CHAPTER THIRTY

Imaging Heterogeneity of Xanthophyll-Independent Non-photochemical Quenching During Photosynthetic Induction in Shade-Grown Leaves of Avocado (*Persea americana* L.)

Kotaro Takayama[1], Barry Osmond[2], and Kenji Omasa[3]

Abstract Leaves of shade-grown avocado plants rich in lutein epoxide (Lx) were used to image heterogeneity in chlorophyll fluorescence quenching and to measure CO_2 uptake and stomatal conductance during photosynthetic induction for 20 min after transition from dark to light, and after changes between 100, 400 and 700 ppm CO_2 at growth irradiance. We found that non-photochemical quenching (NPQ) was initially heterogeneous, with marked differences between cells in tissues defined by minor veins and those adjacent to primary and secondary veins. The duration and extent heterogeneity of transients in NPQ, photosynthetic rate and stomatal conductance were sensitive to CO_2 concentration, whereas PSII efficiency (Φ_{PSII}) was not. There were no changes in the de-epoxidation status of xanthophylls pigments in equivalent treatments, implying that the dynamic, heterogeneous, stomatally-dependent and CO_2-responsive NPQ may involve quenching processes that occur in reaction centres prior to stabilization of heat dissipation in the antennae.

Keywords Avocado, chlorophyll fluorescence imaging, non-photochemical quenching, stomata, xanthophylls

Introduction

Non-photochemical quenching (NPQ) of chlorophyll fluorescence during induction is important in evaluation of photoprotective processes in leaves. Early events of NPQ are of particular interest, especially those associated with development of pH before CO_2 assimilation is fully active, and prior to stabilization of NPQ via de-epoxidation of violaxanthin (Horton et al. 1996; Finazzi et al. 2004). It had been generally assumed in kinetic analyses that NPQ and Φ_{PSII} are relatively uniform in different chloroplasts and cells of the leaves examined. However, chlorophyll fluorescence imaging systems (Omasa et al. 1987; Daley

[1]Laboratory of Physiological Green Systems, Department of Biomechanical Systems, Faculty of Agriculture, Ehime University, 3-5-7, Tarumi, Matsuyama, 790-8566 Japan
[2]Photobioenergetics Group, RSBS, ANU, P.O. Box 475 Canberra ACT 2601, Australia
[3]Laboratory of Biological and Environmental Information Engineering, Graduate School of Agricultural and Life Sciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo, Tokyo 113-8657, Japan

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et al. 1989) demonstrated spatial heterogeneity in the dynamics of stomatal opening in intact leaves (Siebke and Weis 1995), and in PSII efficiency during photosynthetic induction (Bro et al. 1996). Here we report a chlorophyll fluorescence imaging study of photosynthetic induction in the leaves of shade-grown avocado plants. These leaves are unusually rich in lutein epoxide (Lx) under relatively low light intensities (García Plazaola et al. 2007). We show that stomata and CO_2 have large effects on the heterogeneity and extent of NPQ that develops in the first 10–20 min of illumination, well before de-epoxidation of violaxanthin (V) or Lx is detectable.

Materials and methods

Seedlings of avocado (*Persea americana* L., cv edranol) were kept in a shade enclosure (maximum irradiance 90 μM photons m^{-2} s^{-1}) of a temperature-controlled glasshouse (29°C day/18°C night) for 12 months. The petiole of fully expanded leaves was cut under water, kept in water and quickly transferred to the laboratory. Most experiments were performed at laboratory temperature (25–30°C) and [CO_2] (400–500 ppm). In some experiments a small area of the leaf was carefully covered with Vaseline to restrict CO_2 exchange. In others a single leaf was pre-incubated in the dark in an H_2O-saturated, temperature-controlled (25°C) gas stream with 100, then with 400 and 700 ppm [CO_2] in a sealed chamber within the light box of a chlorophyll fluorescence imaging system (Technologica, Colchester UK; Barbagallo et al. 2003) through which the gases were circulated. Leaves were dark adapted for 30–60 min between induction exposures to an orange LED actinic light (100 μM photons m^{-2} s^{-1}) and imaged during saturating flashes at intervals of 20 min and during relaxation in the dark for 5 min. After imaging each induction transient the leaf was transferred to a Licor gas exchange system (Licor Lincoln NE) fitted with a PAM 2000 (H. Walz, Effeltrich Germany) to obtain gas exchange parameters and spot measurements of chlorophyll fluorescence in an identical induction experiment. Pigments were extracted from 1 cm leaf discs taken from comparable leaves before and after transfer to full sunlight in an unshaded part of the glasshouse, and separated by HPLC using protocols similar to those described by Matsubara et al. (2007).

