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Chlorophyll *a* Fluorescence and Carbon Assimilation in Developing Leaves of Light-Grown Cucumber[1]

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ABSTRACT

The development of photochemical activity and carbon assimilation in light-grown cucumber (*Cucumis sativus* L. cv Natsusairaku) leaves was studied to determine the pattern of acquisition and its relationship to leaf growth and expansion. Measurements of chlorophyll *a* fluorescence showed that leaves acquire photochemical function over a period of 6 or more days, and gas exchange studies showed increases in carbon assimilation over a parallel time period. As leaves expand and mature, they undergo a sequential, three-step series of changes in fluorescence response. The initial kinetics show the absence of wholly functional quenching mechanisms. Dynamic imaging of fluorescence kinetics showed that a temporal series of changes occurred within defined areas of individual developing leaves. The spatial acquisition of photochemical activity in leaves was basipetal as is their directional expansion, development of air spaces and stomata, and the cessation of imported carbon.

Biochemical and physiological studies of developing photosynthesis in monocot leaves show that young cells just above the basal meristem are photosynthetically competent as soon as they are in the light (1). The reaction centers of both photosystems are functional, energy passes from PSII to PSI through the chain of electron carriers, and water is split photochemically. Although the basal cells of 4-d-old leaves, but not 8-d-old leaves, are unable to perform state I to state II transitions, photosynthetic activity is otherwise homogeneous throughout the leaf (1). The functional homogeneity may be related to the single locus of origin of most leaf cells, the basal meristem.

Typical dicot leaves grow in a more complex fashion than monocots (15). Cells within the leaf blade have numerous origins and the blade exhibits mosaic growth, *i.e.* meristematic cells scattered throughout the leaf divide and expand to produce clusters of cells until expansion is complete (19). In spite of this mosaic growth, dicot leaves expand anisotropically in banded-sectors. The magnitude of the relative growth rate in each sector increases basipetally (23).

This pattern of leaf growth results in heterogeneity in chloroplast and photosynthetic development within each leaf, consequently making their study more difficult than monocots (14). Older studies have established that the capacity for photosynthesis is attained by certain regions of the leaf early in development, and that the remainder of the leaf becomes competent and may increase in efficiency over time (6). Within developing leaves this is indicated by their patterns of carbon import and export (11, 28) and lack of synchrony in Chl content and Hill activity (8) or Chl and rubisco activity (2, 27). However, a direct demonstration of changes in photochemical activity within regions of a leaf has been lacking.

The objective of this work was to determine if developing cucumber (*Cucumis sativus* L. cv Natsusairaku) leaves and the regions within them acquire the capacity for photochemical function simultaneously or sequentially in a pattern related to their expansion and maturation. Imaging of Chl *a* fluorescence of entire leaves (16) now makes it possible to monitor photochemical activity nondestructively and compare induction kinetics in selected leaf areas. The goal was to localize in the developing leaf the first occurrence of characteristic phases of fluorescence kinetics and to complement this with gas exchange measurements on equivalent leaves to give information on carbon assimilation.

MATERIALS AND METHODS

Plant Materials and Leaf Area Measurements

Cucumber (*Cucumis sativus* L. cv Natsusairaku) plants were grown as previously described (16) for up to 8 weeks. For fluorescence and gas exchange measurements, plants were brought from the controlled-environment greenhouse to the laboratory and allowed to acclimate for about 2 h at 25 C. Plants were dark-incubated for at least 30 min before fluorescence induction. Leaves monitored for fluorescence and assimilation were intact and remained attached until all measurements on the plant were completed. Leaf area was measured with a LI 3100 area meter (LI-COR, Lincoln, NE).

Chl *a* Fluorescence Measurements

A portable Kautsky apparatus (Richard Branker Research Ltd., Plant Productivity Meter, SF-20) was used to monitor fluorescence on the upper leaf surfaces in interveinal areas midway between the midvein and leaf margin. Light of 670 nm at 40 μmol photons m^{-2} s^{-1} was used for excitation and fluorescence was measured at >710 nm. Data were collected at 30 ms intervals over a period of 400 s, stored, and analyzed by computer.

