

Delayed Luminescence of Biophotons from Plant Leaves

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Delayed luminescence of plant leaves was imaged by a 2-D cooled charge-coupled device. We report the delayed luminescence imaging of normal/injured leaves picked and the leaves intact. The luminescent intensity was lower in leaf veins, scars and edge cut. The intensity of delayed luminescence from intact leaves was higher than that of picked leaves. These results indirectly support the argument that the delayed luminescence of a photosynthetic system is closely related to the electron transfer process of PSII in the thylakoid membrane.

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I. INTRODUCTION

Delayed luminescence (DL) is a light-induced photon emission in the optical spectral range (400~800 nm) from biological systems. It differs from common fluorescence or phosphorescence by its extraordinarily long decay time (milliseconds to hours) and ultra-weak luminescent intensity (with a few up to some hundred thousand photons $/(cm^2 \cdot s)$) [1-9]. Furthermore, it has been known that DL shows non-exponential (hyperbolic) relaxation characteristics [1,2,6,7]. This unusual property of DL is sometimes accepted as evidence of macroscopic coherent fields in living systems [1,6-9]. In this perspective, the spontaneous photon emission and delayed luminescence are all categorized as a biophoton emission [1,7-14]. The term of "biophoton" implies that the ultra-weak photon emission reveals critical information of metabolic processes and quantum mechanical cooperative nature within biological systems although some people in the field of biophoton research do not agree with the concept of quantum mechanical coherence of living systems. On the other hand, in the field of fluorescence microscopy, the "delayed luminescence" indicates the phosphorescence and delayed fluorescence from chromophores in living systems. In this case, samples are stained artificially to obtain the microscopic image which shows the dye interactions with specific biomolecules [15-17]. Taking any point of view, it is widely accepted that the delayed luminescence of photosynthetic systems is caused by a back reaction occurring

between the primary photoproducts of PSII reaction centers, which is stimulated by the light-driven electrochemical gradient across thylakoid membranes [18,19]. There have been a few publications on the imaging of DL. The first report by Bennoun and Beál describes the DL from a mixture of wild-type and mutant colonies of *Chlorella sorokiniana*, whose mutants have an altered permanent or light-induced electrochemical gradient across thylakoid membranes [18]. The second report by Parkhomtchouk and Yamamoto presents the DL of whole plants which have white flowers and green leaves. It also contains the imaging of DL from human palms too [20].

In this paper we report the full-scale imaging of DL from leaves for the first time. We investigated the DL imaging of normal/injured leaves and the intact leaves. The DL image was obtained by illumination by a metal halide lamp. The intensity of DL was low in leaf veins, scars and edge. It has a great notion that the DL intensity of intact leaves was higher than that of picked leaves.

II. METHODS AND PROCEDURE

1. Experimental Setup

The two-dimensional charge-coupled device camera (CH250 with Metachrome II (phosphor coated), Photometrics) with a chip of TH7895B (Thomson, back

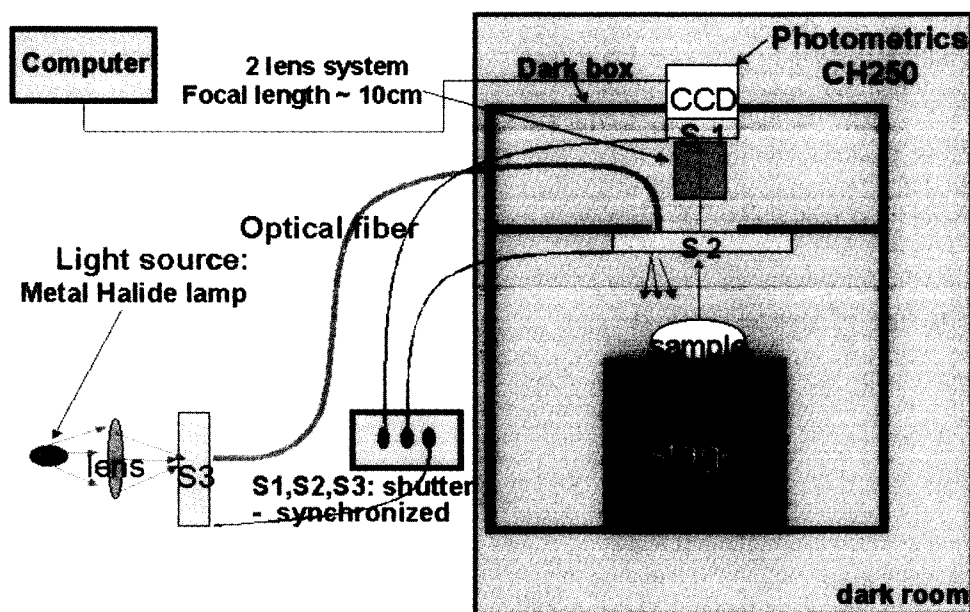


FIG. 1. Schematics of the DL imaging system.

