Utilization of rapid detection kit in measuring Huanglongbing outbreak level: case study in Koto Tinggi, West Sumatera, Indonesia

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Abstract. Huanglongbing (HLB) is a disease of citrus caused by bacterium Liberobacter asiaticus that can lead to plant death. Most farmers are unaware that their plants have been infected since the beginning of plant growth, and rather considers the condition as a result of nutrient deficiency. Detection of HLB usually is conducted in expensive and complicated laboratory test through PCR (polymerase chain reaction). Rapid detection is necessary in order to formulate a strategy to solve the HLB outbreak appropriately. HLB Rapid Detection Kit is an innovation that can identify the presence of HLB easily and inexpensive way, even farmers can do the test by themselves. An observation has done to apply the HLB Rapid Detection Kit in citrus plantation area of Koto Tinggi, Agam district, West Sumatera. The results revealed that the number of citrus plants that affected by HLB disease was 19.22% - 41.84%. HLB outbreak in citrus plantation suspected to be due to the use of citrus infected seedlings. The strategies for restoration citrus areas that have HLB outbreak are eradicating and replanting citrus plantation using certified disease-free citrus seedlings, those implementation should be integrated in citrus area.

1. Introduction
Huanglongbing disease (HLB) or also known as CVPD (citrus vein phloem degeneration) is a disease caused by the bacterium Liberobacter asiaticus, which causes damage to the phloem vessels in citrus plants. Plants affected by this disease will have photosynthetic translocation disorders. In the initial stages even though the plant has been infected by L. asiaticus but it still shows healthy growth. At a later time, the visible symptoms including chlorosis, then getting worse causes the canopy to become stunted where the leaves become stiff and grow straighter, branches grow molested, and has small and asymmetrical fruit, which in turn can cause death plants [1-4].

L. asiaticus can infect since citrus plants are in the seeding phase, because the infection can be through grafting by using entres taken from infected mother trees [5]. The spread of HLB disease can occur through the planting of infected seedlings, healthy looking citrus seedlings can contain HLB bacteria because the incubation period is 3-5 mo, so it is necessary to strengthen the domestic quarantine system in preventing the spread of HLB disease from endemic areas to free areas [1, 6, 7]. This systemic disease spreads rapidly through the insect vector Diaphorina citri, so that the HLB epidemic occurred
very quickly, reaching more than 95% within 3-13 yr after the first symptoms appear [1, 4, 6]. It can be said that HLB epidemic is a threat to sustainability of citrus farming in Indonesia.

Because the first symptom that is seen is chlorosis, so in general farmers think that the plants have a nutrient deficiency, then farmers do more intensive fertilization. But what happens within a period of 2-4 yr after the initial symptoms appear, plants show failure to develop and produce. Decreased fruit production and quality due to HLB attack causes farming to be economically unfeasible within 7-10 yr after planting [4], even citrus fruits produced by HLB-infected plants experience nutrient deficiency and poor quality [8]. However, farmers are reluctant to eradicate infected plants since most of the plants still produce fruits. Whereas in terms of epidemiology, this condition will increase the availability of inoculum sources in the field [4]. Misdiagnosis of the disease causes errors in decision making, which in turn becomes ineffective and inefficient handling, and ultimately causes greater losses for farmers.

Detection of HLB is usually conducted in expensive and complicated laboratory tests through PCR (Polymerase Chain Reaction) analysis [1, 5, 9]. The Indonesian Citrus and Subtropical Fruit Research Institute (ICSFRI) under the Ministry of Agriculture (MoA) has created new methodology to detect HLB diseases. The HLB Rapid Detection Kit is an innovation that can identify the presence of HLB easily and inexpensive way. Even farmers can do the test by themselves with realtime results. HLB Rapid Detection Kit dissemination is conducted at various citrus centers in Indonesia, one of them is in Kototinggi Village, Agam Regency, West Sumatra. Dissemination of this Kit was expected to help farmers and local government to detect the possibility of an HLB outbreak rapidly in citrus area. Dissemination of the HLB Rapid Detection Kit is necessary to ensure there was an HLB outbreak in the citrus area in Kototinggi village, Agam, West Sumatra. It was carried out using observation so that anticipatory steps can be taken to prevent wider infectious.

