Laser-induced Fluorescence Spectroscopy for In-vivo Monitoring of Plant Activities

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Abstract

New spectroscopic method based on laser-induced fluorescence spectroscopy (LIFS) is proposed for the purpose of in vivo plant activity monitoring. To discuss LIFS application to plant monitoring, we developed three different LIF measurement systems which were used properly according to measurement applications. The basic configuration of the LIF system consisted of a laser, a spectroscopic device, a detector and a PC. Here, we report the systems’ details and some plant leaf monitoring results. 1) Tomato leaf growth monitoring by a mobile LIF spectrum monitoring system: LIF spectrum of tomato leaf was generally separated into two wavelength regions from 400 nm to 650 nm (blue-green LIF) and from 650 nm to 800nm (red & far-red LIF). It was suggested that a slope of the blue-green intensity to the red & far-red one could be a parameter for evaluation of leaf growth process. 2) Vitality map of coffee tree leaf by a LIF imaging system: The system made possible to show an intensity distribution as a visualized LIF image with any wavelengths. The intensity was gradually decreased from the root part toward the top part of the leaf. Relation of the LIF intensity to plant vitality as effective light use inside the leaf was discussed. 3) Remote estimation of chlorophyll concentration of tree leaves by a LIF imaging lidar (light detection and ranging): It was found that the ratio of LIF intensity at 740nm to that at 685 nm had a linear correlation to chlorophyll concentration of leaf. Monthly variation in chlorophyll concentration of natural living tree leaves 65 m away from the system was remotely monitored. Through these experimental results, we discuss the effectiveness and adaptability of the LIFS idea and the usefulness of the developed systems for precise monitoring of agricultural-related plant activities.

INTRODUCTION

In vitro chemical treatments have been used to understand plant activities. This can offer very sophisticated physiological information, unfortunately it is not suitable for living materials, i.e. plant and agricultural products, because the treatment needs crash process. Also the system is generally designed for laboratory use. Otherwise in agricultural field, previous experience using information as size, shape, weight, color and so on judges growth stage of agricultural products and determines the harvest time. More objective criteria not depending on individual’s judgment are required.

Plants use the sun light in any growth stage and after using the light energy for living activities, unused energy in that stage is reemitted outside as fluorescence. As
fluorescence reflects the primary process of photosynthesis, information on plant growth status, health and disease, light use and so on can be obtained by detecting the fluorescence, which offers non-destructive in vivo plant monitoring.

Idea of fluorescence use for remote plant monitoring was firstly indicated by Hemphill (1968) who used Fraunhofer Line Discrimination principle (FLD) (Kozyrev, 1956). Recent research of FLD plant monitoring is addressed to imaging (Saito et al., 2003) and aircraft monitoring (Moya et al., 2004). Stoll et al. (1999) proposed a space mission for screening vegetated area. The FLD is a passive monitoring technique. Active fluorescence, i.e. laser-induced fluorescence (LIF), monitoring technique has been also studied. Usage of laser source in place of the sun light induces fluorescence effectively. Laser’s single wavelength as an excitation source makes it easy to analyze fluorescence data. Measures et al., (1973) reported blue fluorescence detection from sugar maple tree at range of 542 feet by a LIF lidar (light detection and ranging). McFarlane (1980) applied LIF information to plant stress detection. Especially, UV laser-induced LIF has been widely studied in plant biology (Cerovic et al., 1999).

In this paper, we describe our developed LIF measurement systems first, then show LIF spectra and LIF images of plant leaf, and finally through the experimental results, we discuss the effectiveness and adaptability of the laser-induced fluorescence spectroscopy and the usefulness of the developed systems for precise monitoring of plant activities.

METHODS AND MATERIALS

Mobile LIF Spectrum Monitoring System and Tomato Seedling

A picture of a mobile LIF spectrum monitoring system is shown in Fig. 1. A LIF excitation source was a pulsed Nd:YAG laser with wavelength of 355 nm, pulse energy of 0.2 mJ, pulse width of 10 ns, beam diameter of 6 mm and repetition rate of 10 Hz. The laser beam was delivered by a transmitting fiber to a tomato leaf. LIF from the tomato leaf was collected and sent to a monochromator with a receiving fiber and the output from the monochromator was detected with an intensified CCD detector. Outlet of the transmitting fiber and inlet of the receiving fiber was set in one united holder. The holder was gently pressed on the leaf through a soft rubber to prevent incoming ambient light. Tomato seedlings were cultivated in a greenhouse of Research Institute of KAGOME Co., Ltd (Nishinasuno-machi, Nasu-gun, Tochigi, Japan).

