlorosis occurs within small sectors of leaves, resulting in an uneven blotchy mottle symptom. At other times, all upstream leaves become uniformly chlorotic, suggesting that phloem blockage occurs in the stem below. Because of the correlation between starch accumulation and disease symptoms, we examined HLB symptom production under conditions of increased photosynthesis. Overall, the continuous light conditions increased symptom expression in infected plants, particularly in more tolerant genotypes, and reduced the time before distinctive symptoms developed, which is an aid to routine biological assays. Because the amount of sugar production due to the extended periods of photosynthesis would be expected to increase, the increase in severity of HLB symptoms under these conditions provides additional evidence of the correlation of disease symptoms and disruption of phloem translocation of carbohydrates coupled with abnormal accumulation of starch in leaves of infected trees.

### Conclusion

- The field trees from citrus orchard of All India Coordinated Research Project on Citrus, Central Campus, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar showed distinct symptoms of leaf mottling, interveinal chlorosis, small upright yellow leaves with dark green spots and vein yellowing, characteristic of the disease.
- Of the nineteen varieties screened for their field reaction, four varieties showed resistant reactions, four exhibited moderately susceptible reactions, two were susceptible and nine varieties /species exhibited highly susceptible reaction.

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