

Spatiotemporal Changes in PRI and NPQ Under Different Light Intensity Gradients on Leaf Surfaces

Atsumi Konishi, Mashashi Munehiro, and Kenji Omasa

Abstract PRI (photochemical or physiological reflectance index) is well known as an indicator of changes of xanthophyll pigments, therefore it is regarded as a useful index to assess the degree of heat dissipation of plant leaves. NPQ (non-photochemical quenching of chlorophyll fluorescence) is also an index of the degree of heat dissipation. We therefore compared spatiotemporal changes in PRI and NPQ on attached leaf surfaces. During photosynthetic induction, heterogeneity of NPQ was clearly compartmentalized at leaf veins, whereas PRI was homogeneously distributed. These results implied that differences of xanthophyll pigments between each compartment during photosynthetic induction are too small to be detected by reflectance imaging. When a large gradation of light intensity was generated in the leaf by illumination from an oblique direction, gradations of both PRI and NPQ corresponding to those of light intensity were observed. However, when the leaf was illuminated from above just after the illumination from an oblique direction was turned off, the gradation of NPQ remained,

while that of PRI disappeared. These results suggest that PRI is affected by physical factors, such as leaf inclination and surface structure, relating to leaf reflectance as well as to physiological factors. Therefore, we should pay careful attention to explanations of spatiotemporal changes of PRI.

Keywords PRI, NPQ, chlorophyll fluorescence, imaging, leaf reflectance

Introduction

Chlorophyll fluorescence imaging is one of the leading methods for assessing photosynthetic activities of plants because it provides information without destruction of, or contact with, the living leaves (Omasa et al. 1987; Daley et al. 1989; Genty and Meyer 1995). ϕ_{PSII} and NPQ, which respectively represent the quantum yield of photosynthetic electron transport (Genty et al. 1989) and the degree of heat dissipation, have been used as the common chlorophyll fluorescence parameters. However, it is difficult to calculate ϕ_{PSII} and NPQ of large leaves and canopy surfaces because a strong light is needed to apply the saturation pulse method.

Graduate school of Agricultural and Life Sciences,
The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo,
113-8657 Japan

PRI (photochemical or physiological reflectance index) is well known as an indicator of changes of xanthophyll pigments (Gamon et al. 1992, 1997; Nichol et al. 2006). The activity of heat dissipation is affected mainly by de-epoxidation of violaxanthin (Horton et al. 1996) depending on ΔpH across thylakoid membranes, therefore PRI is expected to be an alternative method for saturation pulse analysis especially in remote sensing (Evain et al. 2004). A roughly linear relationship between NPQ and PRI has been reported (Evain et al. 2004; Nichol et al. 2006), however the relationship has not been strictly investigated with respect to its spatial distribution on the leaf. In this study, we have therefore compared spatiotemporal changes in PRI and NPQ images on attached soybean leaf surfaces.

Materials and methods

Plant material. Soybean seedlings (*Glycine max* L. cv. Hayamidori) were grown in a growth chamber. The plants were illuminated for 12 h each day with fluorescent lights at a PPF of $200 \mu\text{M m}^{-2} \text{s}^{-1}$. 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 germinations. 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). Attached mature leaves were used in the experiments.

Imaging system. Actinic light for photosynthesis and the saturation light pulse that caused a transient saturation of photosynthetic electron transport were provided with a 180 W metal halide lamp (Sumita Optical Glass, Inc., LS-M180) with a short-pass filter (Corning, $\lambda < 600 \text{ nm}$) through an optical fiber.

Chlorophyll fluorescence and reflectance images were captured by a chilled charge-coupled device (CCD) camera (Hamamatsu Photonics, C5985) equipped with a lens (Nikon, Micro-NIKKOR) and a band-pass filter (Optical Coatings Japan, MIF-W, wavelength = 533, 571 or 682 nm). NPQ was calculated by $(F_m - F_m')/F_m'$, where F_m and

F_m' are respectively the maximum yields of chlorophyll fluorescence in the dark and light, while PRI was calculated by $(R533 - R571)/(R533 + R571)$, where R533 and R571 are the reflectance at 533 and 571 nm, respectively.

Heterogeneities of NPQ and PRI during photosynthetic induction. The F_m image was captured during a saturation pulse of $3,700 \mu\text{M photons m}^{-2} \text{s}^{-1}$ after 60 min dark adaptation. After 10 min in darkness, F_m' images were intermittently captured after the start of actinic light illumination at $500 \mu\text{M photons m}^{-2} \text{s}^{-1}$. R533 and R571 images were captured just before the F_m' measurements. In the experiment, the actinic light and the saturation light pulses provided illumination normal to the plane of the leaf.

Heterogeneities of NPQ and PRI under different light intensity gradient. The F_m image was captured during plane-perpendicular saturation light pulse illumination ($5,000 \mu\text{M photons m}^{-2} \text{s}^{-1}$) after 60 min dark adaptation. In the experiment, the actinic light illuminated the leaf from an oblique direction so as to generate a large light intensity gradient over the leaf from about 100 to $1,000 \mu\text{M photons m}^{-2} \text{s}^{-1}$. The leaf was adapted for 30 min after the start of the actinic light illumination. Thereafter, R533 and R571 were measured under the oblique actinic light and immediately under an illumination of $500 \mu\text{M photons m}^{-2} \text{s}^{-1}$ plane-perpendicular light in place of the oblique actinic light. Just after the measurement, the F_m' image was captured. When the F_m' was measured, the leaf was subjected to the perpendicular saturation light pulse.