2. Methods
This research was carried out in November 2018 in Koto Tinggi Village, Baso District, Agam Regency, West Sumatra. The location was chosen purposively because it’s one of the locations of citrus development programs from the government. The selection of respondents was also conducted purposively consisting of 15 respondents who were representatives of 3 farmer groups. The number of respondents was determined based on the consideration of limited funds and time, and the amount was considered to be sufficiently representative.

Primary data collection was done through farmer interviews in groups or commonly called focus group discussions (FGD), regarding the development of the citrus area and current conditions. While secondary data were obtained from the observations conducted by Research Team from ICSFRI of the possibility of HLB outbreak on citrus plants using HLB Rapid Detection Kit.

Based on FGD and observation result of the level presence of HLB disease, strategy were formulated to restore sustainability citrus plantation area.

3. Results and discussion
Based on information from the FGD, the citrus area in Koto Tinggi village, Agam district, West Sumatra was established since 2008 as a government program. From the initial citrus area of 20 ha, there are only 30% left, while the others were no longer in production and have been replaced by other commodity crops. Based on farmer information, in 2011 citrus plantations produced well with an average productivity reaching 50 kg/tree, and peak production of up to 60-70 kg/tree in 2013-2015. At the end of 2016 to 2017 production began to decline, plants began to show symptoms of nutrient deficiency, as the leaves have chlorosis, shoots and stems turn yellow, the leaves are abnormally small with erect stems (Fig. 1).

At the end of 2017, the plant was not able to grow even though it has been fertilized. These conditions have positively shown HLB infection, but most of farmers have not been alerted. Farmers consider the plants to be damaged and not develop due to nutrient deficiency or have reached physiological age. Even though plants that are not affected with HLB and well cared for can still produce well for more than 20 yr [4].
Figure 1. Citrus plantation infected by HLB disease in Koto Tinggi village, Agam District, West Sumatera. Leaves is chlorosis (a), the leaves are abnormally small with erect stems (b).

Those diseased citrus plants could still produce, but the quality of the fruit was low, the taste was sour and the shape of the fruit was abnormal and its size was small, so it was generally sold as a squeezed citrus, but the price of the squeezed citrus was much lower than the cost of its maintenance. Therefore, many citrus plantations were finally cut down and replaced with other commodity crops. But there were farmers who still maintain the citrus plants because they grow in polyculture with vegetable commodities such as caisim, cabbage, eggplant, tomatoes, and chillies, so they have additional income.

Based on the information from farmers, pests and diseases of citrus plant that were most feared were fruit fly and powdery mildew whose effect were seen directly on the citrus produced, especially on fruit damage, such as rotten fruit. Farmers were not yet aware of the symptoms and fatal consequences of HLB disease, which can actually cause plant death.

The results of HLB disease testing using the HLB rapid detection kit in the citrus area in the nagari Koto Tinggi, Agam district [10] showed in Table 1. From ± 3500 plants originating from citrus plantations in 3 farmer groups, there were 19.22%-41.84% of plants positively infected with L. asiaticus. Moreover, in one of the farmers’ citrus farming based on symptoms that appear indicated 100% infected. However, the discovery of infectious vectors, i.e. Diaphorina citri have not been found. From the results of this observation, the absence of vectors indicates that the number of citrus plants that have been infected with L. asiaticus was because from the infected seedlings.

Thus it could be concluded that the use of healthy seed was very important in ensuring the sustainability of citrus farming. As the results of previous research [6] citrus farming using disease-free certified citrus seedlings was economically more profitable, i.e. 24 - 25.7% compared to those not using disease-free citrus seedlings. The use of disease-free certified citrus seedlings can also provide a higher economic benefit and production surplus compared to those without certified citrus seedlings.

HLB rapid detection kit as seen on Fig. 2, has advantages including; 1) detection process took place quickly, about 45 minutes, from preparation to data interpretation; 2) easy, simple protocols and the application did not require special expertise; 3) inexpensive, did not require special laboratory facilities, sophisticated and expensive equipment; 4) Test results could be observed visually by the occurrence of discoloration that could be seen with the naked eye.
Table 1. Test results of the HLB Rapid Detection Kit in Koto Tinggi citrus development area, Agam District, West Sumatera.a.

| Farmer Groups  | Plant age (years) | Number of citrus plants | Number of citrus plants infected with HLB | % infected with HLB | Vector existence |
|----------------|-------------------|-------------------------|------------------------------------------|---------------------|-----------------|
| Tunas Baru     | 3 - 8             | 951                     | 243                                      | 25.00               | 0               |
| Bumi Harapan   | 5 - 8             | 1336                    | 559                                      | 41.84               | 0               |
| Amanah         | 4 - 15            | 1321                    | 254                                      | 19.22               | 0               |

Data source: [10].

