Aplications of Laser Induced Chlorophyll Fluorescence Imaging to detect Environmental Effect on Spinach Plant

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Abstract. Laser Induced Chlorophyll Fluorescence spectroscopy has been developed and used for many years and recently becomes a promising nondestructive method to detect early symptoms of environmental stresses on plants. An affordable, portable, and effective system of Laser Induced Chlorophyll Fluorescence detection needs to be developed that can be used by most farmers. In this research, a system of chlorophyll fluorescence imaging which consisted of some light sources, some optical components, samples, a CMOS camera as a detector has been applied to detect the effect of sunlight intensity variation on plants of Amaranthus tricolor spinach. The spinach plants were treated by the sunlight intensity variation after 10 days growth. The samples were the detached spinach leaves grown under three sunlight intensity variations and the spinach leaf extract in ethanol grown under two sunlight intensity variations. The detached leaf samples were taken for investigation after 10 days and 18 days treatments. There were 5 plant samples for each treatment. The spinach extraction samples was taken from different spinach plants after 20 day treatment. The variations of sunlight were 90 % using one layer of plastic cover, 40% using a layer of plastic and a layer of dark net cover, and 32 % using two layers of dark net cover, compared to sunlight intensity without cover. Diode lasers and LEDs with different wavelength were used as light sources, performances of both were compared. The detached leaves and the spinach extract in a cuvette were illuminated by laser and LED light and their images were recorded by the CMOS camera. The fluorescence intensities were represented by the maximum intensities of RGB values found from RGB plot using ImageJ software. The research results showed that wavelength diode lasers and LEDS that can give significant differences in the three treatments were 680 nm for LED and 780 nm for diode laser for detached leaf experiment. The 680 nm LED and the 780 nm diode laser, respectively, gave 24.3 % and 29 % different in fluorescence intensities between 90% and 32 % sunlight intensity treatments. The 525 nm LED and 532 nm diode laser did not give significant difference for all treatments. The fluorescence intensities were higher for 525 nm LED 532 nm diode laser than those for 680 nm LED and 780 nm laser because the chlorophyll absorbed more light in NIR wavelength. The fluorescence intensities were higher at 10 day treatment that at 18 day treatment. It could be because the plants had much less chlorophyll since the optimum growth of the spinach is 25 days. The leaf extract method gave better spectrum visualization however it could not differentiate both treatments significantly except at 680 nm wavelengths. Both diode laser and LEDS have the possibilities to be used as the low cost light source for chlorophyll fluorescence imaging.

Keywords: *Amaranthus tricolor L.* spinach, CMOS camera, Chlorophyll fluorescence imaging, sunlight intensity variation, ImageJ

Introduction

Monitoring and early detecting symptoms of plant diseases and environmental stresses for agriculture commodity plants are very important. The detection and monitoring are needed for preventing it from spreading and to know before it appears. Traditional methods are mostly used for early detection such as field survey and to check visual marks on the plants, if found, it will be brought to laboratory for analysis. These methods are less efficent, time consuming and expensive. Sankaran *et al*. [1] have reviewed that there are no real time sensors available commercially which can monitor

ISSN: 978-602-71169-7-9

the health of plants. He has reviewed some techniques used to detect plant diseases and environmental stressess. There are clasified in two categories, direct methods such as molecular method and serology, indirect methods such as spectroscopy and imaging. Both methods have limitations and advantages. Spectroscopy and imaging methods are prefarable because they are nondestructive.

Photosynthesis, chlorophyll, and fluorescence are three terms that can not be separated in plant physiology. When Sunlight is absorbed by chlorophyll molecules inside leaves, three processes happen simultaneously. Light is used in photosynthesis processes by a photochemical process. The light is also emitted in term of fluorescence. Excess of light energy is then converted to heat. These three processes are competitive that efficiency among them are different. By measuring chlorophyll fluorescence, information on the two other processes can be obtained. Even though the amount of light emitted by fluorescence only 1-2 % of the absorbed energy, measuring it in the field is easy using a fluorometer, however, this measurement is an averaging measurements that can hide peculiar results that is needed the most [2]. Spectroscopy and imaging of chlorophyll fluorescence that is induced by laser light become important tools in plan physiology and agriculture because the methods can be used to detect early stress and diseases in plant before any symptoms appear. Some researches have been done to correlate responses of plants on absorption, reflection, and transmission of light, stresses on plant physiology. Environmental stresses can be drought, flooding, freezing, herbicides, competitions between plant and plant diseases [3]. All the cases, the fluorescence at certain wavelength can tell about the stresses. The reason is because the chlorophyll concentration is reduced by the stresses. Therefore, chlorophyll fluorescence can be an indicator if stress happen to a plant. Measurements of reflectance, absorption, transmission of light from plant can tell more information about the environment stresses [4,5].