illuminated) was consisted of 512×512 pixels of size $19 \mu\text{m} \times 19 \mu\text{m}$. (Fig. 1) Its effective image area was $(512 \times 19 \mu\text{m}) \times (512 \times 19 \mu\text{m})$ and the working temperature was maintained at -45°C . An electric shutter (Shutter1; S1) of which operation time could be regulated by PC interface up to 1 microsecond order, was set ahead of the CCD. In front of the S1, a two-lens system was set which enabled one to focus a subject in very short ranges (10~15 cm). The lense system had an NA of 0.18. The illumination light from the light source was focused on the optical fiber which guides the light onto the subject. We used three shutters in the system: the first was located directly in front of the CCD window. The second shutter between a specimen and the two-lens system was for controlling the illuminating light. The third one was located between the light-source side and the optical fiber to block the illumination light. Shutter2 (S2) stayed closed when Shutter3 (S3) was opened and opened when Shutter3 was closed. The light-tight chamber and the stage were made of anodized aluminium.

2. Procedure

A reference image was obtained by 0.1 second exposure under external illumination. A background image which was to be used for background correction was acquired under darkness. First, with the light source being on, S3 was opened while S1 and S2 were closed. This was to prevent illumination effect on lenses. Stray light from delayed luminescence of the apparatus including glass lenses is high enough to ruin the signal to noise ratio (S/N). After illumination, S3 was closed and then S2 and S1 were opened and the

data acquisition started. This process was carried out in one second. The data analysis was performed by a built-in image processor in the CCD system. With the raw image, we subtracted the background image and then analyzed the data by using the relative intensity at each pixel. A Gaussian filtering was mainly used.

Leaves of *Euonymus japonica* were picked from the one selected plant and were adapted in a dark room for 5~10 minutes.

III. RESULTS

1. Imaging of DL from Leaves

We used a neutral white metal halide lamp (Osram Powerstar HQI-R 150 W/NDL with pre-focused dichroic reflector) which had broad band from 370 to 750 nm (peaks at 400, 530, 580 and 660 nm) as an illumination light source. The illumination time was 10~180 seconds

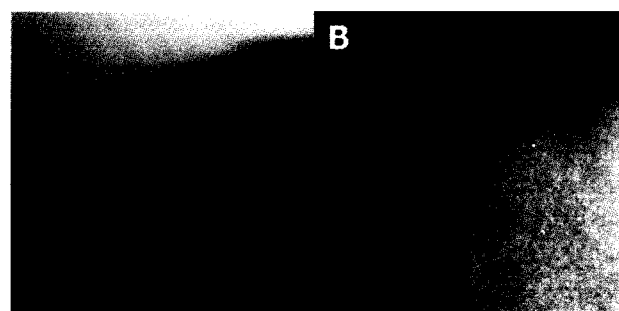


FIG. 2. (A) Reference image of a leaf. The dark part corresponds to the leaf. (B) DL image of the leaf.

and the exposure time was 60 and 180 seconds. The DL image is presented in Figure 2. Fig. 2 (A) is the image for reference. The dark part of the image is the sample leaf. In Fig. 2 (B), the bright part is the DL image of the sample. One can see that the whole part of the leaf emits DL. The average value of photon counts per pixel was 3~4 higher than the background image.

