

## Causes of unusual pomelo leaf after flooding in Nakhon Pathom province

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### Abstract

This study was aimed to investigate the causes of unusual pomelo leaf, wavy leaf, after flooding in Nakhon chaisi district and Sampran district of Nakhon Pathom Province. The wavy leaf symptoms were found in the branches of pomelo plant introduced from other areas, including Prachin Buri, Chai Nat, Samut Songkhram and some local areas in Nakhon Pathom province. Eighty pomelo leaf samples, 53 with wavy leaf symptom and 27 normal leaves were checked for Citrus Tristeza Virus (CTV) and *Candidatus Liberibacter asiaticus* (CLA) by polymerase chain reaction (PCR). Results showed that 29 samples were positive to *Candidatus Liberibacter* while 51 samples were negative including those with wavy leaf symptoms and normal leaf. All samples were negative to CTV detection.

**Keywords:** unusual leaves, pomelo, flooding, Nakhon Pathom

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### 1. Introduction

In Nakhon Pathom Province, pomelo (*Citrus maxima* Merr.) trees are grown in the three main districts: Nakhon chaisi, Sampran and Phutthamonthon. Pomelo production can be exported and sold domestically because the taste and appearance of the fruit is a requirement of the market. The popular pomelo varieties are: Thong Dee and Kao Num Pheong. Before the flood crisis in 2011, the area of pomelo plantation in Nakhon Pathom Province was about 7,000 Rai from the three main districts. During the big flooding in 2011, the pomelo orchards in all three districts were mostly flooded resulting in the decrease in pomelo production area. The main problems that followed after the floods were the lack of money for restoration of the orchard, soil quality problems, family conflict over inheritance problem, disease problems and lack of pomelo shoot cutting for plantation. The problem of plant disease was root rot [1] and fruit drop [2]. Nowadays, farmers have restored the orchard to start growing pomelo tree again but due to there was not enough pomelo shoot cutting from local area therefore they needed to buy pomelo shoot cutting from other sources such as from Prachin Buri province and Chai Nat province. However, 3-4 years after planting, farmers from Nakhon chaisi and Sampran districts had observed an unusual pomelo

leaf, wavy- like leaf. This unusual characteristics of leaf made the farmers worry about their pomelo production because unusual leaves show signs of disease, resulting in poor photosynthesis which affects pomelo yield. The symptoms may be present in young leaf and mature leaf. Sometimes symptoms showed only at the top mature but also at the certain branches. The pomelo farmers in Nakhon Pathom reported that this symptoms had never occurred. After they received the branches (shoot cutting) from various sources then this symptoms of wavy leaf occurred and the area of the symptoms spreaded. Citrus tristeza and greening diseases are the most destructive diseases of citrus industry. The impact of these two diseases is on all domesticated *Citrus* species that caused significant decline in production. Greening disease causes by *Candidatus Liberibacter asiaticus* (CLA) whereas tristeza disease caused by Citrus Tristeza Virus (CTV) [3] and the symptoms of these diseases usually appear on the leaves. Thus, the objective of this research is to study on the causes of unusual characteristics of pomelo leaf after flooding in Nakhon Pathom province.

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## 2. Materials and methods

The research was conducted in the pomelo growing areas in Nakhon Chaisi and Sam Phran districts, Nakhon Pathom province and Prachin Buri province, Thailand during April 2015-April 2016. The methods used are described below.

### 2.1 Survey of the source of shoot cuttings from the orchards showed signs of unusual leaf

Survey and data collection of pomelo shoot cutting sources by questionnaires from farmers of pomelo orchards, Nakhon Chaisi And Sam Phran district where appeared unusual characteristics of pomelo leaf.

### 2.2 Sampling pomelo leaf

Samples source were pomelo orchard in Nakhon Chaisi and Sam Phran district, Nakhon Pathom province and Prachin Buri province. The unusual wavy leaf (53 samples) and also normal leaf (27 samples) were cut from the apex of 20 cm. (three branches per one pomelo tree) for four pomelo tree in each orchard. Leaf samples were placed in the moist chamber before analysis.

### 2.3 Microorganisms analysis

CTV and CLA were investigated for tristeza and greening diseases on unusual and normal pomelo leaf by PCR technique. The samples were identified CTV infection by RT-PCR with specific primers as described by [4]. The greening disease was identified by Nested PCR using specific primer pairs for fD1/fD2 and O11/O12c [5]. All samples were analyzed at Plant Health Clinic, Department of Plant Pathology, Faculty of Agriculture Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus.

### 2.4 Survey of insect vectors and insect pests

Insect vectors and insect pests were investigated by survey in pomelo orchards from Nakhon Chaisi and Sam Phran district.

