CHAPTER SEVEN

Three-Dimensional Chlorophyll Fluorescence Imaging for Detecting Effects of Herbicide on a Whole Plant

Akira Eguchi, Atsumi Konishi, Fumiki Hosoi, and Kenji Omasa

Abstract In this study, a three-dimensional image of chlorophyll *a* fluorescence of a whole plant was generated and examined. After a melon (*Cucumis melo* L.) plant was treated with herbicide containing 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), the two-dimensional image of chlorophyll *a* fluorescence intensity of the plant was captured and mapped onto a three-dimensional image derived from lidar (light detection and ranging) data. From the image, it was observed that increases in chlorophyll *a* fluorescence intensity appeared along the veins of the leaves. DCMU inhibited photosynthetic electron transport, which consequently caused disappearance of the chlorophyll *a* fluorescence quenching. Therefore it was implied that the herbicide containing DCMU was absorbed from the root and reached the veins of the leaves through the stems. The image produced allowed three-dimensional observation of chlorophyll fluorescence intensity of a whole plant from any point of view. Consequently, we could recognize the difference of the intensity at regions along the veins in mature leaves.

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Introduction

Imaging techniques have been widely used for plant analysis. There are several two-dimensional imaging techniques, e.g., thermal imaging, multi-spectral imaging, hyper-spectral imaging and fluorescence imaging. These imaging techniques allow non-destructive detection of photosynthesis, transpiration, stomatal response, and substances in leaves (Omasa 1990; Lichtenthaler et al. 1996; Ustin et al. 1999; Govindjee and Nedbal 2000; Buschmann et al. 2000; Osmond and Park 2002; Omasa and Takayama 2003; Chaerle et al. 2003; Jones 2004; Oxborough 2004). Chlorophyll *a* fluorescence imaging enables us to measure directly the spatial and temporal changes in photosynthetic electron transport in leaves. Diagnosis of the developmental stage of photosynthetic organs and of functional injuries caused by biotic and abiotic stresses are conducted using the technique.

Lidar (light detection and ranging) has been used as a novel active sensing tool for three-dimensional measurement (Vanderbilt 1985; Omasa et al. 2002, 2007; Tanaka et al. 2004; Parker et al. 2004; Hosoi and Omasa 2006). Lidar can measure accurately the distance between the sensor and a target based on the elapsed time between the emission and return of laser pulses (time of flight method) or based on trigonometry (optical probe or light section methods). The three-dimensional structure of plants, including the shape, size, position and tilts of leaves, changes with growth stage and also with stress (Schurr et al. 2006; Omasa et al. 2007). If the three-dimensional structure of plants measured by lidar is combined with a two-dimensional image such as a chlorophyll *a* fluorescence image, it would be possible to correlate information on physiological responses and three-dimensional structures, providing new knowledge that could never be obtained from the two-dimensional image alone. In this paper we combined a three-dimensional image measured by high-resolution portable scanning lidar with a two-dimensional chlorophyll *a* fluorescence image of an individual, herbicide-treated plant.

Materials and methods

Plant material

Melon (*Cucumis melo* L.) seedlings were grown in a growth chamber. The plants were illuminated for 12 h each day with fluorescent lights at a PPF of 200 μM m^{-2} s^{-1}. Air temperature was 26.5°C during the day and 24°C at night. Relative humidity was 70% during the day and 90% at night for about 4 weeks after germination. The pots were filled with artificial soil (mixture of vermiculite and perlite, 2:1, v/v). Plants were watered daily with a nutrient solution (1:1,000 dilution of HYPONex).

Imaging system

Figure 1 shows a chlorophyll a fluorescence imaging system. A metal halide lamp (Sumita Optical Glass, Inc., LS-M180) of the wavelength of 400–700 nm was used as actinic light for photosynthesis. The light intensity was kept at 130–160 μM m^{-2} s^{-1} on the leaves of a whole plant. Chlorophyll *a* fluorescence was measured by a cooled CCD (charge coupled device) camera (Hamamatsu