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Imaging System for Measuring Chl Fluorescence Induction

As previously described in detail (16), this system consists of a video camera with a charge coupled device imager and appropriate filters interfaced to a time code generator and video tape recorder. Images of the entire leaf were collected at intervals of 17 ms, digitized by an analog to digital convertor, stored on an optical disc, and then analyzed by computer. Two xenon lamps (380–620 nm) were used to evenly illuminate (±10%) the entire upper leaf surface at fluxes of 80 and 150 μmol m^{-2} s^{-1}, depending on leaf size; fluorescence was measured at 683 nm. Five data points from each measured area (less than 2 mm^{-2}) were analyzed and the data averaged.

Fluorescence Data

The kinetic data are displayed as single curves on a log time scale. While this time basis changes the overall appearance of fluorescence curves, the presentation gives good resolution of the fast phases of fluorescence and compresses the slow phases of the signal, but without loss of important characteristics of fluorescence induction. The terminology of Lavorel and Ettienne (13) is used to identify the consecutive phases of the fluorescence induction signals (I, D, P, S, M, T[2]), and their interpretation is based on established criteria (12, 17, 24, 30).

Assimilation Measurements

Measurements were made by an apparatus previously described (21). Briefly, air was mixed from N_2, O_2, and 3% v/v CO_2 in air using mass flow controllers. Some of this synthetic air passed through an aluminum leaf chamber. The air flow through the chamber was controlled by a mass flow controller. Some of the synthetic air and air from the leaf chamber was compared for water content and CO_2 content with an IR gas analyzer. Cross sensitivity of the CO_2 measuring section to water was eliminated by condensing the water out of the air as the air passed from the water measuring tube to the CO_2 measuring tube. Leaf temperature was measured with a copper-constantan thermocouple probe. Calculations of evaporation, conductance to gas exchange, photosynthesis, and intercellular CO_2 partial pressure were made according to von Caemmerer and Farguhar (29). Light was provided by a quartz halogen lamp and flux was measured with a LiCor quantum sensor. Water content was measured with a Dew 10 (General Eastern) dew point hygrometer.

RESULTS

Chl *a* Fluorescence of Developing Leaves

Mature leaves of 8-week-old reproductive plants showed typical Chl *a* fluorescence transients, with I, D, P, S, M, and T clearly evident (Fig. 1). However, these points could not be distinguished in young leaves (Fig. 2). The youngest leaf displayed an asymmetric curve consisting of a single, broad P peak, quenched over a 400 sec interval. No other transients were present. The next two leaves showed increased variable fluorescence, a steeper rise to P, but continued slow quenching of P as in the youngest leaf; other transients were absent. The fourth leaf showed the typical pattern of fluorescence kinetics with increased variable fluorescence and the presence of I, D, S, and M transients. The area of the leaves measured in these reproductive plants ranged from 1.5 to more than 300 cm^2.

Dynamic Imaging of Fluorescence Transients within Developing Leaves

Four-week-old cucumber plants also showed progressive changes in kinetics; the changes occurred among leaves and within the base, middle, and tip regions of leaves (Fig. 3). Beginning with leaf 9, just expanded away from the bud, different kinetics were found in the three leaf regions. The leaf tip showed the largest variable fluorescence and the presence of P and an indication of M. The middle and basal regions of this leaf had less fluorescence and only a single, broad P peak that was quenched slowly. The next two older leaves, 8 and 7, showed similar patterns of fluorescence transients, with increases in variable fluorescence, especially in the tip area, but also in the middle region of leaf 7. The P, S, and M transients were more evident and the D to P rise was steeper in leaf 7 than in the younger leaves. In leaf 6, the variable fluorescence decreased in the tip, but the characteristic kinetics remained. The base and middle area of this leaf continued to show a curve with a single peak. In leaf 3, a large, mature leaf of more than 400 cm^2, the characteristic fast and slow phases of Chl *a* fluorescence were present and similar in the middle and basal regions of the leaf. Variable fluorescence declined in the leaf tip. Imaged leaves ranged in area from 10 to 400 cm^2.

