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Rapid detection of chlorophyll-a reduction in tropical fruit leaves using violet laser

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Abstract. Chlorophyll is an important substance in photosynthesis process. Chlorophyll turns CO₂ and water into energy required by trees. Detection of chlorophyll in leaves and trees will give us much information, such as chlorophyll quantity and quality. In this paper, we present a simple, yet powerful technique to observe chlorophyll-a degradation in tropical fruit leaves. We conducted experiment on mango, mangosteen, noni, jackfruit and rambutan leaves. We excited fresh leaves using violet (wavelength at 420 nm) laser and detected chlorophyll emission spectra. We found that there were noticeable differences among chlorophyll spectra from 5 leaves. We analyzed chlorophyll peak wavelength and intensity. We also observed time dependence chlorophyll emission upon continuous excitation to study chlorophyll degradation.

Keywords: chlorophyll, leaves

1. Introduction

Photosynthesis is an important process that usually takes place in leaves. Photosynthesis is basically a process when leaves absorb sunlight to produce energy that can be stored in other parts of trees [1]. Many substances play an important role in photosynthesis, one of them is chlorophyll. In photosynthesis, chlorophyll absorbs energy from sunlight to synthesize carbohydrate by converting CO₂ and water [1, 2]. Carbohydrate, which is one of the chemical energies, is then converted into nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP). These energies are stored in the form of sugar and other organic molecules [3, 4].

Chlorophyll, also known as green pigment, gives green color of the leaves. Chlorophyll may reduce during photosynthesis process [3]. Color change, from green to brown, is an obvious sign of chlorophyll reduction in leaves. These aging leaves indicate final life cycle phase of leaves [2, 5]. Chlorophyll reduction in leaves is mostly due to high photosynthesis process and lack of chlorophyll production in leaves [1].

Several detection techniques have been developed to observe chlorophyll in leaves. One of the most common techniques is UV-Vis absorption spectroscopy. This technique can be done in-situ or ex-situ using leave extract [6]. This technique identifies absorbency peak intensity of chlorophyll. However, sometime absorbency spectrum of chlorophyll may overlap with several substances such as chlorophyll-a, chlorophyll-b and beta-carotene [7]. Another technique is then required to specifically distinguish chlorophyll-a substance in leaves [5]. In this paper, we propose photoluminescence



technique to obtain and study chlorophyll-a reduction in leaves using violet laser as excitation source. We focused on 5 different leaves, which are tropical fruit leaves. They are mango (mangga) leaves, mangosteen (manggis) leaves, noni (mengkudu) leaves, jackfruit (nangka) leaves and rambutan (rambutan) leaves. The purpose of this work is to (1) study chlorophyll content in leaves and (2) to study chlorophyll degradation in leaves.

2. Methodology

This research was done in several steps such as sample preparation, optical setup preparation, and acquisition process and data analysis. Leaves used in this work were mango leaves, mangosteen leaves, noni leaves, jackfruit leaves, and rambutan leaves. Those leaves were collected from trees and freshly measured in the optical laboratory. We studied several parts of parts of the leaves such as center part, leaf tip, and leaf base. All data were measured at least 8 times to confirm repeatability issue.

Photoluminescence setup was comprised of excitation violet laser (continuous wave 420 nm laser at power 60 mW), a laser focusing lens, cuvette stage holder, two lenses acting as emission collector and a fiber-based spectrophotometer (as shown in Figure 1).

The experiment was done based on the following procedure.

- Prepare fresh leaves for photoluminescence experiment.
- Recording chlorophyll-a emission spectra.
- Recording chlorophyll-a reduction (changing of intensity at two specific wavelengths) upon continuous violet laser excitation.
- All experiment was done randomly on entire leaves' surface and repeated at least 8 times per leaf.
- Data fitting analysis using data processing software, including exponential decay fitting for studying chlorophyll-a reduction.

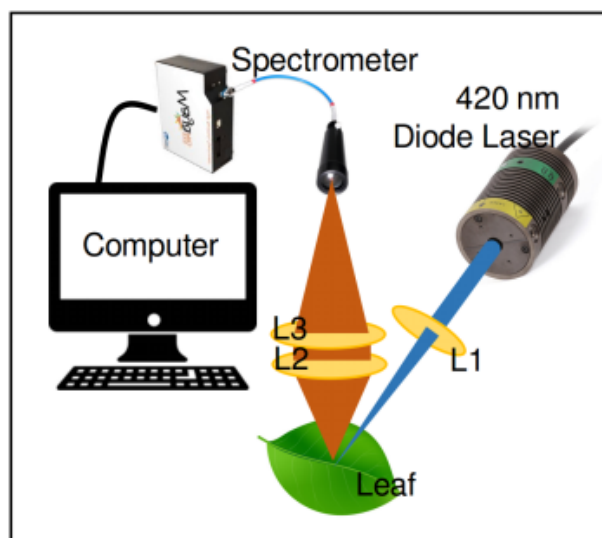


Figure 1. Photoluminescence setup for studying chlorophyll degradation.

