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Research paper

Detecting cabbage seedling diseases by using chlorophyll fluorescence

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ABSTRACT

The objective of this study was to detect cabbage seedling diseases by using a self-developed chlorophyll fluorescence imaging system. Commercially available blue light sources with a wavelength ranging between 430 nm and 480 nm were used to excite fluorescence. A filter with a wavelength of 684 ± 10 nm attached to a near-infrared camera was used to capture the image of fluorescence emitted by the cabbage seedlings. The experimental results indicated that using the chlorophyll fluorescence imaging system, leaf spots on cabbage seedlings were detected 8 h earlier compared with using a charge-coupled device camera. Based on this finding, the proposed chlorophyll fluorescence imaging system can be used to develop early detection systems for cabbage seedling leaf spots.

1. Introduction

Growing seedlings in plug trays has become the mainstream model for culturing seedlings in Taiwan. Plug trays are primarily used because of the associated advantages, such as high production efficiency, high seedling quality, short recovery period for transplanted seedlings, and ease of use. However, diseases typically occur and spread rapidly among seedlings densely planted in plug trays. After a disease outbreak, pathogenic bacteria can spread through sprinkler irrigation systems and infect nearby healthy seedlings in a plug tray, eventually resulting in seedling deaths and serious losses. Moreover, if a disease does not cause immediate death of the seedlings, infected seedlings will exhibit weak growth or dysplasia (Chen and Tai, 2000).

In plant chloroplasts, the energy excited by light passes primarily through Photosystems I and II (PSI and PSII), which comprise reaction centers and antenna systems. During photosynthesis, the PSII antenna first receives light and transmits the light energy to the reaction center to pyrolyze water, generating oxygen and electrons. These electrons are then transmitted to PSI through an electron transport chain. During the transport process, the light energy absorbed by the antenna can be emitted via 3 pathways: (1) photochemical reaction, (2) dissipation in the form of heat, and (3) fluorescence emission. If light energy cannot be dissipated, the excess light energy causes photoinhibition, which reduces photosystem activity, chlorophyll fluorescence, and photosynthetic efficiency (Wong et al., 2011).

Using PSII to detect chlorophyll fluorescence is a nondestructive and highly sensitive method widely employed for detecting physiological stress in plants. Regarding the light source absorbed by leaves, 80%–90% is used for photochemical reactions, 5%–15% for heat dissipation, and 0.2%–2% for fluorescence emission. However, because of the difficulty of detecting photochemical reactions and heat dissipation, fluorescence emission of plant leaves was detected and adopted as a research index. The fluorescence wavelengths emitted by plants typically range between 660 nm and 800 nm (Yao et al., 2002).

Various parameter values of chlorophyll fluorescence (e.g., F0, Fm, Fv/Fm, and ΔF/Fm′) can demonstrate how chlorophyll uses incident light energy in real situations. These parameter values are defined as follows: F0 is the minimum fluorescence measured during dark adaptation; Fm is the maximum fluorescence measured during dark adaptation; and Fv/Fm refers to the optimal quantum yield, and is a common indicator for identifying photoinhibition. The Fv is defined as Fm−F0, indicating variable fluorescence. ΔF/Fm′ represents the effective quantum yield, which refers to the actual efficiency of quantum yield. ΔF = Fm′ − Fs, where Fm′ represents the maximum fluorescence during light adaptation, and Fs represents the reading when the fluorescence curve approaches a steady state during light adaptation.

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Chlorophyll fluorescence analysis is a rapid and nondestructive method for analyzing plant stress. When plant tissues are stressed or damaged, the fluorescence quantum yield is indirectly influenced because of the interrupted photosynthetic system. Many studies have shown that chlorophyll fluorometers can be used to detect relationships between plants and environmental, chemical, or physiological stresses. Therefore, chlorophyll analysis is a feasible method for identifying and predicting plants and fruits under various stresses.

In recent years, numerous scholars have conducted research regarding chlorophyll fluorescence applications. For example, Chaerle and Straeten (2001) indicated that the use of fluorescence images for identifying plant condition under stress is feasible. Krumov et al. (2008) used a chlorophyll fluorescence imaging system to detect the physiological conditions of plants under drought and high-temperature stresses. Lichtenthaler and Babani (2000) employed a chlorophyll fluorescence imaging system to measure plant photosynthesis and condition under water stress. Vargas et al. (2004) investigated animal fecal contamination of strawberries and cantaloupes using multispectral fluorescence imaging techniques. Qin et al. (2008) used hyperspectral reflectance imaging and spectral information divergence to detect citrus canker. Mendoza et al. (2010) integrated hyperspectral scattering characteristics and image analysis techniques to improve predictions of apple fruit firmness and soluble solids content. Chaerle et al. (2007) adopted chlorophyll fluorescence imaging techniques for early detection of tobacco mosaic virus to facilitate the development of prevention preventative measures.