2. DL Image of Leaves Picked

DL image from whole leaf of *Euonymus japonica* showed some interesting results. The first was that there exists lower level luminescence in veins than that in other parts of the leaf (Fig. 3). The reference image which shows the reflectance of the leaf indicates that the veins have higher reflectance than the rest of the leaf. Since the incident angle of the illumination light is all same at the entire region of the leaf and the leaf

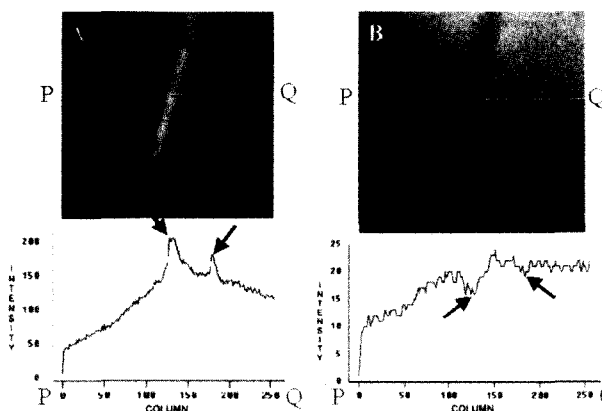


FIG. 3. (A) Reflectance of the leaf (reference image). One can see that the veins have higher reflectance. (B) DL image of the leaf. DL from veins has lower luminescent intensities than that from surrounding parts. The unit of the intensity is counts per pixel.

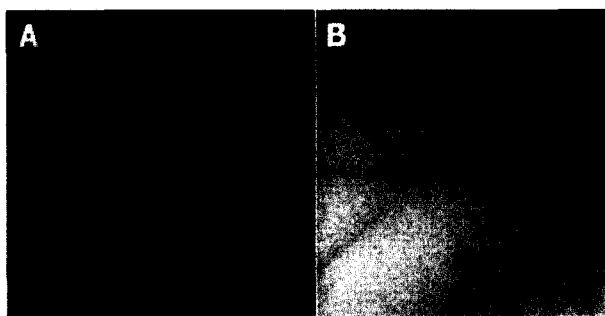


FIG. 4. DL imaging of cross-formed scar on a leaf. (A) Reference image. (B) DL image of the scar. The DL intensity at the scar was about 8.5% weaker than that of non-scarred parts. (The longitudinally centered dark part of the DL image is the major vein.)

was fairly flat, there is no topological effect on reflectance. In contrast to this, the DL from veins has lower luminescent intensities. The second result in this experiment is the DL imaging of the scars on a leaf. A cross-formed scar was made on the upper epidermis and palisade mesophyll by a steel cutter (Fig. 4). The DL intensity at the scar was about 8.5% weaker than that of non-scarred parts (The longitudinally centered dark part of the DL image is the major vein.). Last, DL from edge cut of a leaf was imaged. (Fig. 5) The cutting tool used here was also a steel cutter. There was no special increase or decrease of DL intensity at the edge cut.

3. DL Image of the Leaves Intact

We imaged the DL of a *Euonymus japonica* leaf which was intact with the individual plant. The subject tree planted in a ceramic vessel with leaf mold was

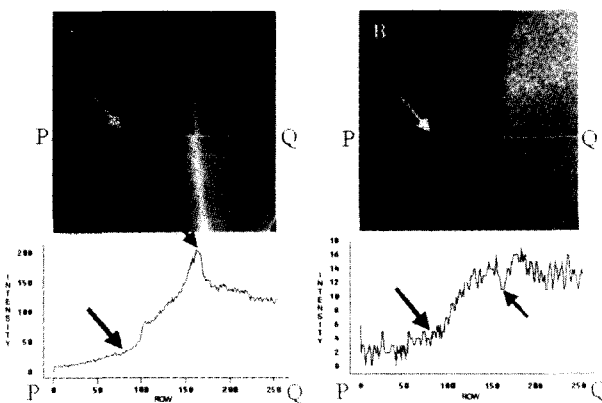


FIG. 5. DL from edge cut of a leaf. (A) Reflectance of edge cut of the leaf. (B) DL image of the edge cut. The DL intensity shows similar tendency to the reflectance. The big arrows indicate the edge cut. The smaller arrows point to the vein part of the leaf. The unit of the intensity is counts per pixel.

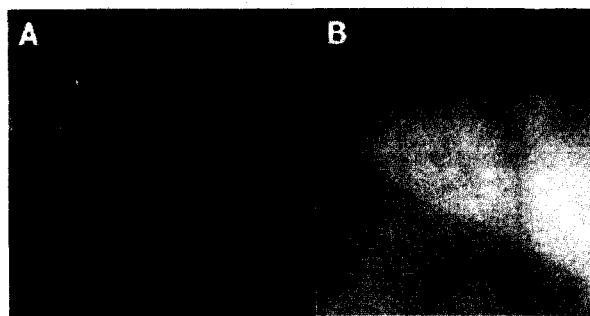


FIG. 6. DL image of an intact leaf. (A) Reference image. (B) DL image of the leaf. DL intensity from the major vascular tissues of the leaf was about 33% weaker than that from the ground tissues.

