Research Article

In Vivo Monitoring on Growth and Spread of Gray Leaf Spot Disease in *Capsicum annuum* Leaf Using Spectral Domain Optical Coherence Tomography

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We have demonstrated the application of optical coherence tomography (OCT) in diagnosis of growth and spread of the gray leaf spot disease in *Capsicum annuum* leaf caused by the fungus *Stemphylium lycopersici*. Using 2D cross-sectional and 3D volumetric images of OCT, *in vivo* study of layer differences between fungus infected leaves and healthy leaves was observed with distinctive features. We observed that the internal layers of the disease-affected parts of the leaf seem to merge forming a single thick layer. The obtained OCT results verify the noninvasive diagnosis ability of fungal growth and spread in *Capsicum annuum* leaves and the applicability of this methodology for other plant diseases.

1. Introduction

Gray leaf spot is a fungal disease in plants caused by two plant pathogens, namely, *Stemphylium solani* and *Stemphylium lycopersici* [1]. It has been observed in various plants like tomatoes, corn, pepper, potatoes, turf grass, and so forth [2]. *Stemphylium solani* causing gray leaf spot on pepper (*Capsicum annuum*) was first observed in natural field in Louisiana by Sinclair et al. in 1958 [1]. Pepper is a widely used crop around the world with key contribution in various cuisines. Because of its wide usage, management of pepper crop is important in agriculture. Gray leaf spot disease mostly affects the infant pepper plant and is often observed in mountainous regions. The infection of gray leaf spot is mostly limited to the leaves, but with favorable environment conditions it infects the stem and petiole. Symptoms start to appear as minute spots of 1 to 2 mm in diameter, with white to gray sunken center and brown edges spreading randomly across the leaves [3]. As disease progresses, it enlarges up to 3 to 6 mm in diameter with gray to white sunken centers

surrounded with brown to dark brown margins. Nearby spots tend to combine and form larger spots as they ages. The disease interrupts with photosynthesis, resulting in severe defoliation. Without proper control measures, it can lead to reduction of harvest.

Detection of gray leaf spot disease is usually done by visual identification of disease spread in leaf. However, visual inspection is a difficult and time consuming process [4]. Moreover, internal changes in leaf cannot be observed in visual inspection without damaging the leaf. Other optical imaging and microscopic imaging techniques to study the internal morphological changes in plants require sectioning [5]; this is a time-consuming process and also this inhibits the possibility for an *in vivo* study. Drawbacks in these conventional inspection methods can be overcome with OCT. OCT is a noninvasive, nondestructive imaging system that uses low-coherence interferometer technique to obtain high-resolution cross-sectional images. OCT was introduced in 1991 [6]. Owing to the advantage of real-time imaging with micrometer resolution, OCT has proven to be a useful

FIGURE 1: Schematic diagram of the SD-OCT system.

imaging technique in biological field. Biological tissues are highly scattering; due to this reason, OCT is a profound biological imaging technique used in ophthalmology, dermatology, endoscope technology, and otorhinolaryngology [7–10]. Applications of OCT also have expanded to electronic devices like LEDs and LCDs for defect inspection and layer thickness measurement, and so forth [11, 12]. OCT has also become popular in the field of plant study and research. It is indeed helpful in studying the morphology and changes in layers of fruits, seeds, and other parts of plants [13–18]. Recently, research work has been started on plant leaves, to study effect of disease in morphological changes of layers in plant leaves [19, 20]. *In vivo* continuous monitoring of *Capsicum annuum* leaf for disease progression of gray leaf spot disease and its relative morphological changes in structure is yet to be done.

This study shows the possibility of *in vivo* monitoring of plants in their natural environment for disease growth and the resulting morphological changes in internal layers of plant leaves without causing any damage to the plant. *In vivo* 2D and 3D images were obtained using spectral domain optical coherence tomography (SD-OCT) for detecting the spread of disease and morphological changes in the layers of *Capsicum annuum* plant leaf, caused by gray leaf spot disease in controlled environment conditions that favor the growth of disease.

2. Method

2.1. Plant Preparation. The *Capsicum annuum* seeds were surface-sterilized after which the seeds were soaked and shaken in 1.2% NaOCl (sodium hypochlorite) for 30 minutes. Then, they were washed with distilled water for 20 minutes and dried at room temperature. The seeds were planted in

pots and kept in plant growth room where 12/12-hour dark and light conditions were maintained at 25°C temperature and 50% humidity.