### 2.5 Plant nutrition concentration analysis

Soil from Sam Phran orchard was collected and taken for plant nutrient concentration analysis. All plant nutrient analysis was done at Soil Plant and Agricultural Material Testing and Research Unit, Central Laboratory, Kasetsart University, Kamphaeng Saen Campus.

## 3. Results and discussion

### 3.1 Survey of the source of shoot cuttings from the orchards showed signs of unusual leaf

Based on the survey and data collection of the pomelo shoot cutting which was taken from the other area for planting on the orchard and appearing the unusual wavy leaf symptom of both Nakhon Chaisi and Sam Phran areas by asking the farmers. The results showed that the sources of pomelo shoot cutting used in

the two districts was come from four areas: 1) Prachin Buri 2) Chai Nat 3) Amphawa district, Samut Songkhram province and 4) original trees in the local area. The big source of shoot cutting was come from Prachin Buri province (>90%). The seedlings which the farmer used for planting from the local area was not appeared crinkly leaves. However, even if the source of seedlings were known (both varieties of Thong Dee and Kao Num Pheang) but to search the original pomelo tree of each seedling was quite difficult because some farmers purchased seedling from many sources and brought together then they sold the seedlings to other farmers. There was only some farmers who purchased seedling from the source by themselves were able to know the original source but not original tree. Only some groups know which branches come from which orchard. It can not be determined from the beginning. As a result, tracking the rootstock of the leaf is difficult. The wavy leaf occurred after planting for about 2 years. Moreover, farmers provide the information on the use of cuttings from original trees in the area, which survived from the flood. If the farmer used a branch from the original tree in the area, the wavy symptoms of the leaf did not appear.

### 3.2 Sampling pomelo leaf

A survey of leaf characteristics in Nakhon chaisi and Sampran districts. It can be observed clearly from the mature leaf and young leaf, especially from young leaf at the top of branch. The results showed that the symptoms of the leaf was wavy which found on the leaves from the shoots down to about 15-20 centimeters. Most of the wavy leaves were found in the varieties of Thong Dee. While the Kao num pheang varieties was very rare. The unusual leaves, normal leaves of Thong Deas and normal leaves of Kao Num Pheang showed in Figure 1. Pomelo tree (Thong Dee) grew for 2-3 years which showed unusual leaf at the top of branches and normal leaf at the top of branches (Fig. 2)

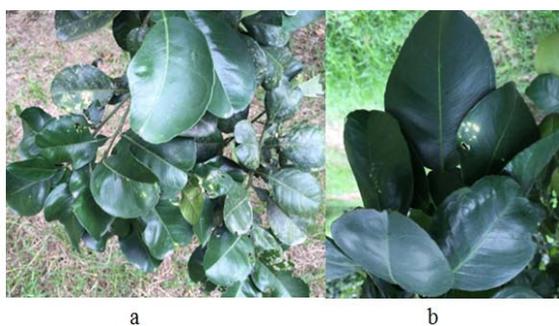


**Figure 1** Wavy leaf of Thong Dee (a), normal leaf of Thong Dee (b), normal leaf of Kao Num Pheang (c) from pomelo orchard of Nakhon Pathom province

To Survey and collect samples of pomelo leaves which appear as unusual leaf of the seedling orchard at Prachin Buri province. The results of leaf observation showed that there were both of wavy leaf and normal leaf (Fig. 3).



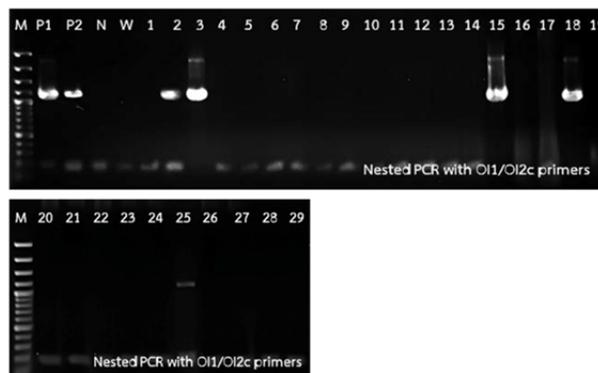
**Figure 2** Wavy leaf of Thong Dee (a), normal leaf of Thong Dee (b), at the top of brunches from orchard of Nakhon Pathom province



**Figure 3** Wavy leaf of Thong Dee (a), normal leaf of Thong Dee (b), from original pomelo tree at the Prachin Buri orchard

### 3.3 Detection of major citrus diseases (tristeza and greening disease)

A total of 6 wavy leaf samples from Nakhon Pathom province were collected for the detection of tristeza and greening diseases which caused by Citrus Tristeza Virus (CTV) and *Candidatus Liberibacter asiaticus* (CLA), respectively for preliminary result. The results showed that all samples were not infected CTV. Then the symptoms of unusual leaf of pomelo in Nakhon Pathom Province are not caused by CTV. The positive sample leaf for detection of CLA was 1 (16.6%) and 5 samples were CLA (83.4%) (no figure). However, since *Candidatus Liberibacter asiaticus* was detected, Total 74 samples of two times sampling (29 and 45 of normal and wavy leaf samples were collected from pomelo orchard, Nakhon Chaisri and Sam Phran District for CLA detection. The results of the analysis were shown in Figures 4-5.