Spectroscopy and imaging method has been developed and become a promising methods to detect plant diseases and stresses because it is undestructive method. With advances in semiconductor technology, diode laser and LED (light emitting diode) become anternative light sources. They are available comercially in wide range of wavelengths and powers. Thie beam quality of both liht sources are getting better. These opportunities can make laser or LED induced chlorophyll fluorescence imaging wich is low cost, portable and efficient visble.

In this research, two optical systems were used to investigate the effect of sunlight intensity variation on grown spinach. The optical systems are the fluorescence imaging using detached spinach leaves and fluorescence imaging using the exract of spinach leaves. Lasers or LEDs were used to induce the fluorescence. The spinach plants, Amaranthus tricolor spinach were treated by the sunlight intensity variation after 10 days growth. The samples were the detached spinach leaves grown under three sunlight intensity variations and the spinach leaf extract in ethanol grown under two sunlight intensity variations. The detached leaf samples were taken for investigation after 10 days and 18 days treatments. There were 5 plant samples for each treatment. The spinach extraction samples was taken from different spinach plants after 20 day treatment. The variations of sunlight were 90 % using one layer of plastic cover, 40% using a layer of plastic and a layer of dark net cover, and 32 % using two layers of dark net cover, compared to sunlight intensity without cover. The detached leaves and the spinach extract in a cuvette were illuminated by laser and LED light and their RGB images were recorded by the CMOS camera. This research was conducted in an undergraduate laboratory with small research budget, advanced spectrometers or fluorometers are not available to use for quantitative measurement. For qualitative measurement, the fluorescence intensities of samples after illuminated by laser or LED were represented by maximum intensities of the RGB plots of the images calculated using the ImageJ software.

Materials and Methods

There are two schemes used for this experiment, optical system for chlorophyll fluorescence imaging of detached spinach leaves and of spinach leaf extract. Both systems used diode lasers and LEDs with differents wavelength as the light sources. The diode lasers used were 405 nm, 532 nm, 650 nm, and 780 nm in wavelength. The LEDs used were 450 nm, 525 nm, and 680 nm in wavelength. A usb, 3 Megapixel, RGB CMOS camera was used as the imaging detector. The camera was equipped with a 35 mm camera lens with f/20 maximum aperture. The samples of this research was the green spinach *Amaranthus tricolar L.* The plants were grown in polybag with 15 cm in diameter, filled with dirt and compost with ratio 1:1 as shown in Figure 1. The plants were watered twice a day with 125 mL water/polybag.

Figure 1. Amarathus Tricolor L. Spinach grown under sunlight intensity variations

The plants were given treatments after 10 day growth. The treatments were the variations of sunlight intensities which were 90 % using one layer of plastic cover, 40% using a layer of plastic and a layer of dark net cover, and 32 % using two layers of dark net cover, compared to sunlight intensity without cover. For detached leaf samples, the three variations were used while for the extract samples, two variations were used. For each treatment, there were 5 plants or polybag

Figure 2a was the optical system for the experiment of chlorophyl fluorescence imaging using the detached spinach leaves. This experiment used dode lasers with 532 nm, 780 nm, and LEDS with 525 nm and 680 nm wavelength. The power for each light sources were 5 mW, 20 mW, 7 mW, and 5 mW respectively. Both system were built in a light tight box to minimize room light. The whole image for each leaf after illuminated by light was recorded by the CMOS camera. Each of the 2nd leaf for each polybag were taken after 20 days and 28 days to laboratory for investigation using the chlorophyll fluorescence imaging. Each sample leaf was placed for 30 minutes in the dark box before illuminated by laser or LED light to minimize quencing process. The images were processed using mageJ software. *Image-J* displays the fluorescence spectrum in RGB plots, RGB intensity versus distance or position in pixel.

