

# The simple and rapid diagnosis of citrus huanglongbing (Citrus greening) by Scratch method.

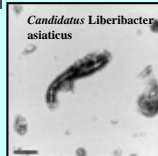
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## Abstract

We have demonstrated a rapid and simple diagnostic method (Scratch method) for HLB, which detected starch in citrus leaves by iodine-starch reaction, because it was revealed that starch granules accumulate abnormally in HLB infected leaves (Schneider, 1968). Average quantity of starch of three citrus cultivars was 514.2 mg/kg in HLB infected leaves and 85.6 mg/kg in healthy leaves (Welch's *t*-test  $p < 0.01$ ). Significant difference was shown in quantity of starch between disease and healthy leaves. Based on this result, we devised Scratch method that uses sandpaper for HLB diagnosis. Accuracy of the Scratch method diagnosis in field showed more than 90% of agreement rates to PCR assay. In addition, Scratch method did not show HLB positive reaction in healthy and nourishment deficiency or another diseased leaves, *Citrus tatter leaf virus* and *Hop stunt viroid*. Therefore, Scratch method must be useful as rapid and simple diagnosis of HLB in the field.

## Introduction

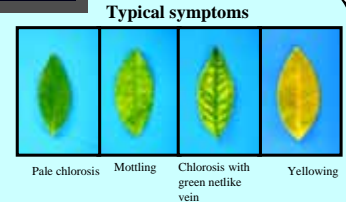
Citrus huanglongbing (HLB) caused by '*Candidatus Liberibacter asiaticus*' is one of the most destructive diseases in citrus growing areas of Okinawa Prefecture. Rapid, responsive and accurate diagnostic test of HLB is an important issue to control the disease, because it is difficult to identify HLB symptom from those of physiological disorder, or nourishment deficiency. In the control guidance of Okinawa Prefecture, PCR assay is suggested for diagnosis of HLB. But PCR assay is expensive, not suitable for a large number of samples, and time-consuming, thus making HLB infected trees left in fields for a long time and delays control. Therefore, it is necessary to develop efficient, quick and simple diagnostic method for HLB.



## Materials and Methods

### 1. Quantitative analysis of starch in citrus leaves.

Quantity of starch of three citrus cultivars (*Citrus depressa*, *C. reticulata*, *C. oto*) was analyzed by using colorimetric determination (Sugiyama and Ooshiro, 2001). Citrus leaves for test were collected in several typical symptoms (Nasu *et al.*, 2003).

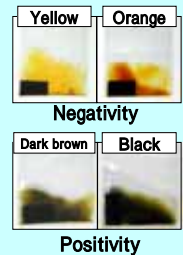


### 2. Scratch method by iodine-starch reaction.

Comparison of the diagnosis of field samples by Scratch method and PCR assay.

#### Procedure of Scratch method

- 1) Scratch a leaf table 20 times with a sandpaper (120) cut in 1 × 2 cm for sampling tissue.
- 2) Put the sandpaper into 1ml water in a vinyl pack, and lightly rubbed.
- 3) Add to 25 μl 0.05M iodine solution for dyeing starch.
- 4) Observation of dyeing of water solution



## Results

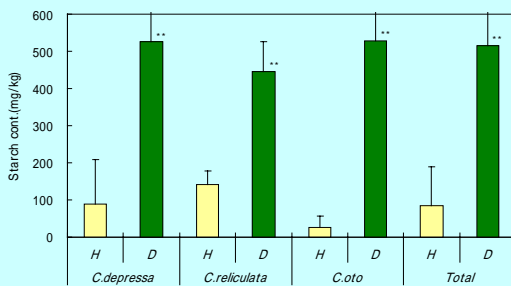


Fig. 1. Comparison of starch content in citrus leaves. H: Healthy leaves, D: HLB infected leaves. \*\* indicate significant differences at  $P < 0.01$  as determined by Welch's *t*-test.

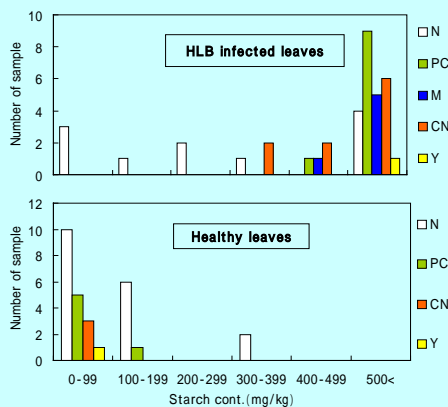


Fig. 2. Comparison of starch content in citrus huanglongbing-diseased leaves and in healthy leaves. N: No symptom, PC: Pale chlorosis, M: Mottling, CN: Chlorosis with green netlike vein, Y: Yellowing.

Table 1. Diagnosis of citrus huanglongbing by Scratch method and PCR on field samples from *C. tankan*.

	Number of trees	Symptom <sup>1)</sup>	Number of leaves		Positivity(+)		Negativity(-)		Not-clear(±)		Agreement rate with PCR(%)		
			PCR	Scratch	PCR	Scratch	PCR	Scratch	Trees	Leaves			
Uruma city	24	N	23	0	23	23	0	0	91.7	96.2			
		PC	25	1	2	24	23	0					0
		CN	23	1	1	22	22	0					0
		Y	7	2	2	5	4	0					1
Ishigaki city	7	N	5	0	5	5	0	0	100.0	90.0			
		PC	8	1	1	7	6	0					1
		M	3	3	3	0	0	0					0
		CN	2	0	0	2	2	0					0
Y	2	0	0	2	2	0	0						

1)N: no symptom  
 PC: pale chlorosis  
 M: mottling  
 Y: yellowing  
 CN: chlorosis with green netlike vein

Table 2. Diagnosis of citrus huanglongbing by Scratch method and PCR on CTLV and HSVd infected leaves.

Variety	Symptom	Sample leaves	Positivity(+)		Negativity(-)		Not-clear(±)		
			PCR	Scratch	PCR	Scratch	PCR	Scratch	
HSVd leaves	<i>C. amakusa</i>	N <sup>1)</sup>	5	0	0	5	5	0	0
		PC	10	0	0	10	10	0	0
CTLV leaves	<i>C. reticulata</i>	Y	5	0	0	5	5	0	0
		PC	10	0	0	10	10	0	0

1)N: No symptom  
 PC: Pale chlorosis  
 Y: Yellowing

Accuracy of the Scratch method diagnosis in field samples showed more than 90% of agreement rates to PCR assay (Table 1). Scratch method did not show HLB positive reaction in another diseased leaves, *Citrus tatter leaf virus* and *Hop stunt viroid* (Table 2).

## Conclusion

Scratch method must be useful as rapid and simple diagnosis of HLB in the field.



Diagnosis kit

HLB infected leaves showed high accumulation of starch than healthy leaves in three citrus cultivars (Fig 1).

Typical symptoms leaves showed high accumulation of starch than no symptoms in HLB infected leaves. The healthy and nourishment deficiency leaves did not show high accumulation of starch (Fig 2).