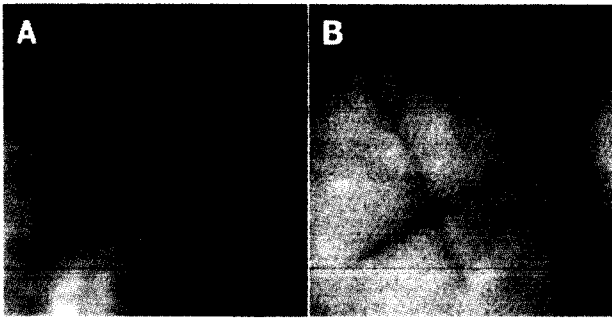


FIG. 7. DL imaging of the scar on an intact leaf. (A) Reference image. (B) DL image of the scar. DL of the scar was about 30% weaker than that from undamaged parts.

taken into the light-tight chamber. A selected sample leaf was fixed on an anodized aluminium stand for the purpose of stable imaging. DL intensity from the major vascular tissues of a leaf was about 33% weaker than that from the ground tissues (Fig. 6). DL of scar was about 30% weaker than that from undamaged parts (Fig. 7).

IV. DISCUSSION

In Fig. 3, the DL intensity from veins has a lower value than that of the other parts. This result can be related to the distribution densities of chlorophylls which is regarded as a source of DL [18,19]. Leaf vascular tissues contain few chloroplasts within themselves in comparison to the ground tissues (mesophylls) [15] (Fig. 8). Since it is widely accepted that the electron transport processes in the chloroplast membrane induced by external light stimulation cause luminescent processes in the visual range, the local chlorophyll distribution differences could be a direct reason for the inhomogeneity of DL image from a leaf.

In Fig. 4 and Fig. 5, one can find that the DL intensities at the scar and the edge cut are weaker than that of non-scarred parts. This result is also interpreted by the local difference of chlorophyll distribution den-

sities on a leaf since the spongy mesophyll cells are loosely packed and less chlorophylls in comparison to the palisade mesophyll cells [21]. It is worth to remind that the palisade layer was damaged at the scar. Previous report of ultra-weak photon emission imaging from injured plants by Suzuki *et al.* is compatible to this result [22]. They imaged a cross-injured adzuki seedling and reported that the higher intensity of biophoton emission was at the injured region which indicated (according to their point of view) the defense mechanism of the plant to injury or infection. However, our experiment showed that lower photon emission intensities were localized at the injury in the case of DL. This implies that the mechanism of spontaneous photon emission and delayed luminescence of plants may be different [23]. As an additional remark, it seems that the tendency of reflected light and that of DL are mostly correspond.

The DL images of the leaves intact, Fig. 6 and Fig. 7, the experimental results are very similar to those of picked leaves. It is noteworthy that the overall DL intensities of intact leaves were higher than that of picked leaves in general. This may due to higher water content and more active chlorophylls in the leaves intact.

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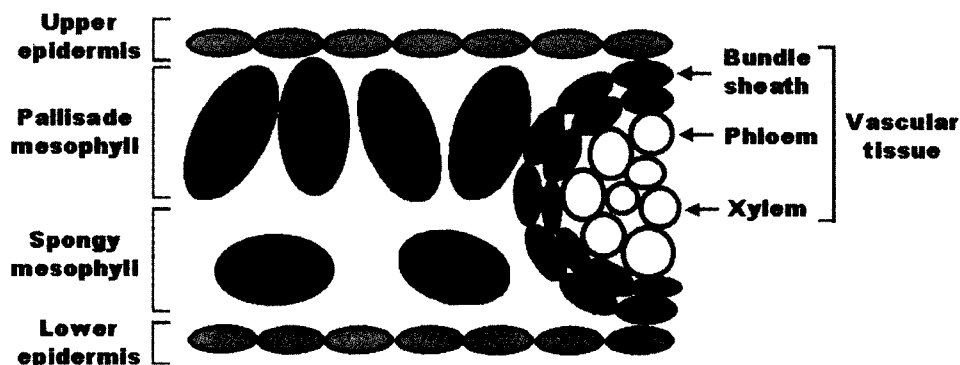


FIG. 8. Schematics of the cross-section of a plant leaf. Each oval or circular object represents a cell organizing the leaf.

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