Results and discussion

Heterogeneities of NPQ and PRI during photosynthetic induction

During photosynthetic induction under perpendicular actinic light illumination, differences of stomatal opening caused differences in the amount of CO_2 in each compartment (Bro et al. 1996), therefore heterogeneity of NPQ was generated (Siebke and

Weis 1995). In Fig. 1, NPQ on the leaf was heterogeneously observed (A), while PRI was homogeneously distributed (B). NPQ distribution was clearly compartmentalized at leaf veins because bundle sheaths of the heterobaric leaf inhibited gas diffusion (Bro et al. 1996). These results imply that differences in xanthophyll pigments (Gamon et al. 1992, 1997) during photosynthetic induction were too small to be detected by reflectance imaging.

Heterogeneities of NPQ and PRI under different light intensity gradients

To generate larger differences of xanthophyll pigments on the leaf, the oblique light illumination was used. When a large gradation of actinic

light intensity was generated on the leaf by oblique illumination, gradations of both PRI and NPQ were observed to correspond to those of light intensity (Fig. 2A, B). When the leaf was illuminated perpendicularly just after the oblique illumination was turned off, the gradation of NPQ remained, meanwhile that of PRI disappeared (Fig. 2C). It seemed that the NPQ gradation on the leaf indicated changes in the xanthophyll pigments and that these pigments were not changed within 1 s because their changes were caused by enzyme reactions. Therefore, when the vertical light illuminated the leaf, the pigment gradation on the leaf remained. In spite of the relatively large NPQ gradation, the pigment changes were not large enough to be detected by PRI.

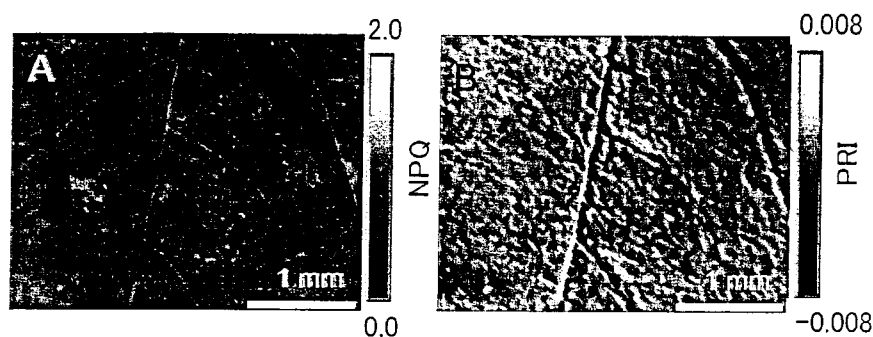


Fig. 1 Images of NPQ (A) and PRI (B) at 10 min after the start of the actinic light illumination during photosynthetic induction

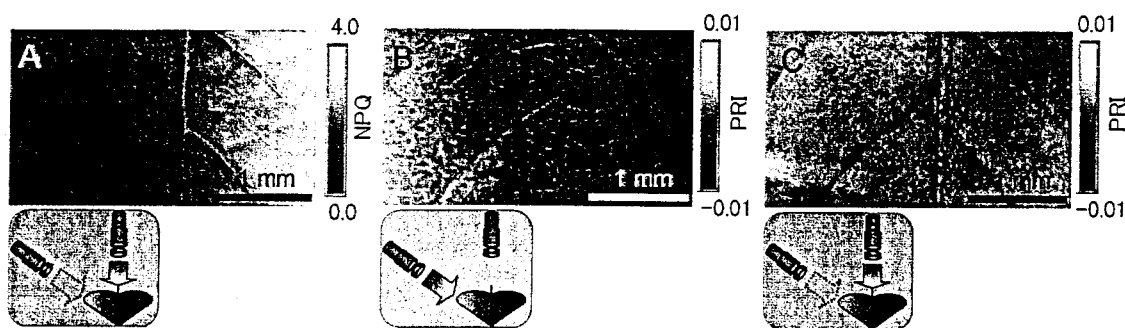


Fig. 2 Images of NPQ (A) and PRI (B and C). The oblique actinic light illuminated the leaf in all experiments. Saturation light pulses illuminated the leaf at 90° and the oblique actinic light was turned off when F_m and F_m' were measured (A). The oblique measuring lights illuminated the leaf as R533 and R571 were measured (B). The perpendicular measuring light illuminated the leaf and the oblique actinic light was turned off when R533 and R571 were measured (C)

These results suggest that PRI was affected by physical factors, such as leaf inclination and surface structure, relating to leaf reflectance as well as physiological factors. Especially in the cases where the photosynthetic activities of individual plants and vegetation are assessed by PRI, it is essential to take these physical factors into account. Therefore, we should pay more careful attention to explanations of spatiotemporal changes of PRI, and further study is needed.

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