Figure 2b showed the optical system of chlorophyll fluorescence imaging for spinach leaf extract. The diode lasers used were 405 nm, 532 nm, and 650 nm in wavelength. The LEDs used were 450 nm, 525 nm, and 680 nm in wavelength. The ND filters were used to make the excitation light intensity of each light source impinged on the cuvette equal. The CMOS camera recorded each spectrum obtained on the extract after illuminated by excitation light. The extracts of spinach leaf were obtained from spinach plants grown under two sunlight intensity variations, 90 % using a plastics cover and 40 % using a layer of plastic cover plus a layer of dark net cover. There were five samples for each treatment. The extracting of chlorophyll from spinach leaves was done in a biology laboratory, the extracts were kept in black bottle to avoid light contamination. The concentations of chlorophyll extracts used were 1gr/40ml.

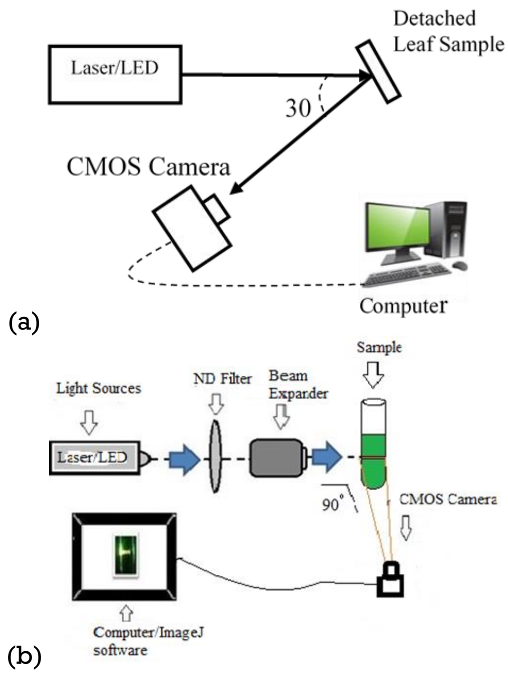

Figure 2. Optical system for chlorophyll fluorescence imaging of detached leaves (a) and for leaf extract (b), (not scaled).

ISSN: 978-602-71169-7-9

Results and Discussions

Results of this experiment were fluorescence images of spinach leaves and chlorophyll extracts recorded by the CMOS camera. The data were analyzed using ImageJ software and presented by RGB value histogram and also using graphs of maximum RGB values. The maximum RGB value is related to the fluorescence intensity. Variations of data were based the variations of sunlight intensities and wavelength of laser and LEDs used. There were 5 plants for each treatments. The maximum intensity obtained for each treatment was the average for 5 plants or polybags.

1. Analysis of Chlorophyll Fluorescence Intensities for detached leaf samples

Figure 3 showed the maximum fluorescence intensity represented by the maximum intensity in RGB plot. The graph showed the maximum fluorescence intensities of detached spinach leaves after 10 day treatment. The intensities were varied for each treatment and for each excitation light. The longer the wavelength was, the less the maximum intensity. There were two things that could cause linear decrease of the intensity, first is the absorption characteristics of a and b chlorophylls which absorb more NIR light, second is the sensitivity of the CMOS camera which less sensitive in NIR light. Futher investigation using a calibrated spectrometer is needed.

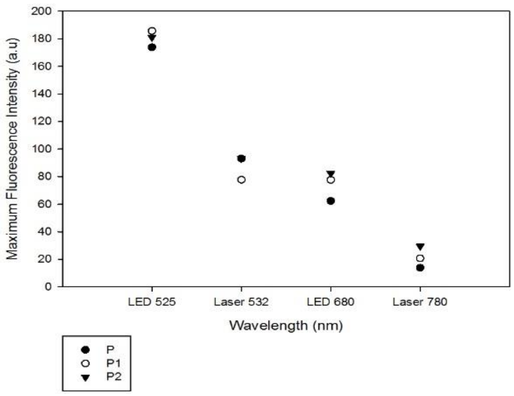

Figure 3. Maximum Intensities versus wavelength for detached leaf After 10 days treatments

