S15-009

Comparison of the induction method with the saturation pulse method in chlorophyll fluorescence imaging

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Keywords: chlorophyll fluorescence imaging, herbicide, induction method, saturation pulse method

Introduction

Chlorophyll fluorescence is the re-emission of light energy absorbed by the antenna chlorophyll, which is not used in the photochemical and nonphotochemical reactions in photosynthesis. Chlorophyll fluorescence imaging, developed by Omasa et al. (1987) and Daley et al. (1989), has been widely used as a sensitive and a non-destructive way to diagnose the photosynthetic dysfunctions caused by abiotic stresses factors such as air pollutants, low concentrations of O_2, water deficit, high or low light, UV light, chilling, and agricultural chemicals (Omasa et al. 1987; Daley et al. 1989; Omasa and Shimazaki 1990; Siebke and Weis 1995a, 1995b; Rolfe and Scholes 1995; Takayama et al. 2000), and biotic stress factors (Balachandran et al. 1994; Osmond et al. 1998).

The chlorophyll fluorescence induction (CFI) method and the saturation pulse method have been used for the chlorophyll fluorescence imaging. The CFI method analyses the rapid changes in the intensity of chlorophyll fluorescence emitted form photosystem II (PSII) antenna chlorophyll during a dark-light transition (Kautskey effect; chlorophyll fluorescence induction). The CFI method elucidates the state of the photosynthetic electron transport system (Omasa et al. 1987; Govindjee 1995). Meanwhile, the saturation pulse method quantitatively estimates photochemical and nonphotochemical quenching under light using saturation light pulses (Daley et al. 1989; Balachandran et al. 1994; Siebke and Weis 1995a, 1995b; Rolfe and Scholes 1995; Osmond et al. 1998; Takayama et al. 2000).

In the present paper, using the CFI method and the saturation pulse method simultaneously, the effects of a commercially available urea herbicide including DCMU on attached *Cucumis sativus* L. leaves were analysed.

Materials and Methods

Cucumber (*Cucumis sativus* L. cv. Hokushin) plants were grown in a controlled-environment chamber at 27/23ºC (day/night) temperature and 50/70% (day/night) RH under fluorescent light (photosynthetic photon flux (PPF) 300 μmol photons m^{-2} s^{-1}) in pots containing a 1:1 (v/v) mixture of vermiculite and perlite for 4 weeks. Nutrient solution was supplied daily. Nekosogi-ace, a commercially available urea herbicide including 3-(3,4 dichlorophenyl)-1,1-dimethylurea (DCMU), was applied on the surface of the soil in the pot.

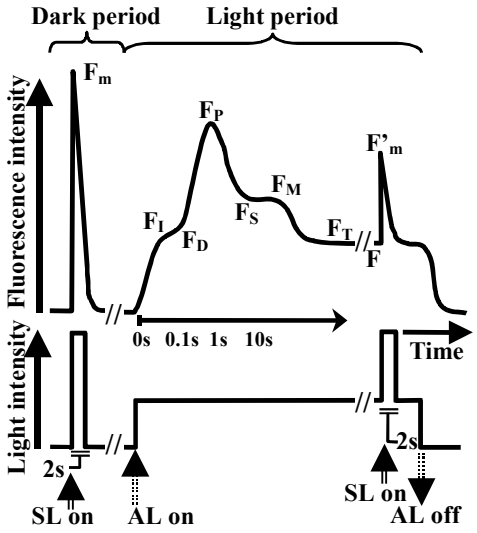

Figure1. Schematic diagram of the consecutive measurement of CFI method and saturation pulse method. All experiments were performed on attached cucumber leaves.

Figure 1 shows a schematic diagram of the measurement. A dark-adapted leaf is irradiated with the saturation light pulse (PPF 4000 μmol photons $m^{-2}s^{-1}$) and F_m image is obtained. Then the leaf is irradiated with the actinic light (PPF 300 μmol photons $m^{-2}s^{-1}$). CFI images (F_I at I, F_D at D, F_P at P, F_S at S, F_M at M, and F_T at T) are obtained during the transition from dark to light (Kooten and Snel 1990). After confirmation of steady-state fluorescence under light, F and F'_m images are obtained just before and during the irradiation of the saturation light pulse. In the saturation pulse method, NPQ and Yield images were calculated from the chlorophyll fluorescence images (Krause and Weis 1991). NPQ, which equals $(F_m - F'_m) / F'_m$, quantifies nonphotochemical quenching of chlorophyll fluorescence under light (Bilger and Bjőrkman 1990). Yield, which equals $(F'_m - F \times \text{Ratio}) / F'_m$, estimates the yield of PSII photochemistry (Genty et al. 1989; Genty and Meyer 1995; Meyer and Genty 1996). Ratio was derived from the ratio of the intensity of actinic light and saturation light pulse. Simultaneous measurements of the stomatal conductance and the assimilation rate were performed with diffusion porometer measurements. The measurements as above were performed on an attached cucumber leaf before and 48 h after the herbicide treatment.