Assimilation in Developing Leaves

The upper surface of cucumber leaves had modest levels of assimilation regardless of their developmental age and posi-

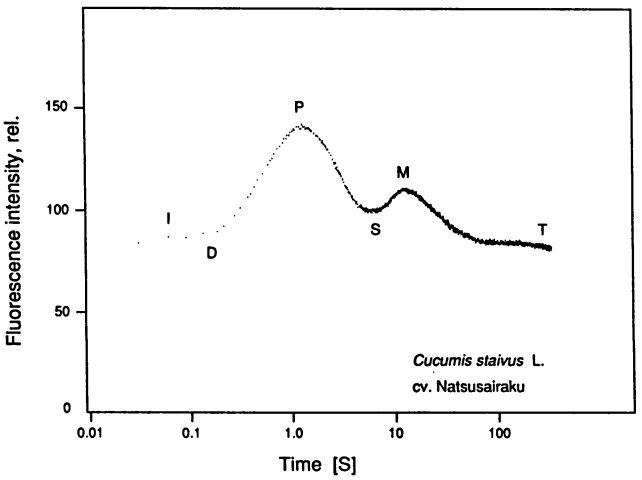

Figure 1. Chl *a* fluorescence transients measured in midregion of a mature leaf, greater than 300 cm^2 in area, on an 8-week-old cucumber plant.

[2] Abbreviations: I, D, P, S, M, T, inflection points of Chl *a* fluorescence kinetics where I is intermediary level, D is dip, P is peak, S is quasi-stationary level, M is second maximum, and T is terminal level.

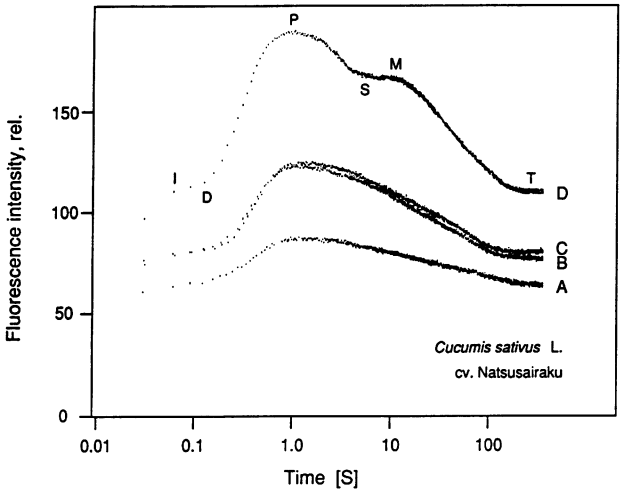

Figure 2. Fluorescence induction transients in the four youngest leaves of an 8-week-old, reproductive cucumber plant. The areas of leaves A to D were 1.5, 3.5, 8, and 70 cm^2, respectively; fluorescence measurements were made in the middle of each leaf.

tion on these vegetative plants (Fig. 4). In contrast, assimilation on lower leaf surfaces was minimal in the youngest leaf, then rose to a peak in the middle leaves, and declined in older, fully expanded leaves.

DISCUSSION

Cucumber leaves were not photochemically competent as soon as exposed to light. Characteristic Chl *a* fluorescence kinetics developed (Fig. 2) during a period of six or more days. Since the leaves are fully illuminated during this time, developmental factors rather than light play the major role in regulating the development of photochemical activity. The lengthy development of photochemical activity in cucumber is difficult to reconcile with the expression of photosynthetic genes which is temporally regulated initially and begins when leaves are within the bud and shielded from light (3, 5, 9, 10, 20). Pea leaves also require several days of light before fluorescence transients are typical of mature leaves (26). In cucumber, this pattern of development was found in vegetative, 4-week-old plants (Fig. 3) and reproductive, 8-week-old plants (Fig. 2), indicating that the development of the photochemical apparatus follows the same course regardless of plant age or reproductive status.