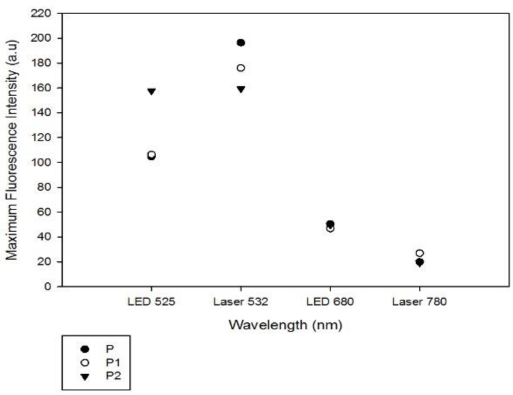

Figure 4 Maximum Intensities versus wavelength for detached leaf after 18 days treatments

Figure 4 showed the maximum intensity versus wavelength for detached leaves after 18 days treatments. As compared to Figure 3, after 18 days treatment, the plants started decreasing its chlorophyll concentration. It can be seen for each wavelength except for 532 nm laser. Treatment P stands for sunlight intensity variation using plastic cover only, followed by P1 and P2 using plastic plus dark net, plastic plus two dark net. After 10 day treatment, the diffrences in treatment can be seen significantly using lights with wavelength 680 nm and 780 nm, but at 18 day treatments, they were equal. The reason could be because the leaves were not sensitive to the NIR wavelength due to lacking chlorophyll content. The 680 nm LED and the 780 nm diode laser, respectively, gave 24.3 % and 29.5 % different in fluorescence intensities for 90% and 32 % sunlight intensity treatments. The 525 nm LED and 532 nm diode laser did not give significant difference for all treatments.

2. Analysis of Chlorophyll Fluorescence Intensities for samples of spinach leaf extracts

The experiment on the spinach chlorophyll was not as intensive as the detached leaf experiment. The chlorophyll extracts were obtained after extract 20 day treatments. The spinach plants were different than the spinach plants of the detached leaf experiment. After 20 day treatment, the spinach leaves started to change their color to yellowish color.

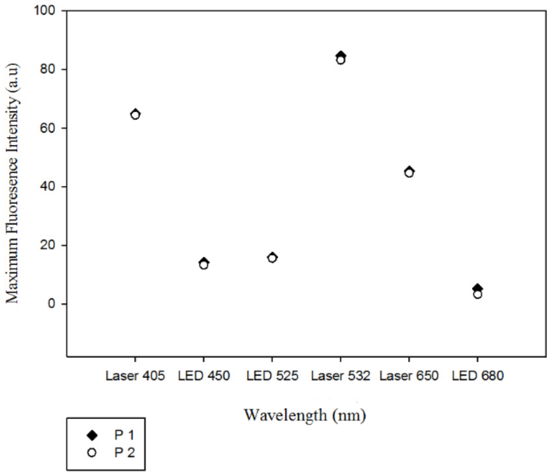

Figure 5 Maximum Intensities vs. wavelength for chlorophyll extract after 20 days treatments

Figure 5 showed the maximum intensity versus wavelength of the excitation light. The intensities were higher for laser lights than for LED lights because the laser lights used had higher excitation light intensities. The fluorescence spectrums of the chlorophyll extracts contained some excitation light which can bee seen from the highest peak in the RGB plot. The maximum intensity n Figure 5 was the second highest peak in the RGB plot. There is no significant difference could be seen between both sunlight intensity variations. This could be because most of the leaves were at the same state, the same chlorophyll content.

Summary

The experiments of chlorophyll fluorescence imaging for detached leaves and for extract spinach has been performed. There are some findings can be written for the experiments. The fluorescence intensities were higher for 525 nm LED 532 nm diode laser than those for 680 nm LED and 780 nm laser because the chlorophyll absorbed more light in NIR wavelength. The wavelength diode lasers and LEDS that can give significant differences in the three treatments were 680 nm for LED and 780 nm for diode laser for detached leaf experiment. The 680 nm LED and the 780 nm diode laser, respectively, gave 24.3 % and 29 % different in fluorescence intensities between 90% and 32 % sunlight intensity treatments. The 525 nm LED and 532 nm diode laser did not give significant difference for all treatments. The fluorescence intensities were higher at 10 day treatment that at 18 day treatment. It could be because the plants had much less chlorophyll since the optimum growth of the spinach is 25 days. The leaf extract method gave better spectrum visualization however it could not differentiate both treatments significantly except at 680 nm wavelengths.

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