The pathogen *Stemphylium lycopersici* was grown in V8 agar medium, 3 gm of CaCO₃ (calcium carbonate), 20 gm of agar, and 800 mL of distilled water with 200 mL of V8 juice, in a chamber under 12-hour light conditions at 20°C and 12-hour dark conditions at 15°C. 7 mL of sterile distilled water was poured into incubated fungal plate and spores were gently scrapped off. This suspension was filtered through 3-layered cheese cloth and spores were adjusted to 5×10^3 spores/mL and sprayed on abaxial and adaxial surface of leaves. The pots were then incubated in 12/12-hour light and dark conditions at 20°C during the day and 15°C during the night to aid the disease growth.

2.2. Optical Coherence Tomography Setup. The healthy plant and the disease inoculated plant were imaged using SD-OCT. The schematic diagram of the SD-OCT system is shown in Figure 1. The system was operated with a broadband light source (BroadLighters T-850-HP, Superlum) with 860 nm center wavelength and full width at half maximum (FWHM) of 165 nm. Since the leaf samples have high scattering coefficient, therefore high power is needed in sample arm to obtain intensity image with high depth penetration. Hence, a 9:1 coupler (FC830-90B-APC, Thorlabs) is used. 90% of optical power is directed to sample arm and 10% to reference arm. 2D galvo scanners (GVS102, Thorlabs) are used to scan the samples. A 3 mm × 3 mm scanning range is used. The detection part for the system consists of diffraction grating (HD1800 L/mm, Wasatch Photonics), 100 mm focusing lens (AC508-100-B, Thorlabs), and 4096-pixel line scan camera (spl4096-140 km, Basler). The laboratory built SD-OCT system has a frame rate of 32 frames per second. Therefore,

FIGURE 2: Photographs of *Capsicum annuum* plant leaves and OCT cross-sectional images. Healthy plant used for imaging is shown in (a) along with its respective 2D OCT image (b). Disease-induced plant pot used for imaging is shown in (c) with its 2D OCT image (d), highlighted with white box as disease infected region.

each 2D image takes 31.25 milliseconds and similarly one 3D image takes 15 seconds. The lateral and axial resolutions of the system were $11.4\ \mu\text{m}$ and $3.5\ \mu\text{m}$ in air. A total of 500 A-scans were used to produce one 2D image and 500 consecutive 2D images were obtained to make a 3D image. We employed A-scan analysis for the detection of disease. A-scan is an abbreviation for axial depth scan (1D scan). A-scan signals are extracted from the 2D OCT images. By analyzing normalized A-scans, thickness of leaves layers can be measured quantitatively from width of A-scan peaks, for comparison between healthy and disease-affected regions in leaves. By using A-scan analysis, the morphology of layers with precision is obtained which helps in comparing healthy and diseased infected plant leaves.

A set of leaves were randomly selected from disease inoculated plants. The selected portions of leaves were scanned every day as the disease spread. The OCT scanning was carried out for 20 days; throughout the experiment the temperature and humidity were maintained in favor of disease growth. The obtained 2D and 3D images were used to identify and locate the region of interest where the disease was spread.

3. Results

3.1. Two-Dimensional Image Analysis of Healthy and Diseased Plants. The disease infected *Capsicum annuum* plant leaf and healthy *Capsicum annuum* plant leaf along with photographs and cross-sectional depth images are shown in Figure 2. A scan range (area) of $3\ \text{mm} \times 3\ \text{mm}$ was used for scanning the sample. Initially, before the appearance of the disease symptoms, the entire leaf was scanned in sections. As the disease progressed and when the morphological changes in leaves started to appear in 2D OCT images, only disease infected regions are chosen and scanned throughout the experimental duration. Figure 2(a) shows the healthy plant pot used for imaging and Figure 2(c) shows the disease-induced plant pot used for imaging. All leaves were imaged on adaxial surface. In Figure 2(b), the layers of cuticle, upper epidermis, and palisade layers are visible in healthy plant leaves and healthy regions of disease infected leaves. In

Figure 2(c), region affected by fungi is highlighted with white box as disease infected region. As shown in Figure 2(d), a single thickened layer is visible in disease infected region that indicates the degraded and merged layers of leaf due to the fungi infection, whereas the healthy part of the disease infected leaf still appears with its individual layers as in healthy plant. Figures 2(c) and 2(d) are taken on day 6, after disease inoculation in plant pot.

