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Hyperspectral reflectance imaging combined with carbohydrate metabolism analysis for diagnosis of citrus Huanglongbing in different seasons and cultivars

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ABSTRACT

Huanglongbing (HLB) is a highly destructive disease to citrus that is threatening the global citrus industry. It is a great challenge for HLB disease detection at various stages due to the long asymptomatic period and the similar symptom to nutrient deficient trees. This research was aimed to propose an effective method for HLB detection in different seasons and cultivars based on hyperspectral imaging coupled with carbohydrate metabolism analysis. It was found that sucrose accumulated more steadily than starch, glucose and fructose in infected leaves through the hot and cool seasons, but nutrient (Fe) deficient leaves presented a reverse pattern to HLB infected leaves. Spectral and textural features from optimal wavelengths and principle component images were well linked to the HLB fingerprint. The three-class classification models for healthy, HLB infected (asymptomatic and symptomatic), and nutrient deficient leaves achieved 90.2%, 96.0%, and 92.6% accuracy for the cool season, hot season, and the whole period, respectively, using least squares-support vector machine (LS-SVM) classifier. Additionally, the robustness of classification model was validated by a different citrus cultivar using model transfer strategy with the overall accuracy of 93.5%. These results demonstrated the potential of hyperspectral imaging combined with carbohydrate metabolic analysis for HLB detection in different seasons and cultivars.

1. Introduction

Citrus Huanglongbing (HLB), also known as citrus greening disease, is a systemic disease to citrus trees, causing great economic losses to the worldwide citrus industry. HLB infection is associated with three non-culturable phloem-restricted gram-negative bacteria, including *Candidatus Liberibacter asiaticus* (*Las*), *Candidatus Liberibacter americanus* (*Lam*) and *Candidatus Liberibacter africanus* (*Laf*). It can cause blotchy mottle leaves, lopsided fruit, and eventually the death of trees. The disease is transmitted by the Asian citrus psyllid as well as grafting, and the symptom appears in infected flushes ranging from months to several years depending on the host age, cultivar, and environmental conditions, which poses a great challenge for diagnosis [1]. So far, there are no effective ways for HLB treatment due to the complicated nature of the pathogen, and thus early detection and timely removal of the infected trees are necessary to prevent the rapid spread of the disease.

Infield survey and laboratory analysis are currently two most widely used methods for identifying HLB infected trees. Infield survey usually relies on the visual inspection of the symptomatic leaves and lopsided fruit, which is a subjective evaluation with relative low accuracies [2]. Moreover, such initial screening can only be performed in a specific season. Laboratory analysis based on the DNA test by using polymerase chain reaction (PCR) technique can qualify the bacterium proliferation in infected leaves, and it is considered as a more accurate method for HLB detection. However, PCR is a time-consuming and expensive method, and requires well trained and experienced operators. Meanwhile, the sampling process can easily result in false negatives due to an uneven bacterium distribution in the infected trees [3].

There is a growing interest in developing non-invasive and rapid technologies for plant disease detection [4–6]. Reported studies have

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demonstrated that spectroscopic techniques such as visible and near-infrared (Vis/NIR), fluorescence, and Raman spectroscopy have the potential for citrus HLB detection by finding specific spectral and/or photosynthetic features related to HLB disease [7–9]. Although reasonable detection results (83–97%) were obtained, spectroscopic measurement can only collect information at limited sampling points of a leaf, which is not efficient for the infield disease screening. The detection accuracy also significantly decreased when identifying asymptomatic leaves [10]. Considering the large variability and heterogeneity of HLB disease spreading from a single leaf to the entire orchard, point to point or one dimensional (1D) measurement could cause high uncertainty in disease detection. Two or three dimensional (2D or 3D) optical imaging techniques use light photons in the wavelength range from ultraviolet to near infrared to acquire images that contain information of optical-biological interaction in the spatial domain. They have been employed for high throughput characterization of plant physiological, biochemical and pathological states, such as quantification of phenotypic traits in plant breeding [11,12], analysis of pathogen infection in grapes [13,14], and diagnosis of nitrogen content in cucumbers [15], as well as detection of citrus diseases [16,17]. For the HLB disease evaluation, Sankaran et al. (2013) investigated the potential of vehicle-based Vis/NIR multispectral and thermal imaging system for detecting HLB disease in the field, and a reasonable result was obtained with the overall classification accuracy of about 87% [18]. Wetterich et al. [19–21] used fluorescence imaging technique to identify HLB infected symptomatic leaves from healthy ones, and achieved the detection accuracies of 90–95%. However, the accuracy decreased to only 61% when using the same fluorescence features for HLB infected samples from different orchards [19–21]. Pourreza et al. [2,23] proposed a polarized imaging technique to detect HLB disease based on the plane rotation of the polarized light caused by accumulated starch in HLB infected leaves [22,23], but the accuracy was largely influenced by the starch concentration and the lighting condition.