LIF Imaging System and Coffee Tree

A picture of a LIF imaging system is shown in Fig. 2. A sample leaf set in a dark box was irradiated by a violet laser diode with wavelength of 398 nm, average (CW) power of 30 mWmax. The beam diameter was expanded by a beam expander to cover the whole area of the leaf. A liquid crystal tunable filter was used for selection of a desired wavelength from broad LIF spectrum of the leaf. The wavelength selection from 420 nm to 750 nm could be easily and rapidly done by voltage tuning. This tuning method was ideal for image analysis, because it had no mechanical moving which sometimes made difficulties for precise positioning of pixels. An image intensified CCD camera (1024 x 1024 pixels) detected LIF and made it visible as an image with the selected wavelength. Sample was a coffee tree leaf. Coffee trees were cultivated at UCC UESHIMA COFFEE Co., Ltd. Hawaii Farm (Kona, Hawaii, U.S.A.) under natural weather conditions.
LIF Imaging Lidar System and Natural Tree

A picture of a LIF imaging lidar system (Saito et al., 2002) and experimental configuration are shown in Fig. 3. The system was developed to investigate the feasibility of long-range plant monitoring. A pulsed Nd:YAG laser beam coming from a laboratory building irradiated target trees. Laser was with wavelength of 532 nm, pulse energy of 10 mJ, pulse width of 6 ns, beam diameter of 6 mm and repetition rate of 10 Hz. The beam was magnified by a negative lens so that the beam could cover the whole area of the target tree. LIF from tree leaves was collected by a 42 mm diameter camera lens, and in front of the lens three band-pass filters were inserted alternately to obtain chlorophyll fluorescence (685 nm and 740 nm) and scattered light (532 nm) from the leaves. They were detected by a gated image intensified CCD camera (510 x 492 pixels). The gate with 40 ns was opened at 413 ns after the laser was fired. This synchronized delay setting with only a short opening period allowed the system to obtain weak fluorescence signal only from the tree and to reduce ambient light. The target trees were poplar (Populus nigra var. italica), ginkgo (Ginkgo biliba Linn) and hiba (Thujaopsis dolabrata var. Hondae Makino) growing under natural conditions in our campus, which was located about 65 m away from the system.

RESULTS AND DISCUSSION

Growth Stage Monitoring of Tomato Leaf

Using the mobile LIF spectral monitoring system, LIF spectrum of the tomato leaf was measured. The spectrum shown in Fig. 4 was separated into two wavelength regions; blue-green fluorescence which was shorter than 650 nm and red & far-red fluorescence which was from 650 nm to 800nm with two peaks at 685 nm and 740 nm. The same spectral shapes also obtained in many plant leaves (Saito et al, 1998), so the spectrum shape was a common feature of plant leaf. Figure 4 shows a slope between the blue-green intensity (peak) to the red & far-red one. The leaf at the middle part of the seedling had larger gradient, and at the bottom part the slope took the opposite gradient. It was suggested that the top part leaves were under growing, the middle part ones were mature, the lower part ones were older, and the bottom part ones were withered or dead. Cerovic et al. (1999) reported that main origins of the blue-green fluorescence were ferulic acid derivatives, other phenylpropanoids, and NAD(P)H, and that of the red & far-red fluorescence was chlorophyll a. The slope reflected the balance of those pigments which changed according to the growth stage. Direction of the slope (positive or negative) together with the entire LIF shape can be a good parameter for estimation of the leaf growth process related to those pigments.

Vitality Map of Coffee Tree Leaf

LIF images with different wavelengths of a coffee leaf are shown in Fig. 5. The wavelengths of 460 nm, 685 nm and 740 nm corresponded to the featured one of LIF spectrum (see Fig. 4). Each image at each wavelength contains information on pigments mentioned above. A distribution pattern in the LIF intensity image was clearly seen even in one leaf. This means that living status was different depending on the leaf area. As a common feature, the intensity in every image gradually decreased from the root part toward the top part of the leaf. Two possible considerations on this were considered; 1) if fluorescence is a process of dissipation of absorbed light energy which should be used for photosynthesis activity, the leaf top where emitted fluorescence was very low used the
absorbed light energy more effectively in large quantity and photosynthesis worked better at the top area than other parts, or 2) if fluorescence intensity increases with the amount of pigment, the root part contained larger amount of the pigment, so that overall activity of photosynthesis was higher at the root part. Possibility of vitality mapping related to light use and photosynthesis activity can be shown by using LIF image data.