3. Result and Discussion

Emission spectra of chlorophyll-a for 5 leaves is given in figure 2. Based on the emission spectrum, we can notice there are two emission peaks, the first peak (P1) exists around 680 nm wavelength and the second peak (P2) occurs around 730 nm wavelength. The spectrum of each leaf was an average spectrum of 8 measurements. In general, we can see that rambutan leaves and mangosteen leaves emit

strongest chlorophyll-a emission, indicating abundant chlorophyll-a content in those leaves. On the other hand, mango leaves show the least chlorophyll emission.

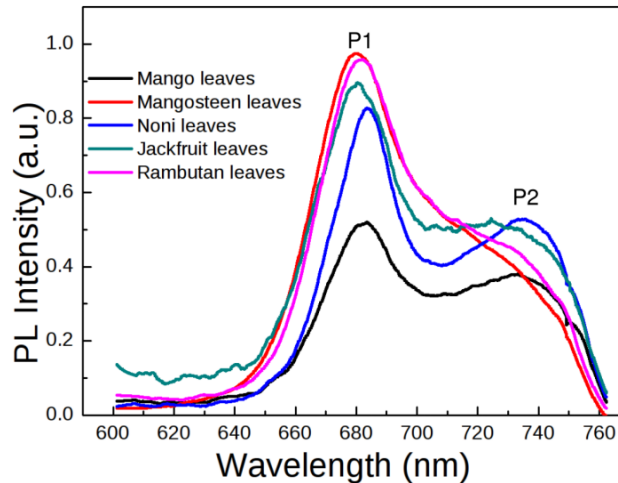


Figure 2. Chlorophyll emission spectra for 5 different leaves.

We notice that peak intensities of P1 and P2 are different. In general, P1 is higher than P2. We also notice that peak wavelength varies. Figure 3a indicates peak wavelength for P1 and P2 for 5 different leaves. We observed for P1; all leaves have similar peak wavelength around 681 nm. The peak wavelength difference among leaves is not obvious. However, P2 show significant difference among leaves. P2 of rambutan leaves has peak wavelength at 723 nm. Meanwhile, P2 of noni leaves has peak wavelength at 733 nm. In the future, this peak wavelength difference can be utilized to determine type of leaves.

In addition, we also notice that peak intensity between P1 and P2 is not always constant for all leaves. As shown in figure 3b, the photoluminescence peak intensity ratio P1 ($I_{\lambda 1}$) and P2 ($I_{\lambda 2}$). Peak ratio varies from 1.3 for mango leaves until 1.9 for jackfruit leaves. In the future, this peak intensity ratio can also be utilized to determine the type of leaves.

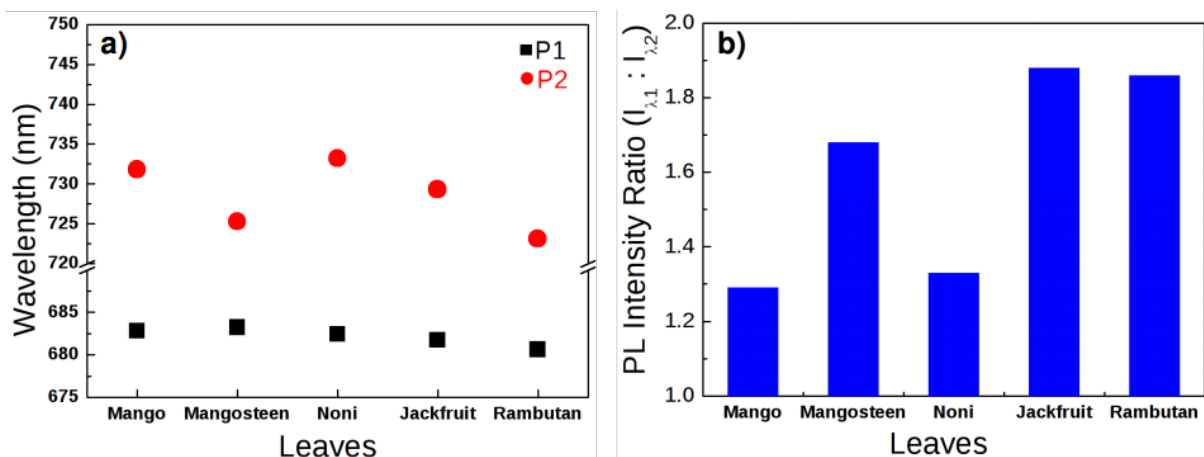


Figure 3. Peak wavelength for P1 and P2 (a) and peak intensity ratio (b) for 5 leaves.