Vis/NIR hyperspectral imaging is a well-established chemical sensing technique that provides a broadband spectrum of each pixel in the image, and offers important information related to the chemical compositions and physical structures of plant materials in multiscale levels [24]. The technique has been successfully used in evaluating various plant diseases [25–27]. It has been applied also in detecting citrus HLB disease at the tree canopy level by using hyperspectral imaging-based aerial platform, although the detection accuracies varied greatly in different studies (58–87%) [28–30]. Despite progress in applying hyperspectral imaging for analyzing and diagnosing HLB and other plant diseases, there are still many challenges that need to be addressed before moving the technology from the laboratory-based studies to the more practical applications. Little is known about the applicability of hyperspectral imaging for detecting HLB disease at different infection degrees (from asymptomatic to symptomatic stages). Few robust statistical models based on the spectral image data have accurately identified citrus HLB infected trees by considering citrus cultivars and seasonal factors. Moreover, alteration of carbohydrate metabolism is a common feature of plant-pathogen interactions. It would be interesting to interpret hyperspectral chemical images with dynamic biochemical processes that are responsible for the interconversion of different carbohydrate contents in different seasons.

Therefore, this study was aimed to investigate the potential of hyperspectral imaging coupled with carbohydrate metabolism analysis to detect the citrus HLB disease in different seasons and cultivars. The specific goals were to (i) understand the dynamics of carbohydrate metabolism in HLB infected leaves from asymptomatic to symptomatic stages; (ii) explore HLB fingerprints from hyperspectral reflectance images and develop monthly and whole-seasonal classification models for citrus HLB detection; (iii) validate the robustness of the classification model using a different citrus cultivar with calibration model transfer.

2. Materials and methods

2.1. Leaf sample collection

Three healthy and three HLB infected trees of Satsuma from a commercial citrus orchard (Orchard 1) in Linhai, Zhejiang Province, China, were selected by HLB experts. Citrus branches with three of each were detached from four orientations (east, south, west and north) of each tree from June to November 2016, covering the vegetative and reproductive growth stages through the hot and cool seasons. After sampling, they were immediately placed into a cool box to avoid desiccation, and six leaves were then detached from each branch in the lab right before the image acquisition. 432 ($3\times4\times6\times6 = 432$) leaves at four orientations of six trees were collected every month, and a total of 2592 leaf samples were obtained in the entire experimental period. Due to the symptom similarity between HLB infected and nutrient deficient leaves, 231 Satsuma leaves including 201 Fe deficient and 30 healthy leaves were also collected from another commercial orchard (Orchard 2) in Linhai on August 10th, 2017, which were used to investigate the differences of carbohydrate metabolism and hyperspectral images between HLB infected and nutrient deficient leaves. Meanwhile, 330 Ponkan leaves including 90 healthy, 60 HLB infected asymptomatic, 60 HLB infected symptomatic and 120 nutrient deficient (60 Fe deficient and 60 Zin. deficient) obtained from a citrus orchard in Lishui (Orchard 3), Zhejiang Province on September 10th, 2016 were used to validate the robustness of the classification model developed from the Satsuma dataset. Leaf samples collected in June, July, August, September, October and November were considered as spring flushes, early summer flushes, late summer flushes, early autumn flushes, mid-autumn flushes and late autumn flushes, respectively. More detailed sample information was summarized in Table 1.

Table 1
Description of citrus leaf samples from three experimental orchards.

Orchard	Orchard 1		Orchard 2		Orchard 3			Growth stage	
Cultivar	Satsuma		Satsuma		Ponkan				
	Healthy	HLB infected	Healthy	Fe deficient	Healthy	HLB infected	Fe/Zn deficient	Leaf	Fruit
June	216	108*	–	–	–	–	–	old mature flushes	young fruit
		108	–	–	–	–	–	spring flushes	
July	216	216	–	–	–	–	–	early summer flushes	early enlargement
August	216	216	30	201	–	–	–	late summer flushes	mid-enlargement
September	216	216	–	–	90	120	60/60	early autumn flushes	late enlargement
October	216	216	–	–	–	–	–	mid-autumn flushes	coloring
November	216	216	–	–	–	–	–	late autumn flushes	full ripe

* 108 old mature HLB infected flushes were collected in June due to the lack of samples from emerging flushes in HLB infected trees in Orchard 1.