Results and discussion

Stomata are involved in transient heterogeneity of NPQ during induction

Induction experiments in air showed that NPQ became heterogeneous, in clearly defined intervein areas over the leaf, within 30 s (Fig. 1A), and transients usually lasted 2–10 min. Avocado leaves are heterobaric (data not shown) and heterogeneity of NPQ was clearly defined by vascular elements. Vaseline treatment showed that CO_2 transfer across secondary and tertiary vascular elements was extremely slow and that development of NPQ in areas in which stomata were occluded was greater (NPQ = 2), more rapid, and sustained (Fig. 1 B–D). Interestingly, NPQ adjacent to the mid-vein and primary veins of the treated area was lower after 15 min (Fig. 1D), perhaps suggesting supply of dissolved CO_2 from other areas of the leaf via the transpiration stream. NPQ relaxed to low, homogeneous values after 5 min in darkness (Fig. 1E).

Duration and extent of transient heterogeneity in NPQ responds to [CO_2]

Heterogeneity of NPQ was compared during induction transients at different [CO_2]. As above, NPQ was high and distinctly heterogeneous between 3 and 7 min into the induction in 400 ppm CO_2, and low and homogeneous after 20 min (Fig. 2). In 100 ppm NPQ was initially higher and less heterogeneous at these times, but remained high and more heterogeneous after 15 and 20 min, whereas in 700 ppm CO_2 NPQ was generally lower and most heterogeneous after 3 min. In general, spot measurements of NPQ with the PAM in each of the three areas of leaf enclosed in the gas exchange system closely followed the transients detected by

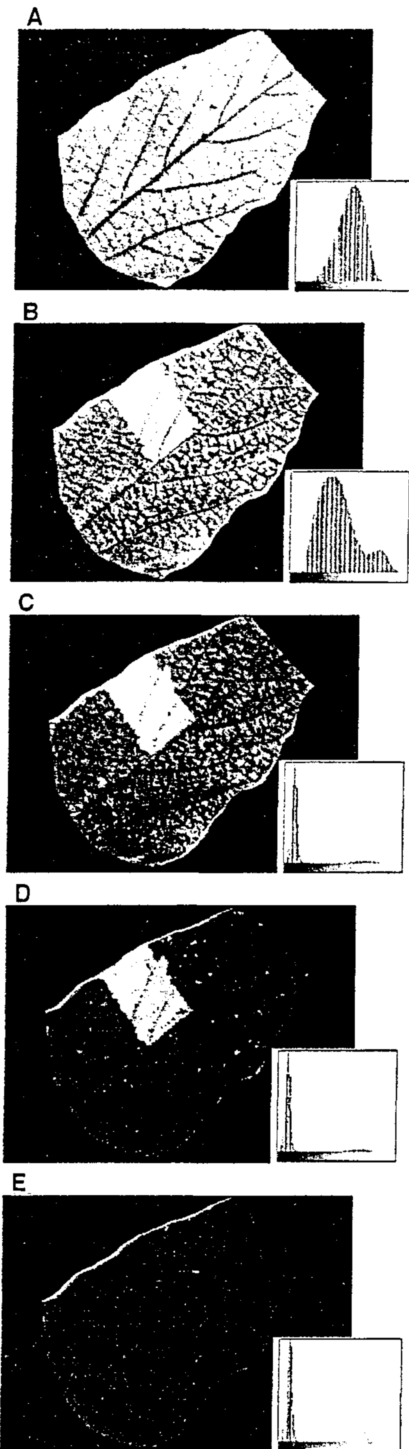

Fig. 1 Effects of local occlusion of stomata on the underside of a detached *P. americana* leaf with Vaseline on heterogeneity of NPQ during photosynthetic induction in air. Images were captured at 30 s, 3 min, 5 min and 15 min after turning on the actinic light and a relaxed image was obtained after 5 min in darkness. The gray-scale bar shows a range of 0–2 NPQ

the imaging system for the leaf as a whole, and were within the range of values measured by the imaging system (Fig. 2). The greatest disparity was found in the 700 ppm CO_2 treatment.

During the induction protocols with this leaf the greatest increase in stomatal conductance with time occurred in the 100 ppm CO_2 treatment, was less at 400 ppm, and remained low at the initial dark level in 700 ppm CO_2 (data not shown). Assimilation rate remained negative throughout induction at 100 ppm, became positive within 1 min at 400 ppm and slowly increased to near saturation after 20 min, and saturated within 5 min at 700 ppm (data not shown).