Hsieh (2006) used saturation pulse chlorophyll fluorescence to develop an imaging system for detecting and analyzing plant condition under light-heat stress. Hsu (2007) used high-resolution chlorophyll fluorescence images to examine the photosynthetic performance of phalaenopsis. Chang et al. (2005) used chlorophyll fluorescence to monitor the physiological responses of grafted vegetable and fruit seedlings. They used a chlorophyll fluorometer to measure the chlorophyll fluorescence of grafted seedling leaves and explored the leaf performance of grafted seedlings under various environmental stresses. Previous researchers working in the same laboratory where this study was conducted also engaged in related studies of nondestructive detection using chlorophyll fluorescence images. Chiu and Chen (2011) developed an apple (e.g., Golden Delicious) bruise detection system that used chlorophyll fluorescence images. Chen (2011) developed an online system for detecting apple bruising based on chlorophyll fluorescence images.

A review of the aforementioned literature confirmed that chlorophyll fluorescence imaging techniques have potential applications in the early detection of diseases. The purpose of this study was to detect and analyze cabbage seedling diseases by using a chlorophyll fluorescence imaging system. Fluorescence spectrophotometers were used to determine the relationship between cabbage seedling diseases and chlorophyll fluorescence. In addition, chlorophyll fluorometers were used to detect and verify seedling condition and facilitate physiological disease detection of the seedlings. The primary goal was to devise a chlorophyll fluorescence imaging system capable of filming and identifying leaf diseases that cannot be seen with the naked eye. This study also explored whether the proposed system can detect diseases earlier compared with the traditional naked-eye identification method to achieve early prevention of seedling diseases.

2. Materials and methods

2.1. Chlorophyll fluorescence imaging detection system

This study adopted the chlorophyll fluorescence imaging system developed by Chiu et al. (2013) as the research basis. Comparing to the spectrometer which can only detect and calculate the fluorescence quantity value point by point on the leaf, the chlorophyll fluorescence image system can detect a region on the leaf and display the fluorescence quantities by values as well as by color so the operator can easily observe the distribution of the fluorescence variation.

The chlorophyll fluorescence image system measures the fluorescence intensity from the leaves was affected by the leaf physiological condition which is usually expressed by photosynthetic capability, growth vigor, or carbohydrate accumulation. The variances of leaf fluorescence intensity usually rely on the conditions of environmental factors and have been considered to be used as reliable values to reflect the real time responses of the leaves. Therefore, we used the leaf fluorescence intensity as an index of the leaf physiological condition expression. The original color of the leaf fluorescence image was only monochrome, from which fluorescence intensity values could be acquired. However, it was not easy for naked eye identification. For this reason, the original monochrome pictures were colored just for naked eye identifying convenience, not related to the fluorescence intensity measurements.

The overall system structure comprised a sampling box, excitation light sources, an emission light extraction system, and control and analysis systems, as shown in Fig. 1. The system light source was provided by 4 high-brightness blue light-emitting diodes (LEDs), with a power supply of 12 VAC, power consumption of 5 W for each unit, and a brightness of 8 lm. A PCI-1411 image capture card was combined with a near-infrared high-sensitivity camera (produced by Japan Analytical Industry, Japan), with a resolution of 752 (H) × 582 (V) pixels, a 0.5-in sensor chip, and a 1/10000 electronic shutter. Compared with conventional color cameras, the camera used in this study exhibited higher photoelectronic responses to the chlorophyll fluorescence emitted by plants, facilitating observation of chlorophyll fluorescence intensity. The lens focal length was 17 mm, and the aperture value was 5.6. In addition, a 684 ± 10-nm bandpass filter (Onset Electro-Optics Co. Ltd.) was used to capture the fluorescence emitted by cabbage leaves. The 2009 version of LabVIEW graphic-based programming language developed by National Instruments was adopted to establish a chlorophyll fluorescence imaging detection system and human−machine interface.

2.2. Experiment regarding the fluorescence characteristics of cabbage seedlings

To verify whether chlorophyll fluorescence can be used to detect cabbage seedling diseases, a Hitachi F-4500 fluorescence

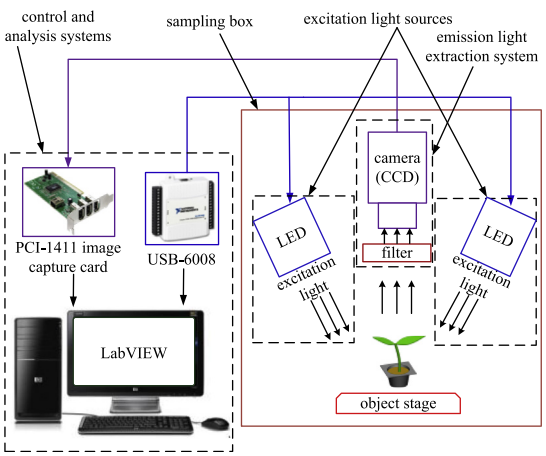

Fig. 1. Structure of the chlorophyll fluorescence imaging detection system (Chiu et al., 2013).

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