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Effect of Ozone Exposure on the Photosynthetic Activity at Cellular Level: Chlorophyll Fluorescence Microscopic Imaging Analysis

By

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S u m m a r y

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Ozone is one of the most major global air pollutants, inducing various forms of damage to plants. The concentration of surface ozone is increasing in many industrial countries. In this study, microscopic images of fluorescence parameter Φ_{PSII} from sunflower (*Helianthus annuus* L.) leaves were used to assess the effect of ozone exposure on the photosynthetic activity at cellular level. Φ_{PSII} gives theoretically the operating quantum efficiency of photosystem II (PSII) photochemistry, therefore, it can be a relative measure of the photosynthetic electron transport through PSII.

At a photosynthetically active photon flux of 300 μmol m^{-2} s^{-1} and 75 % relative humidity, chlorophyll fluorescence and Φ_{PSII} of the upper surface were uniformly distributed across the leaf surface before ozone exposure. After 12h exposure to 300 ppb ozone at a PPF of 300 μmol m^{-2} s^{-1}, chlorophyll fluorescence intensity of ozone injured leaves showed large heterogeneity across the leaves. However, the fluorescence intensity had no relevance to the distance from stomatal pores which are believed to be a main pathway of ozone on the leaf surface. On the other hand, Φ_{PSII} were uniformly distributed across the leaf surface and did not decrease significantly compared with the Φ_{PSII} within control leaves, suggesting that decreases in operating quantum efficiency of photosystem II had not occurred by the ozone exposure.

Consequently, ozone penetration seems to affect the chlorophyll fluorescence intensity independently of the distribution of stomata, and not to affect the photosynthetic electron transport.

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Introduction

In stratosphere, ozone works as a natural screen from the harmful effects of ultraviolet radiation (NOUCHI 2002). Although, tropospheric ozone is one of the most major global air pollutants, causing serious vegetative damage and forest decline. Stratospheric ozone is decreasing due to destruction by human-made chlorofluorocarbons and NO_x, while the concentration of ozone in the ground surface is stable or increasing. Increases in the surface ozone concentration in many industrialized countries are largely caused by photochemical oxidant pollution, produced in the atmosphere by complex photochemical reactions involving NO_x and hydrocarbons (NOUCHI 2002, OMASA & al. 1981).

For nondestructive assessment of photosynthetic activity, chlorophyll *a* fluorescence measurement is very effective (KRAUSE & WEIS 1991, GOVINDJEE 1995, LICHTENTHALER 1988). Furthermore, the application of imaging techniques makes the spatial analysis of chlorophyll fluorescence possible (OMASA & al. 1987, DALEY & al. 1989, LICHTENTHALER & MIEHE 1997, GOVINDJEE & NEDBAL 2000, OMASA & TAKAYAMA 2003). Recently, microscopic fluorescence imaging systems have been developed for cellular-level, and even chloroplast-level, analyses (OXBOROUGH & BAKER 1997, ENDO & al. 2002, ENDO & OMASA 2004). OXBOROUGH & BAKER 1997 observed chlorophyll fluorescence images of individual stomata of intact leaves and calculated the photosystem II (PSII) photochemical yield, Φ_{PSII}, which is a measure of linear electron transport activity through PSII.

In this study, we report the effect of the ozone exposure on photosynthetic injuries of plants cell. Inhibition of photosynthetic activities by the ozone exposure was analyzed by using a reflected light images, fluorescence intensity images and Φ_{PSII} images.

Material and Methods

Seeds of a sunflower (*Helianthus annuus* L.) were grown in a controlled environment chamber, and the plants were grown for 4 weeks. The plants were illuminated for 12 h each day with fluorescent lights at a PPF of 300 μmol m^{-2} s^{-1}. Air temperature was 26.5 °C during the day and 20.0 °C at night. Relative humidity was 45 % during the day and 60% at night. Plants were watered daily with a nutrient solution (1:1000 dilution of HYPONex). In the experiment, the plants were exposed to ozonated air containing 300 ppb (an intermediate concentraion) for 12h. The ozone concentration inside the chamber was monitored continuously with a monitor (MODEL 1200, Dylec).