The CO_2 responsiveness of the extent and duration of heterogeneous NPQ during photosynthetic induction clearly reflects local differences in the internal and external supply of this terminal acceptor for photosynthetic electron transport in the different treatments (Meyer and Genty 1999). Internal sources (photorespiration) predominate in the absence of net CO_2 exchange, and, in spite of stomatal closure in response to 700 ppm CO_2, supply of CO_2 is sufficient to rapidly reduce the heterogeneity, extent and duration of the NPQ transient.

Transient, CO_2-responsive NPQ in avocado is independent of xanthophyll de-epoxidation

There were no significant changes in concentrations of xanthopylls during the first hour of treatment when light intensity increased to 200 μM photons m^{-2} s^{-1} (Table 1). However, after a further 3 h, when light intensity increased to 1,300 μM photons m^{-2} s^{-1}, de-epoxidation of both V and Lx occurred and the pools of V+A+Z and Lx+L increased. Neoxanthin was unchanged throughout, as were the pools of α- and β-carotene and the ratio of Chl *a/b* (data not shown). Evidently the dynamic, heterogeneous, stomatally-dependent and CO_2-responsive NPQ may involve quenching processes that occur in reaction centres prior to stabilization of heat dissipation in the antennae by de-epoxidation of xanthophylls pigments in either the V- or Lx-cycles (García-Plazaola et al. 2007). We propose that these processes involve

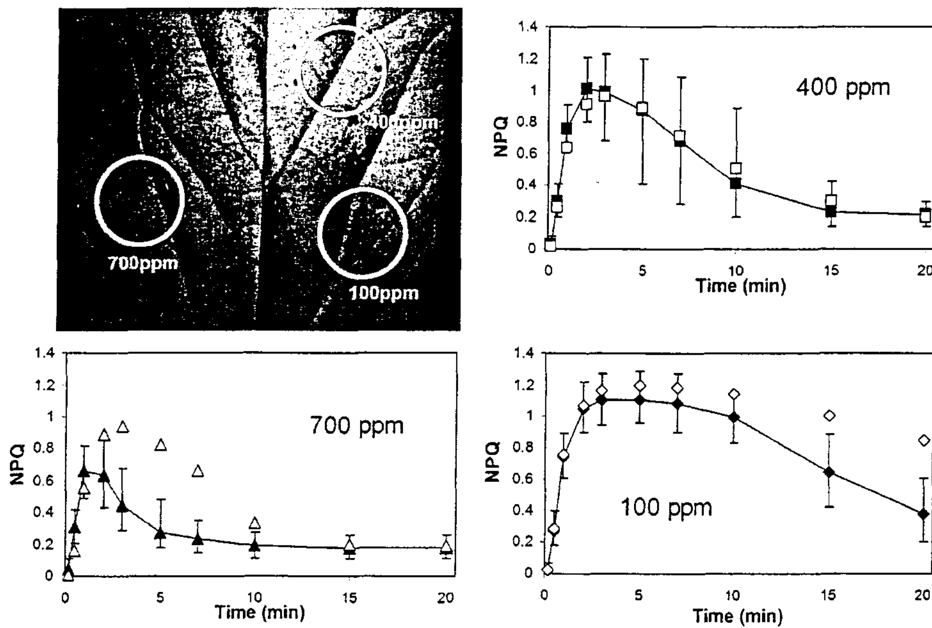

Fig. 2 Correspondence of the NPQ transients measured by imaging (closed symbols showing the range of values obtained at each time point) and those measured independently by chlorophyll fluorescence detection in a gas exchange system (open symbols) on a single leaf of *P. americana* under three different [CO_2]

Table 1 Pigment composition of avocado leaves grown in deep shade and after transfer to morning sunlight (mean ± SE; n = 4)

Light intensity (μmol photons m^{-2} s^{-1})	30	200	1,300
Time after transfer (min)	0	60	240
	mmol mol^{-1} chlorophyll		
Neoxanthin	39.5±0.2	39.9±0.2	39.2±0.3
Violaxanthin (V)	32.9±1.3	33.5±1.3	21.5±0.5
Antheraxanthin (A) + Zeaxanthin (Z)	0	0	20.9±1.0
Lutein epoxide (Lx)	27.8±1.3	28.0±1.7	23.5±0.5
Lutein (L)	118±2.6	112.9±3.5	129.4±1.6

pH-dependent events in reaction centres (Finazzi et al. 2004). We recommend close attention to CO_2 supply and heterogeneity in comparative evaluations of NPQ kinetics in-vivo.

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