Remote Estimation of Chlorophyll Concentration of Natural Tree Leaf

Feasibility study of outdoor long-range LIF measurement was attempted. Our attention was focused on remote estimation of chlorophyll concentration. The idea that the ratio of LIF intensity at 740 nm to that at 685 nm was proportional to chlorophyll concentration (Saito, 2002) was applied to the image data at each of the two wavelengths obtained by the LIF imaging lidar. Chlorophyll concentration itself was quantified by a high performance liquid chromatograph (HPLC). Experiments were done only at night. Results are shown in Fig. 6. Chlorophyll concentration of living tree leaves 65 m away from the system was remotely estimated. Seasonal variation of the concentration was clearly seen. Poplar tree leaves (the left of each image) had higher concentration than ginkgo tree leaves (the center of each image). Hiba tree leaves, which was an ever green tree, kept high concentration even in November. Information about difference of productivity on tree species could be visualized. It should be added that daytime measurements were also possible, but signal to noise ratio was less than that of the night time experiments at that point of time (Saito et. al, 2000).

CONCLUSIONS

Use of the laser-induced fluorescence as a parameter to monitor plant activities was investigated. To show its usefulness experimentally, three LIF monitoring systems were developed so they could answer different requirements of plant monitoring. The LIF spectrum offered information on overall growth status of plants. The LIF image suggested the difference of plant activity depending on the leaf area, and application of LIF information to vitality evaluation was suggested. Remote estimation of chlorophyll concentration was successfully done by the LIF imaging lidar and feasibility of long-range and large-sized plant monitoring was also shown. In the next step, cooperation experiments with another instruments such as CO₂ monitor and water-vapor monitor which can offer direct photosynthesis information is considered to ensure the LIF(S) usefulness for plant activity monitoring.

Our future plan is to combine both of the LIF spectrum monitoring and the LIF imaging system. Such system should be a mobile-vehicle system, so that the monitoring will be performed at anywhere and anytime.

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**Literature Cited**


Figures

**Fig. 1.** Mobile LIF spectrum monitoring system

**Fig. 2.** LIF imaging system
**Fig. 3.** LIF imaging lidar system and experimental configuration

**Fig. 4.** LIF spectrum of tomato leaf depending on location
**Fig. 5.** LIF image of coffee tree leaf with different wavelength

**Fig. 6.** Remote estimation of chlorophyll concentration of natural tree leaves
Spectroscopie de la fluorescence induite par laser pour le suivi in-vivo de l’activité des plantes

Mots clés: Spectre, imagerie, camera CCD, feuille, tomate, café, arbre, LIDAR

Résumé
Une nouvelle méthode de spectroscopie basée sur la fluorescence induite par laser (LIFS, ou Laser-Induced Fluorescence Spectroscopy) est proposée pour le suivi de l’activité des plantes. Pour évaluer l’application de la LIFS au suivi des plantes, nous avons développé trois systèmes de mesure LIFS et les avons utilisés en adéquation avec différentes applications. La configuration de base du système LIFS comprend un laser, un composant spectroscopique, un détecteur et un PC. Nous en présentons ici le détail ainsi que des résultats de mesures sur feuilles. 1) Suivi de la croissance de feuilles de tomate par un système LIFS mobile : le spectre de la fluorescence induite est généralement séparé en deux domaines respectivement de 400 à 650 nm (fluorescence bleu-vert) et de 650 à 800 nm (fluorescence rouge et rouge lointain). Il est suggéré que la pente entre l’intensité dans le bleu-vert et celle dans le rouge et rouge lointain pourrait être un paramètre d’évaluation du processus de croissance des feuilles. 2) Cartographie de la vitalité d’un arbre cafété par un imageur LIFS : le système permet de visualiser sous forme d’image la distribution des intensités de la fluorescence induite pour n’importe quelle longueur d’onde. L’intensité décroît progressivement de la base au sommet de la feuille. La relation entre cette intensité et la vitalité de la plante, en terme d’usage effectif de la lumière à l’intérieur de la feuille, est discuté. 3) Estimation à distance de la concentration en chlorophylle de feuilles d’arbre par un système LIFS à imagerie LIDAR (détectio de lumière et mesure de distance). Il a été trouvé que le rapport des intensités à 740 nm et à 685 nm de la fluorescence induite est corrélé linéairement à la concentration en chlorophylle de la feuille. La variation mensuelle de la concentration en chlorophylle de feuilles d’arbres en conditions naturelles a été mesurée à 65 m de distance. A travers ces résultats expérimentaux, nous discutons l’efficacité et l’adaptabilité du concept LIFS et l’utilité des systèmes développés pour le suivi précis de l’activité de plantes agricoles.