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Analysis of effects of abscisic acid on *Cucumis sativus* L. leaves using chlorophyll fluorescence imaging and thermal imaging

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Introduction

Recent advances in imaging of physiological functions of intact plants to diagnose early abnormal symptoms are remarkable (Omasa and Aiga 1987; Hashimoto et al. 1990; Omasa 1990; Lichtenthaler 1996; Chaerle and Van der Straeten D 2000). For example, chlorophyll fluorescence imaging and thermal imaging have been applied to spatial analysis of stomatal response and photosynthesis.

The techniques of image analysis of chlorophyll *a* fluorescence of plant leaves *in situ*, originally developed by Omasa et al. (1987) and Daley et al. (1989), have been widely used as a sensitive and nondestructive way to assess the functional state of the photosynthetic apparatus. These techniques are used for early detection of changes in patchy stomatal response and photosynthetic activity caused by abiotic stress factors such as air pollutants, low concentrations of O_2, water deficit, UV light, chilling, and agricultural chemicals (Omasa et al. 1987; Daley et al. 1989; Omasa and Shimazaki 1990; Genty and Meyer 1995; Rolfe and Scholes 1995; Siebke and Weis 1995a,b; Omasa 1998; Takayama et al. 2001) and biotic stress factors (Balachandran et al. 1994; Osmond et al. 1998).

Meanwhile, the thermal image provides information on stomatal response and gas exchange under steady-state thermal environments (Omasa 1981a-c, 1990; Omasa and Croxdale 1992; Jones 1999). Omasa et al. (1981a-c) quantitatively evaluated heterogeneous changes in spatial distributions of stomatal resistance (=1/stomatal conductance), transpiration rate, and absorption rate of air pollutants all over the attached leaf from leaf temperature.

In the present paper, a system capable of simultaneous and quantitative analysis of chlorophyll fluorescence images and thermal images of attached plant leaves has been introduced and the effects of ABA on cucumber (*Cucumis sativus* L.) leaves were analysed to clarify the relationship between chlorophyll fluorescence and stomatal response.

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 (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.

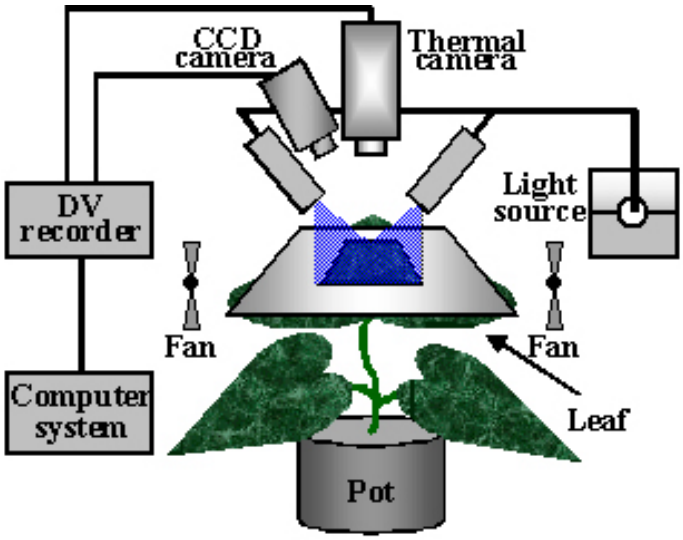

Figure 1. Schematic of a system used for simultaneous measurements of chlorophyll fluorescence images and thermal images. All experiments were performed on attached cucumber leaves.

Figure 1 shows a schematic of a system capable of simultaneous measurement of chlorophyll fluorescence images and thermal images. An attached cucumber leaf was placed in a thin plastic sheet (5×5 cm^2) cut in a square shape and held horizontally. Air current on the surface of the leaf was maintained uniformly using two fans. Measurements of chlorophyll fluorescence images and thermal images were performed on the leaf before and during 10^{-3} M ABA treatment.

Chlorophyll fluorescence imaging was performed as described in Takayama et al. (2001). Two metal-halide lamps (Sumita optical glass LS-M180) controlled by a personal computer provided a saturation light pulse (PPF 2700 µmol photons $m^{-2}s^{-1}$) and an actinic light (PPF 300 µmol photons $m^{-2}s^{-1}$) filtered with a 620 nm cut-off filter (Corning 4-96). A CCD (charge coupled device) (Sony FCB-IX470) camera filtered with a 640 nm long pass filter (Corning 2-64) and an interference filter with a peak transmission of 682.5 nm and half-width of 10.5 nm (Nihonshinku) imaged the chlorophyll fluorescence of the leaf surface. Chlorophyll fluorescence images were obtained from the dark-adapted material during the application of the saturation light pulse (F_m), from the material adapted by the actinic light (F at steady-state), from the light-adapted material during the application of the saturation light pulse (F'_m) before and during the ABA treatment. The parameter images such as NPQ and Yield were calculated from the fluorescence images. NPQ, equals $(F_m - F'_m) / F'_m$, quantifies nonphotochemical quenching of chlorophyll fluorescence under light (Bilger and Bjőrkman 1990). Yield, equals $(F'_m - F \times \text{Ratio}) / F'_m$, estimates the yield of PSII photochemistry and is often correlated with the quantum yield of CO_2 assimilation in suitable experimental conditions (Genty et al. 1989). Ratio was derived from the ratio of the intensity of actinic light and saturation light pulse.