Results and Discussion

Figure 2 shows CFI images and CFI transients of an attached cucumber leaf obtained before and 48 h after the herbicide treatment. The typical and almost identical CFI transients were observed in any location of leaf area before the herbicide treatment (Site-1). At 48 h after the herbicide treatment, abnormal CFI transients were observed at the intercostal sites (Site-2) and at the sites around the major veins (Site-3). At Site-2, the slight suppression of ID decline, the delayed rise of DP, and the suppression of PS decline were observed. Since ID decline in CFI reflects reoxidation of Q_A, a primary electron acceptor of PSII, by the plastoquinone (PQ), the slight suppression of ID decline suggested the slight suppression of the electron flow from Q_A to PQ. Since the DP rise reflects photoreduction of Q_A through reductant from H_2O, a delayed rise of DP was consistent with the inactivation of the water- splitting enzyme system □ (Shimazaki K et al. 1984). Since the PS decline depends on nonphotochemical quenching, the suppression of PS decline suggested the depression of formation of intrathylakoid pH gradient due to the inactivation of the water-splitting enzyme

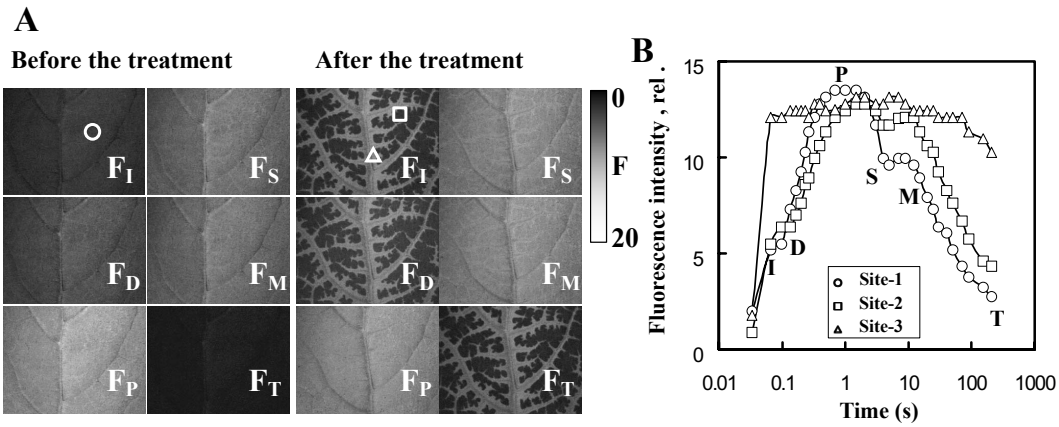

Figure2. CFI images (A) and CFI transients (B) before and 48 h after the herbicide treatment. ○ Site-1, intercostal site before the treatment, □ Site-2, intercostal site after the treatment, Δ Site-3, site located near the vein. Grey scale indicates chlorophyll fluorescence intensity.

system. At Site-3, fluorescence intensities at I and D were increased to P level. Then fluorescence intensity was maintained at a high level. Because fluorescence intensity in the early induction phase is regulated by the redox state of Q_A, the increased fluorescence intensity at I and D suggests that almost all of the Q_A were brought to reduced state. This showed that the inhibition of electron transport from PSII to PSI was caused by DCMU included in the herbicide.

Figure 3 shows NPQ and Yield images calculated from chlorophyll fluorescence images obtained before and 48 h after the herbicide treatment. At 48 h after the treatment, sites around the major veins, similar to sites with high fluorescence intensity in CFI images (see F_I, F_D, and F_T in Figure 2), showed a remarkable decrease in NPQ compared to noninjured sites. The decrease in NPQ suggested that the decrease in the ability of chloroplasts to generate an intrathylakoid pH gradient and to sustain electron transport (Osmond et al. 1998). At sites with low NPQ, decreases in stomatal conductance and assimilation rate were confirmed with diffusion porometer measurements (Table 1). A decrease in Yield was detected only at Site-3. This result showed that the Yield image could not accurately estimate the severe photosynthetic injury caused by the herbicide as shown in Table 1.

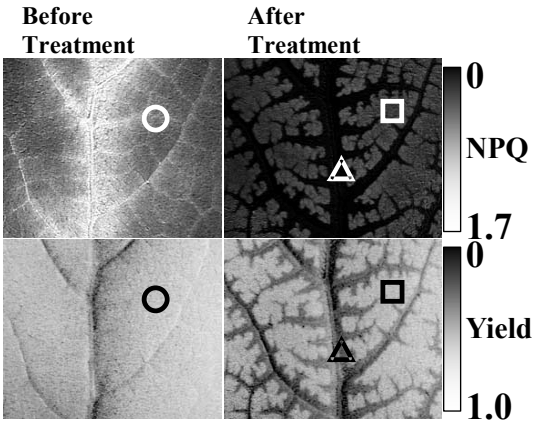

Figure3. NPQ and Yield images calculated from chlorophyll fluorescence images obtained before (A), and 48 h after (B) the treatment. ○ (Site-1), □ (Site-2), and Δ (Site-3) represent the sites defined in Figure 2. Grey scales indicate NPQ and Yield value, respectively.

The NPQ and Yield make it possible to quantitatively estimate photosynthetic activity under light (Genty and Meyer 1995; Meyer and Genty 1996; Osmond et al. 1998). However, NPQ showed high values in the sites around the major veins before the herbicide treatment

(Figure 3). This result showed that NPQ might not indicate exact values at uneven leaf surface, especially, at sites around the major veins. Moreover, Yield was not reduced as much as the decrease in assimilation rate (Figure 3). When we estimate photosynthetic activity using NPQ and Yield, we must pay attention to the problems above.

Table 1 Changes in stomatal conductance and assimilation rate after the herbicide treatment. The values are means of 6 leaves ± SE.

	Time after the commencement of herbicide feeding	
	0 hour	48 hour
Stomatal conductance (mmol $m^{-2}s^{-1}$)	183.3 ± 8.3	155.1 ± 19.0
Assimilation rate (μ mol CO_2 $m^{-2}s^{-1}$)	12.8 ± 0.3	3.8 ± 1.1

As another aspect of the methods, there is a problem with the measurement area. The saturation pulse method, which requires measurement under an evenly distributed high-level of photosynthetic active radiation (PAR) (saturation light pulse), was limited to measuring a small leaf area. In contrast, the CFI method does not require high PAR. Therefore, it is possible to apply the measurement to a larger leaf area.

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