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Imaging of the Red and Far-red Chlorophyll Fluorescence of the Ozone Injured Plant Leaf

By

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S u m m a r y

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Chlorophyll fluorescence imaging at red and far-red wavelength regions was used to assess the effects of ozone exposure to sunflower (*Helianthus annuus* L. cv 'Sun-hope') leaves. Chlorophyll fluorescence images at red and far-red wavelength regions were obtained using two band-pass filters. Φ_{PSII} and NPQ which indicate the quantum yield of photosystem II and the extent of the ability to dissipate excess excitation energy as heat, respectively, were calculated using the saturation pulse method. After 24 hours exposure to 300 ppb ozone at a PPF of 300 μmol $m^{-2}s^{-1}$, visual images, chlorophyll fluorescence intensity images, Φ_{PSII} images and NPQ images were obtained and compared.

Ozone exposure induced both visible injuries and non-visible symptoms that were non-uniformly distributed on the leaf. Visible injuries were bleaching of chlorophylls. Non-visible symptom was decreasing the photochemical quantum yield of photosystem II by increasing non-photochemical quenching. The sites of the non-visible symptoms did not necessarily correspond to the visible injured sites. In far-red chlorophyll fluorescence imaging, the chlorophyll fluorescence intensities and the values of Φ_{PSII} slightly decreased while a slight increase in the values of NPQ was observed at the injured sites. On the other hand, in red chlorophyll fluorescence imaging, changes of values of Φ_{PSII} and NPQ were not observed at the visible injured sites. Far-red chlorophyll fluorescence imaging detected both the visible injuries and damages in photochemistry, while, red chlorophyll fluorescence imaging detected non-visible photochemical damages avoiding the effect of chlorophyll bleaching by ozone exposure.

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Introduction

Ozone is a major phytotoxic air pollutant (NOUCHI 2002). Ozone exposure to a plant leaf induces stomatal closure, decreases photosynthetic rate, and causes heterogeneous visible injury on the leaf surface (OMASA & al. 1981, 1983, SCHMIDT & al. 1990). It was found out that changes of the quantum efficiency of photosystem II by the ozone exposure cannot be detected with a conventional and integrating fluorometer (LEIPNER & al. 2001) because ozone is highly reactive and is thought to react rapidly with a range of compounds associated with cell walls and membranes (HEATH 1994).

Chlorophyll fluorescence imaging is a simple, rapid, and non-invasive mean to assess the heterogeneous photosynthetic activity across the green plant surface (OMASA & al. 1987, DALEY & al. 1989, OSMOND & al. 1998, TAKAYAMA & al. 2003, OMASA & TAKAYAMA 2003, ENDO & OMASA 2004). Chlorophyll fluorescence spectrum has two peaks at red and far-red wavelength regions. The red fluorescence is largely reabsorbed by the chlorophylls, while the far-red one is hardly reabsorbed (PFÜNDEL 1998, VOGELMANN & HAN 2000, PETERSON & al. 2001).

In this study, chlorophyll fluorescence imaging was used to assess the effects of ozone exposure to sunflower (*Helianthus annuus* L. cv. 'Sun-hope') leaves. We have also carried out a comparative analysis of the characteristics of red and far-red chlorophyll fluorescence imaging in this study.

Material and Methods

Sun Hope plants (*Heleanthus annuus* L. cv.) were grown in a growth chamber for 4 weeks after sowing in pots. The pots were filled with artificial soil (mixture of vermiculite and perlite, 1:1 v/v). The plants were illuminated for 11 h each day with white fluorescent lights (PPF 300 μmol $m^{-2}s^{-1}$). Air temperature was 26 °C during the day and 20 °C at night. The plants were watered daily with a nutrient solution (1:1000 dilution of HYPONex). Mature *in situ Heleanthus annuus* L. leaves were used in the experiments.

The ozone treatment was performed in controlled environment chamber (Dylec, OES-10A). The ozone concentration inside the chamber was continuously monitored using an automatic monitoring device (Dylec, MODEL 1200). For this experiment, the O_3 concentration was set to 300 ppb. The plants were exposed to the ozone for 24 h under illuminations (PPF 300 μmol $m^{-2}s^{-1}$).

The chlorophyll fluorescence imaging system installed in our laboratory was used in the study. The measurement method is called "Saturation pulse method". Actinic light (PPF 300 μmol $m^{-2}s^{-1}$) and saturation light pulse (PPF 3800 μmol $m^{-2}s^{-1}$ for 1 s) were provided with four metal halide lamps (Sumita Optical Glass, LS-M180) equipped with a short-pass filter (Corning, 4-96; $\lambda <$ 600 nm) through an optical fiber. Chlorophyll fluorescence was captured by a chilled charge-coupled device video camera (Hamamatsu Photonics, C5985) equipped with a red or far-red wavelength band-pass filter (Optical Coatings Japan, $\lambda = 680$ or 740 nm). Reflectance images were captured with the system without band-pass filter as visual images.