Thermal imaging and calculation of stomatal conductance were performed as described in our previous study (Omasa et al. 1981a-c; Omasa and Croxdale 1992). Thermal images were obtained from the light-adapted material using a thermal camera (Nippon Avionics TVS-8500) with an InSb FPA (focal plane array) detector (3.5 to 4.1 and 4.5 to 5.1 µm). The detected signals from the thermal camera are converted into 14-bit digital signals (256H × 236V) and analysed by a computer system. The spatial resolution, the temperature resolving power, and the frame rates are within 0.3 mm, 0.025ºC, and 120 frames/second, respectively.

Images of stomatal conductance were calculated from thermal images before and during the ABA treatment.

Results and Discussion

Figure 2 shows images of stomatal conductance, NPQ, and Yield, obtained before (A) and 75 min after (B) ABA treatment. Before the treatment, stomatal conductance, NPQ, and Yield were distributed uniformly in the intercostal area. After the ABA treatment, the stomatal conductance immediately decreased, and then fluorescence parameters changed gradually in the ABA treated area. The decrease in stomatal conductance was significant in the center of the ABA treated area. In NPQ and Yield images, sites with high NPQ and low Yield were observed in the intercostal areas of the treated area. The decrease in Yield represents a slight decrease in photochemical efficiency of PSII (Genty et al. 1989). The increase in NPQ represents an increase in the ability of chloroplasts to generate a high intrathylakoid pH gradient and to dissipate excess excitation energy as heat (Krause and Weis 1991; Siebke and Weis 1995a; Osmond et al. 1998). Those sites showed patchy distribution in the inner side of the low stomatal conductance area and did not necessarily coincide with the low stomatal conductance areas.

It was shown that ABA directly induced stomatal closure in leaves, however stomatal closure did not directly induce an increase in NPQ and a decrease in Yield. At the sites where stomatal conductance is low but fluorescence parameters did not change, intercellular CO_2 mole fraction (C_i) did not seem to be affected in spite of restricted CO_2 supply caused by stomatal closure. Consequently, changes in fluorescence parameters such as an increase in

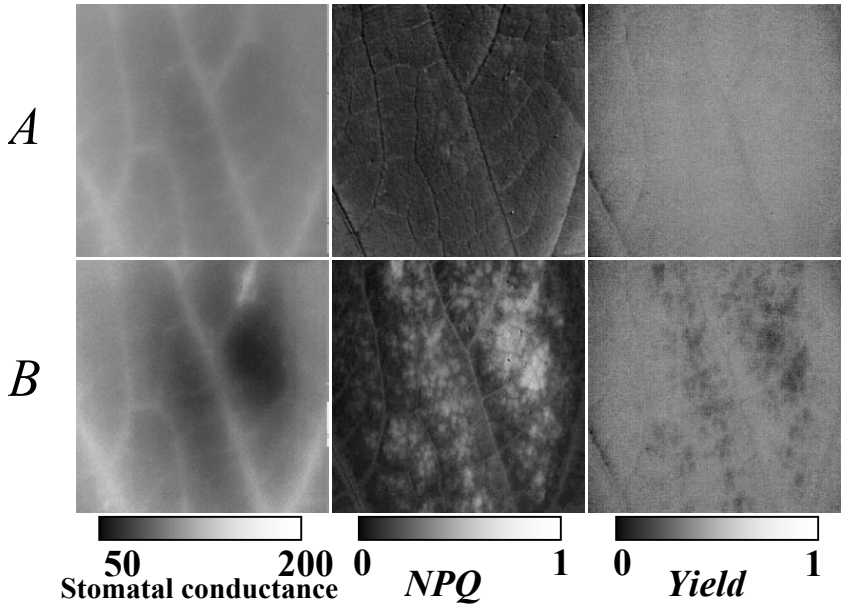

Figure 2. Images of stomatal conductance, NPQ, and Yield, obtained before and 75 min after 10^{-3} ABA treatment. No visible changes observed in the treatment. Environmental conditions: Air temperature, 25ºC; humidity, 30% RH; shortwave radiation, 0.98×10^{-3} cal cm^{-2} s^{-1}; longwave radiation, 2.50×10^{-2} cal cm^{-2} s^{-1}; boundary layer resistance to heat transfer, 0.96 s cm^{-1}. Bars indicate stomatal conductance, NPQ, and Yield value, respectively.

NPQ or a decrease in Yield, reflect change in C_i but not that in stomatal closure and CO_2 supply to tissues of leaf via stomata.

In conclusion, simultaneous measurement of chlorophyll fluorescence images and thermal images proved to be effective for analysing the heterogeneous response and patchy

distribution of photosynthetic activity and stomatal aperture. Such a system would allow new understanding of the relationship between chlorophyll fluorescence and stomatal response.

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