The computer-aided microscopic imaging system for analyzing chlorophyll a fluorescence uses an optical microscope with a 10× objective lens (Mitutoyo, BD Plan Apo 10). A halogen lamp (Nikon, PSM-1520) with a 620-nm cut-off filter (Corning, 4-96) and heat-absorbing filters provides 300 μmol photons m^{-2} s^{-1} of actinic light. A metal-halide lamp (Sumita, LS-M180), filtered as described above, provides a 2-s saturating light pulse of 2000 μmol photons m^{-2} s^{-1}, which causes a transient saturation of PSII photochemistry. Chlorophyll a fluorescence excited by these lights is imaged by a CCD camera (Roper Scientific, The Photometrics Cascade 650).

As soon as ozone fumigation, the plants were moved to a stage of the microscopic imaging system. After 20 minutes adaptation period, fluorescence intensity image (Φ_F) was captured at a PPF of 300 μmol m^{-2} s^{-1}. Just after that measurement, a fluorescence intensity image $\Phi_{Fm'}$ was

measured during a 2-s saturation light pulse during steady-state photosynthesis. Finally, the Φ_{PSII} was calculated ($\Phi_{PSII} = (\Phi_{Fm'} - \Phi_F) / \Phi_{Fm'}$) (GENTY & al. 1989).

Results and Discussion

Microscopic Φ_F and $\Phi_{Fm'}$ images have been acquired (Fig.1). Φ_F and $\Phi_{Fm'}$ images showed that chlorophyll fluorescence were non-uniformly distributed across the field-of-view on the abaxial leaf surface. Fluorescence intensity from the bleached chloroplasts of the plant cells in the spongy tissue was low, on the contrary, fluorescence intensity from the non-bleached chloroplasts was high. Differently from the former cases, Φ_{PSII} were uniformly distributed across the field-of-view on the abaxial leaf surface, with values in the ranges of 0.45 to 0.63, except at the veins (Fig.1).

The results of the reflected image and fluorescence intensity images showed that the ozone exposure damaged the chloroplasts close to leaf surface, probably by making an invasion through stomata. As a result, the chloroplasts damaged by ozone may have got degraded color or bleached, therefore intensity of chlorophyll fluorescence emitted from the chloroplasts got extremely decreased. Consequently, these images proved clearly that certain photosynthetic injuries on the plant leaf occurred non-uniformly by the ozone exposure.

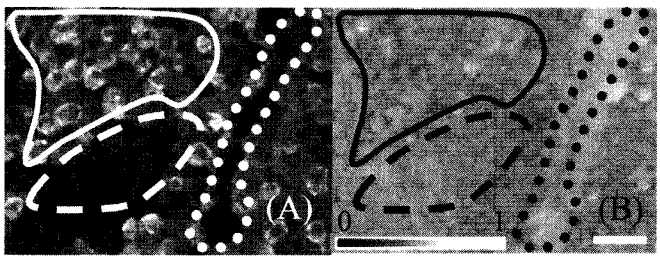

Fig. 1. Φ_F image (A) of a measurement area of an attached *Helianthus annuus* L. abaxial leaf used for the experiment performed at a PPF of 300 μmol m^{-2} s^{-1} and Φ_{PSII} image (B) calculated from Φ_F and $\Phi_{Fm'}$ images, after 300 ppb ozone exposure . The region within the solid line is no-injured plant cells region at the inner part of the spongy tissue. The region within the broken line is an injured plant cells region at the upper part of the spongy tissue. The region within the dotted line is a leaf vein. Scale bar = 30 μm.

On the other hand, Φ_{PSII} were uniformly distributed, with values in the ranges of 0.45 to 0.63, except at the veins. Φ_{PSII} is theoretically proportional to the operating efficiency of the PSII photochemistry, and has been widely used to estimate the relative quantum efficiency of linear electron transport through PSII. Homogeneous distribution of Φ_{PSII} shows that the efficiency of the electron transport of the chloroplasts were not different, even if plant cells were located at the outer

or inner part of the spongy tissue. As stated previously, Φ_{PSII} can represent the efficiency of photochemistry from absorbed light energy. However, the amount of the absorbed light energy cannot be estimated by Φ_{PSII} values.

Consequently, the result of Φ_{PSII} image indicated that the ozone exposure does not decrease the efficiency of photochemistry from absorbed light energy, but decrease the absorbance of the light energy by making the chloroplasts bleached.

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