**Figure 4** Nested PCR with O11/O12c primer for CLA detection of 29 normal and wavy pomelo leaf samples (method from [4])

M = standard DNA (100 bp DNA ladder plus, 0.5  $\mu\text{g}/\mu\text{l}$ , Fermentas®)

W = Water

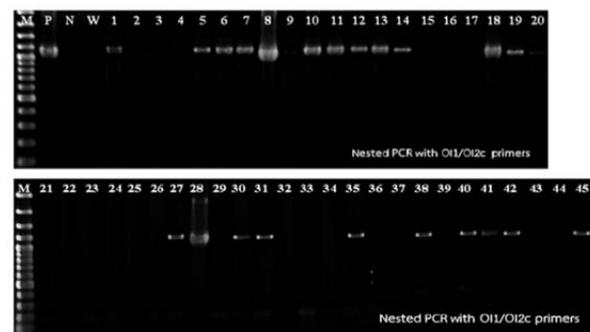
N = Negative

P1, P2 = Positive ( DNA of CLA caused greening disease, 1,200 bp DNA)

Lan 1-14 = Normal pomelo leaf samples

Lan 15-29 = unusual (wavy) pomelo leaf samples

The results of CLA detection by Nested PCR from figure 4 showed 5 leaf samples were infected with CLA that caused Greening disease, 2 CLA positive results from the normal leaf samples and 3 CLA positive results from the wavy leaf samples.



**Figure 5** Nested PCR with O11/O12c primer for CLA detection of 45 normal and wavy pomelo leaf samples (method from [4])

M = standard DNA (100 bp DNA ladder plus, 0.5  $\mu\text{g}/\mu\text{l}$ , Fermentas®)

W = Water

N = Negative

P1, P2 = Positive ( DNA of CLA caused greening disease, 1,200 bp DNA)

Lan 1-10 = normal pomelo leaf samples

Lan 11-45 = unusual (wavy) pomelo leaf samples

**Table 1** Soil chemical properties in pomelo orchard in Sam Phran district, Nalhon Pathom province

sample	Organic matter (%)	Soil nutrient concentration (mg kg <sup>-1</sup> )				
		Phosphorus	Potassium	Calcium	Magnesium	pH
1	2.0	465	395	3112	770	6.0
2	2.05	450	378	3178	789	6.1
3	2.1	468	396	3266	769	5.9

The results of greening detection by Nested PCR from figure 5 showed 22 pomelo leaf samples showed CLA positive, 6 CLA positive results from normal leaf samples and 16 CLA positive results from the wavy leaf samples.

The results of CLA detection of total 74 samples showed 27 CLA infected samples (36.48%) of normal pomelo leaves (8 samples, 10.8%) and wavy pomelo leaves (19 samples, 25.68%) while some samples of normal pomelo leaf (17 samples, 23%) and some wavy pomelo leaf (30 samples, 40.54%) showed no evidence of greening disease (47 samples, 63.51%). Therefore it was possible that greening disease was not the cause of unusual leaf found in pomelo orchard in Nakhon Pathom area. However, *Candidatus Liberibacter asiaticus* is the microorganism which caused greening disease in the citrus plant. Usually, the greening disease symptom show, Zn-deficiency-like symptom and reduced form of leaves, vein clearing, leaf cupping, leaf mottle, blotchy mottle and new emerging leaves with yellow [5],[6]. However, the unusual (wavy) pomelo leaf from the orchard of Nakhon Chaisi and Sam Phran districts were not shown like greening disease symptoms.

Moreover, at the research period or 5 months before started the research work, there was no evidence of the outbreak of insect vectors (*Citrus psyllid*, *Diaphorina citri* Kuway) and insect pests at the research areas.

The results of soil sampling for nutrient uptake, pH and physical characteristics of soil from district, it was found that the soil had high nutrient contents. The soil is acid-alkaline. The organic matter is low to moderate, quite tight and low drainage capacity. (Table 1)

It was found that nutrient from Sam Phran pomelo orchard was very high in all soil. The soil is acid but with organic matter. However, soil characteristics are densely suitable for growing crops and the porosity of the soil is quite good and good drainage [7].

#### 4. Conclusion

The unusual (wavy) pomelo leaf of Nakhon chaisi and Sampran pomelo orchard areas may be caused by growing with shoot cutting from the other areas, CTV and CLA may not the main causes of the unusual symptom. However, unusual characteristics of pomelo leaf found in Nakhon Pathom might not effect on pomelo yielding.

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