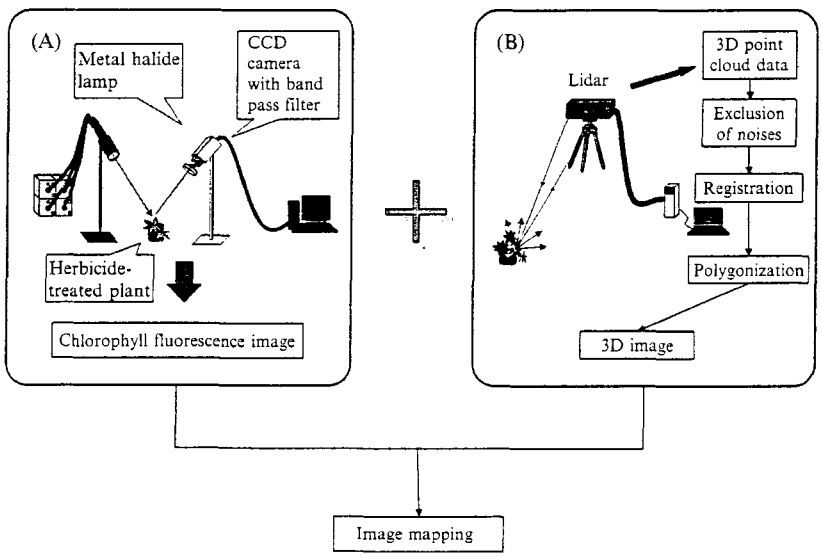

Fig. 1 Schematic view of combining chlorophyll *a* fluorescence image with three-dimensional lidar image. A: procedure of chlorophyll fluorescence image. B: procedure of three-dimensional lidar image

Photonics, C5985) with band pass filter (Optical Coatings Japan, MIF-W, central wavelength = 755 nm and half band width = 8 nm). The measurement was started just after the treatment of the aqueous solution of the Nekosogi-ace which is a commercially available herbicide including 3-(3,4 dichlorophenyl)-1,1-dimethylurea (DCMU) to soil in a potted plant.

For the three-dimensional measurement of the plant, a high-resolution portable scanning lidar (Pulstec Industrial Co., Ltd., modified type of TDS - 130L) was used (Fig. 1B). The lidar is based on the light-probe method and is able to obtain three-dimensional point cloud data of an object within the range of 3.5–10 m. The spatial resolution is 1.7 mm to y axis, 1.2 mm to x axis. The accuracy of range measurement is 0.5 mm at a distance of 3.5 m and 1 mm at a distance of 5 m.

As plants have complicated shapes, self-occlusion within the plants often occurs. Thus, the plant was scanned from four measuring points around it. In this way, all leaves were captured without a blind area. Since the measured point cloud data included noise, this were excluded by median filtering. After that, the lidar data obtained from different measurement points were co-registered into the same orthogonal coordinates. Then, the point cloud data was converted into a polygon image, in which three-dimensional surfaces of leaves are expressed as irregular triangle meshes determined uniquely by arrangement of each point. Finally, the two-dimensional chlorophyll fluorescence image was mapped on the lidar-derived three-dimensional image by the texture-mapping technique (Heckbert 1986). The accurate selection of the corresponding points between the two-dimensional and the three-dimensional images is of great importance because of the complicated structure of the plant. The corresponding points were chosen on the distinctive points of each leaf (e.g., the axils and edges), and the accurate three-dimensional chlorophyll *a* fluorescence image was obtained.

Results and discussion

As shown in Fig. 2, the chlorophyll *a* fluorescence intensity image at 2 h after the herbicide treatment could be accurately mapped onto the lidar-derived three-dimensional image. The difference in the chlorophyll fluorescence intensity was observed in mature leaves from the three-dimensional image produced. The image then showed that increases in chlorophyll fluorescence intensity appeared along the veins of the leaves. It was noted that DCMU inhibited photosynthetic electron transport, consequently the chlorophyll fluorescence quenching disappeared (Maxwell and Johnson 2000). Therefore, it was implied that the herbicide containing DCMU was absorbed from the root and reached the veins of the leaves through the stems.

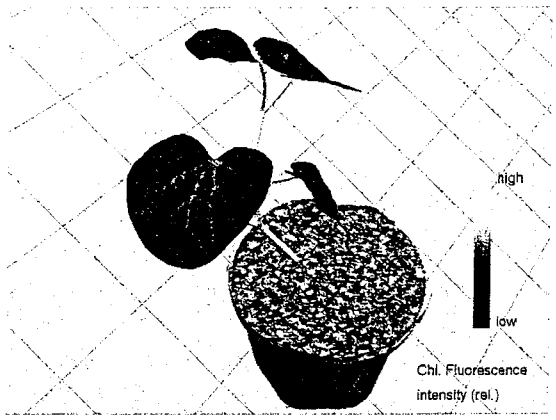

Fig. 2 An example of three-dimensional chlorophyll *a* fluorescence image of a melon plant (*Cucumis melo* L.) at 2 h after treatment of a herbicide containing DCMU

The image produced allowed three-dimensional observation of chlorophyll fluorescence intensity of a whole plant from any point of view. Consequently, we could recognize the difference of the intensity at sites along the veins in mature leaves. Besides the information of the intensity, the image contained detailed structural information, e.g., vertical placement of leaves and stems, leaf inclination, spatial patterns of veins and veinlets in the leaf, etc. Such information within the image could be applied for studying the relationship between physiological and structural characteristics of plants. Also, the chlorophyll fluorescence image can be replaced to other two-dimensional images, such as thermal image and PRI (photochemical reflectance index) image (Omasa et al. 2007). The three-dimensional

composite images will increasingly improve our spatial understanding of biotic activities of plants and their responses to stresses.

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