After ozone exposure, the leaf was dark-adapted for an hour, then F_m images at the red and far-red wavelength regions were captured. After the photosynthesis reached a steady state, F_m' and F images at those regions were captured. Using the relative fluorescence yield images, images of the photochemical quantum yield of photosystem II (Φ_{PSII}) was computed using the following formula: $\Phi_{PSII} = (\Phi F_m' - \Phi F) / \Phi F_m'$ (GENTY & MEYER 1995).

Results and Discussion

Fig. 1 shows the distribution of Φ_{PSII} and chlorophyll fluorescence intensity (F_m) when nonphotochemical quenching was zero, at red and far-red wavelength regions on the leaf surface and visual (reflection) appearance of the leaf after ozone exposure. In the control leaf, heterogeneity was not observed except for veins. In the leaf after ozone exposure, non-uniformly distributed visible injuries can be observed. In the visible injured sites, the changes of Φ_{PSII} and F_m at the red wavelength region were not observed, whereas Φ_{PSII} and F_m decreased at the far-red wavelength region. Significant changes of Φ_{PSII} and NPQ (data not shown) were observed at both wavelength regions. The sites where Φ_{PSII} and NPQ changed significantly did not necessarily correspond to the visible injured sites.

It seemed that chlorophylls were bleached at the visible injured sites (OMASA & al. 1981, 1983, SCHMIDT & al. 1990). There are two peaks in the chlorophyll fluorescence spectrum at the red and far-red wavelength regions. Red chlorophyll fluorescence was reabsorbed by chlorophylls, but in the far-red one, it was hardly reabsorbed (PFÜNDEL 1998, VOGELMANN & HAN 2000, PETERSON & al. 2001). In the bleached site, light absorption decreased, so emission and reabsorption of red chlorophyll fluorescence also decreased, therefore the changes of the chlorophyll fluorescence intensities at the red wavelength region were obscured. On the other hand, because the effects of re-absorption were small at the far-red wavelength region, the effect of decreasing light absorption was conspicuous.

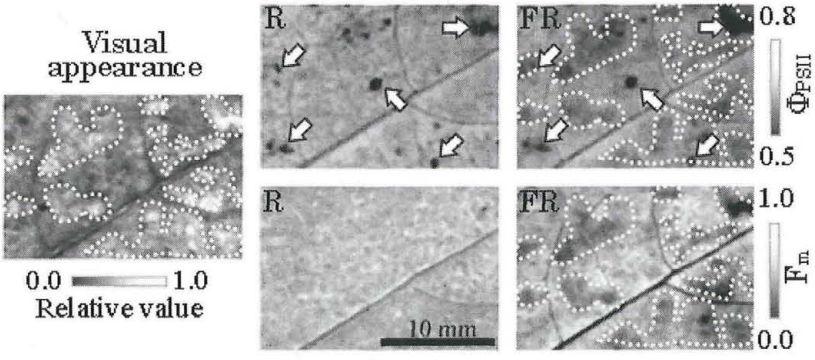

Fig. 1. Effects of the ozone exposure on a *Heleanthus annuus* L. leaf. Images of visual appearance, Φ_{PSII} and chlorophyll fluorescence intensity (F_m) at red (R) and far-red (FR) wavelength regions after ozone exposure. The sites surrounded by white dashed lines represent the visible injured areas. Arrows represent the typical sites where Φ_{PSII} significantly decreased.

NPQ which is calculated from the formula: $NPQ = (\Phi F_m - \Phi F_m') / \Phi F_m'$ (DALEY & al. 1989) represents the extent of the ability to dissipate excess excitation energy as heat. At both wavelength regions, Φ_{PSII} decreased and NPQ in-

creased in the same sites, which implied the increase in nonphotochemical quenching caused the decrease in photochemical quantum yield of photosystem II at the sites (TAKAYAMA & al. 2003).

These results indicated that ozone exposure of sunflower leaf caused both visible and non-visible symptoms. Imaging analysis was needed to detect the damages, because the symptoms were non-uniformly distributed on the leaf. It was observed that the photochemical quantum yield of photosystem II did not necessarily decrease in the visible injured areas. It was possible to detect non-visible photosynthetic damages avoiding the effect of visible injuries i.e. bleaching of chlorophyll with red chlorophyll fluorescence imaging.

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