Based on ICSFRI report [10] HLB rapid detection kit was considered to meet ASSURED criteria (affordable, sensitive and specific, user-friendly, rapid and robust, deliverable to end users). There were 9 testing parameters that compare the advantages of the HLB kit rapid detection over the PCR and RT-PCR technique (Table 2). HLB rapid detection kit were more affordable for users than PCR and RT-PCR, validation was based on respondents. From sensitivity criteria, HLB kit rapid detection is more sensitive than PCR, meaning that with a small concentration the kit is able to detect HLB, and its sensitivity is proportional to RT-PCR. HLB rapid detection kit is specific, only reads the HLB target. The other main advantages were HLB rapid detection kit could be applied by people who do not have the expertise, anyone could do the testing. Moreover, it has rapid and robust criteria, which is the process only takes 45 min, and economic, the kit does not require expensive laboratory equipment as PCR and RT PCR methods, so they could be utilized in remote areas with minimal laboratory facilities. HLB kit is available directly to users.

Table 2. The advantages of the HLB Rapid Detection Kit compared to PCR and RT-PCR methods.a.

| Parameter          | PCR | Kit Detection | RT-PCR | Validation          |
|--------------------|-----|---------------|--------|---------------------|
| Affordable         | +   | +++           | -      | Respondent          |
| Sensitivity        | ND  | +++           | +++    | Genie II            |
| Specificity        | ND  | +++           | +++    | Genie II            |
| User-friendly      | -   | +++           | +      | Referensi           |
| Rapid and robust   | +   | 45´          | ++     | Genie II            |
| Economic           | +   | +++           | +      | Economy Analysis    |
| • Laboratoium      | +++ | -            | +++    | Economy Analysis    |
| • Equipment        | +++ | +/-          | +++    | Economy Analysis    |
| Deliverable to end user | -  | +++          | -      | Economy Analysis    |
| False negatif      | < 10| < 5          | < 1    | Participant         |
| False positif      | < 10| < 5          | < 1    | Participant         |

Data source: [10].
Based on the results of the FGD and the results of observations of the HLB outbreak rate, the following recommendations that needs to be done:

1. It is important to provide farmers with knowledge and understanding of the specific HLB disease symptoms and its harmful effects on the sustainability of citrus plantations.

2. Strategy for restoration of citrus areas that have been infected can be done by eradicating infected plants and replanting citrus plants using certified healthy seeds. Handling of HLB and improving plantations must be integrated in an area.

3. The use of healthy seeds is important, because it is necessary to anticipate the HLB disease since the seedling phase. However, currently the number of certified citrus seeds is very limited, so it is necessary to establish seedling institutions at the farm level. The acceleration of technology transfer of disease-free citrus seedlings to farmer/breeder groups needs to be done, so that the availability of healthy citrus seeds can continue.

4. At present ICSFRI is the only institution for citrus research which has the responsibility in producing and distributing disease-free mother trees. In the context of developing citrus agribusiness in Indonesia through the development of citrus areas, and realizing the independence of seeds at the farm level, the ICSFRI can arrange institutional models for farmers’ seedling and provide technological assistance for healthy citrus cultivation. The seeds produced by farmers can be used to revitalize the citrus farmers’ own estate, or can be sold so that it can become an additional income.

5. Optimizing the role of quarantine institutions between regions to prevent the spread of HLB through citrus seedling deployment.

6. The local government should assist in procurement of HLB Rapid Detection kit for farmers and field officers in citrus centers. So that farmers and field officers can detect HLB infection earlier, so they can immediately make decisions for controlling the disease.

4. Conclusion
The use of HLB Rapid Detection kit in Koto Tinggi, Agam district, West Sumatera could detect the level of HLB outbreak on citrus plantations as early as possible. In most of the cases the infection was caused by the use of citrus seedlings that have been infected by L. asiaticus, pathogen of HLB disease.

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