In addition to spectrum analysis of chlorophyll-a emission, we also conducted a study of chlorophyll-a reduction in leaves. Upon continuous excitation of violet laser, we detected intensity of

P1. We recorded peak intensity every 1 second. For all leaves, we found that chlorophyll-a emission intensity significantly reduces.

Time-dependence chlorophyll intensity is then well fitted using single exponential decay. An example of a decay curve of chlorophyll emission is shown in figure 4. Using single exponential fitting, we can obtain decay time for chlorophyll-a reduction. In figure 4, fitted decay time was 9.04 seconds.

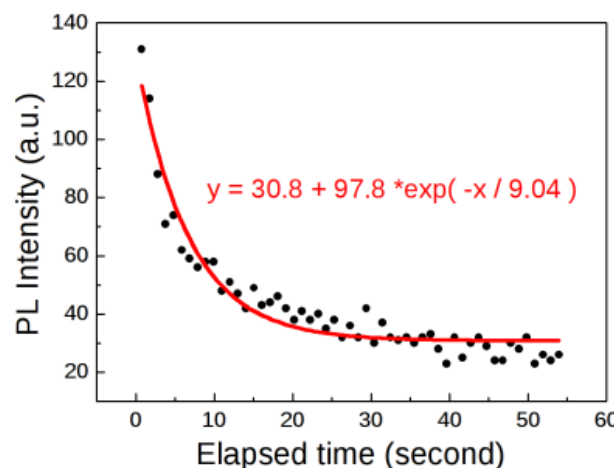


Figure 4. Chlorophyll emission intensity upon continuous violet laser excitation and decay fitting using single exponential fitting.

Figure 5 shows decay time for different leaves. We can see that mangosteen and jackfruit leaves have longer chlorophyll decay time. It means that chlorophyll-a can exist longer in those leaves. Short decay time, such as rambutan leaves indicates that chlorophyll in leaves is easily converted into energy and chlorophyll-a production in leaves was limited since we used fresh leaves picked from the tree.

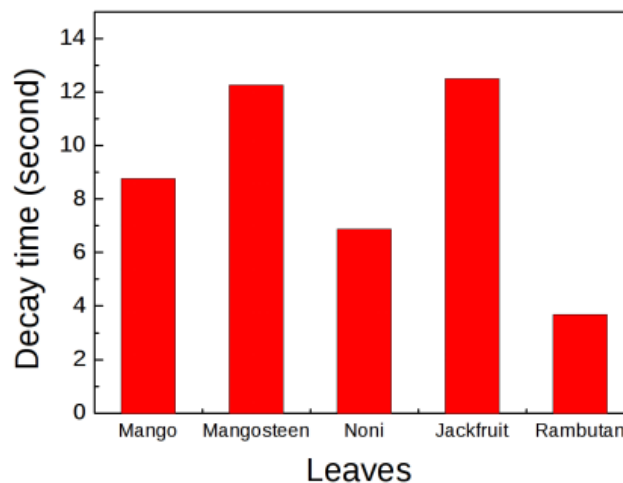


Figure 5. Decay time of chlorophyll emission for 5 different leaves.

The reduction of chlorophyll-a in response to aging which results in natural aging naturally occurs in leaves. Although aging is a genetically controlled program, environmental or external factors can accelerate or suppress aging. External factors cause changes in gene expression, which begin aging. Chlorophyll-a reduction is widely used to describe the loss of green quality in plants such as vegetable crops. Leaf or plant organ detachment is a condition that can accelerate the aging process [8, 9]. Some

chemical treatments may affect the content of chlorophyll in separate leaves. Although the inherent leaves can recover the contents of chlorophyll-a, chemically contaminated leaves can affect the contents of chlorophyll after leaves are released from the plant [9, 10].

4. Summary

We have shown a simple technique to detect chlorophyll in leaves using photoluminescence by utilizing violet laser. We have found that chlorophyll-a has two emission peaks. Different leaf has different characteristic regarding those two emission peaks. The ratio between P1 and P2 can be used to differentiate the leaves. Furthermore, chlorophyll-a reduction in leave is easily monitored. We found that chlorophyll-a reduction curve follows the exponential decay curve so that chlorophyll-a reduction can be characterized using its decay time. Rambutan leaves have shortest decay time, and jackfruit leaves have longest decay time. Decay time can be correlated to chlorophyll-a content and photosynthesis process.

5. Acknowledgments

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