3.2. Two-Dimensional Image Acquisition of Growth and Spread of Disease. Figure 3 shows cross-sectional OCT images of an infected leaf. The growth and spread of the disease were monitored for 20 consecutive days to observe them. The white color rectangular box region in Figures 3(a)–3(e) was taken from day 1 to day 5, these images show disease spread from a noninfected region of leaf in disease infected plant. Day 1 leaf image looks similar to healthy leaf image as shown in Figure 2(b). In day 2 image, the layers of leaf show reduction in top layer thickness. On day 3, the top layer of the leaf looks damaged by the disease infection and on day 4 the bright spot indicates the merging of layers due to disease. The white box region in leaf indicates these changes and it is quite difficult to identify them using visual inspection due to micrometer size. On day 5, all the layers of leaf seems to be damaged and merged into a single thick layer due to disease infection. Figure 3(f) shows the 3D OCT image of the same leaf taken on day 10 marked with midrib and vein of the leaf. The red box region indicates the scanned area for the obtained 2D images from Figures 3(a)–3(e).

3.3. Three-Dimensional Imaging of the Spread of Disease. Figure 4 shows the 3D images obtained from an infected leaf. Figures 4(a)–4(e) display images taken on alternative days from day 2 to day 10. White regions in the 3D OCT images indicate the disease infected areas. The blue dashed box regions show disease growth from day 2 to day 10. Compared with day 2 image, day 10 and day 20 images show a wide spread of disease infection throughout the leaf surface. This can be observed in 3D OCT images. Due to disease saturation, not much significant difference is observed in disease growth from day 10 to day 20.

FIGURE 3: 2D and 3D SD-OCT images. 2D images of disease progression and growth are illustrated through image (a) to image (e) which were taken on consecutive days from day 1 to day 5; (f) is a 3D image of the same leaf showing the area scanned highlighted with red box, and midrib and vein of leaf are indicated in the 3D image taken on day 10.

FIGURE 4: 3D SD-OCT images. Images (a) to (f) are 3D images highlighted with blue dashed box region of same position showing disease growth; all 3D images are processed with 2D images.

3.4. Normalized A-Scan Analysis of Disease Infected Region and Healthy Region of Disease Infected Leaves. The normalized A-scan analysis was done between the disease infected region and healthy region of same disease infected leaf. The 2D cross-sectional OCT image along with the A-scan plot is shown in Figure 5. Figure 5(a) is taken on day 8 after disease was inoculated in plant pot. Equal areas of healthy region and disease infected region were used for A-scan analysis. From the obtained 2D image, raw data is demodulated and processed. To compensate for speckle noise in A-scan signals, a median filter is applied to avoid erroneous peak detections. Ten successive A-scan signals are

extracted from the 2D image. First peak is determined by getting the maximum value intensity peak in depth direction of 2D image. The processed A-scan signals are averaged. A-scan signal intensities are normalized by dividing them by maximum value. This is done in order to acquire stabilized intensity profile [19]. Ten A-scan signals obtained within the healthy regions and disease infected regions that were used for analysis are shown in Figure 5(a). In Figure 5(b), normalized A-scans from the respective region of interests were plotted. The blue graph represents the healthy regions and the three blue arrows 1st, 2nd, and 3rd peaks are indicating the three layers of leaves observed in 2D image.

FIGURE 5: A-scan analysis of healthy and disease infected regions of leaf. 2D OCT image of disease infected leaf is shown in (a) marked with healthy region and disease infected region used for A-scan analysis and its corresponding A-scan analysis are shown in (b).

As compared to baseline, the 2nd and 3rd peak intensities of the healthy A-scan (blue color graph) are significantly distinguishable, and such clear peaks cannot be observed in the diseased A-scan plot (red color graph). Thus, the appearance of the clear peaks confirms the morphological layer information of the leaf and the disappearance of the peaks confirms the morphological changes occurred in the leaf due to the disease. The dotted red graph represents the disease infected region; the broadened first peak indicates the presence of only one layer (resultant from merging of layers) and it is thicker than layers of healthy region. Using normalized A-scan analysis, the measured widths of peaks, which represents the thickness of the 1st, 2nd, and 3rd layers in healthy leaf, are 13 μm , 30 μm , and 90 μm , respectively. The broadened disease infected region has a thickness of 80 μm .

4. Conclusions

In this study, we have investigated the possible applications of OCT for *in vivo*, noninvasive, and nondestructive identification of the disease infected plant leaf from a healthy plant leaf. Cross-sectional OCT images give the information

to detect disease infection in early stages, which is difficult to observe and identify visually by naked eye or other techniques. Therefore, OCT, being a fast growing field, may offer more applications in early diagnosis of other types of disease infected crops and can aid in crop maintenance.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Naresh Kumar Ravichandran and Ruchire Eranga Wijesinghe came up with the proposed research idea; Naresh Kumar Ravichandran designed and carried out the experiments. Seung-Yeol Lee helped to prepare the plant material and disease inoculation. Muhammad Faizan Shirazi and Kibeom Park helped to prepare the paper. Mansik Jeon, Hee-Young Jung, and Jeehyun Kim provided general guidance during the whole research.

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