Changes in Chl *a* fluorescence in developing leaves occurred in a three-step sequence. First, only a single, broad peak, P, was present. The shallow rise to P was accompanied by a lengthy quenching period. Due to the functional connections of PSII with other photosynthetic components, this might signify limited development and function of PSII components (17), a deficiency of one or more PSII acceptors or plastoquinone (12), and the absence or inefficiency of quenching mechanisms (12).

Since the photoreduction of Q through reductant from water is reflected in the DP rise (12, 17) and a diminished rise can be induced by inhibiting the water-splitting enzyme sys-

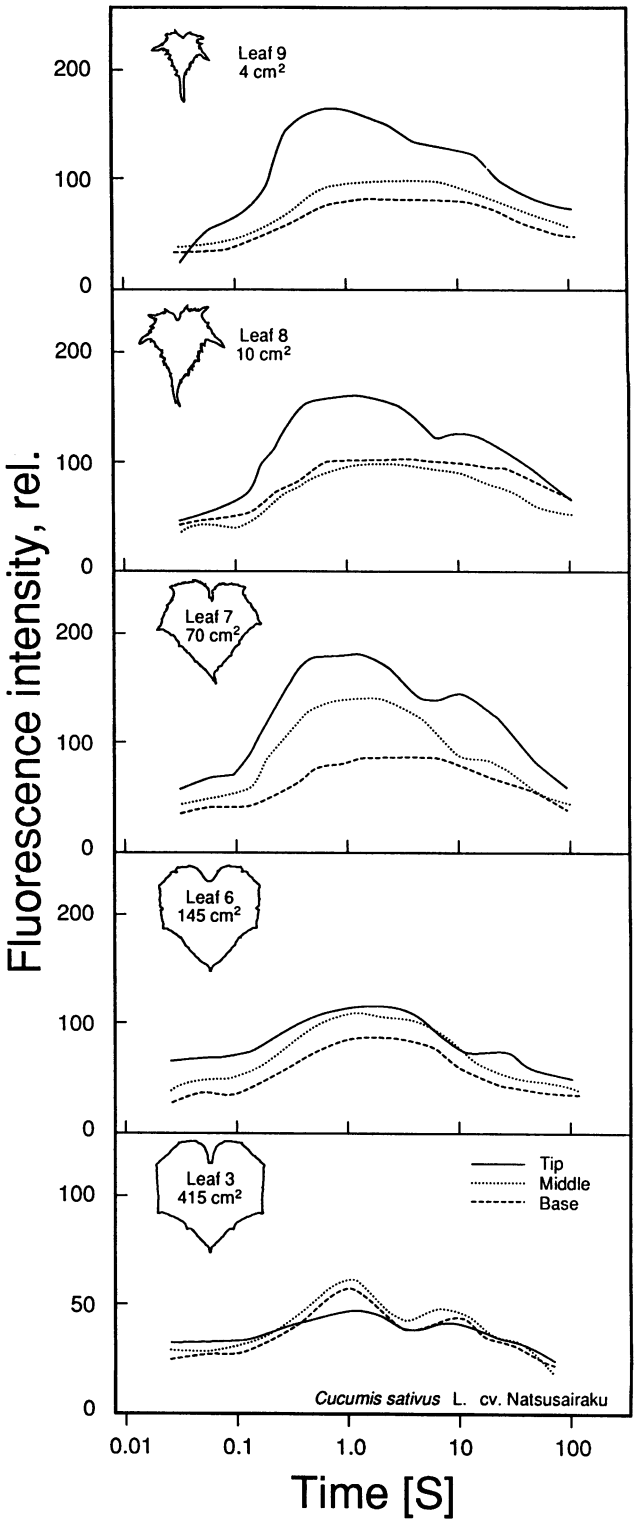

Figure 3. Dynamic imaging of Chl a fluorescence in the tip, middle, and base regions of leaves along the stem of a 4-week-old, vegetative plant of cucumber. Leaves are numbered according to age with leaf 9 being the youngest leaf, just expanded away from the bud. Plants were dark-incubated for a minimum of 30 min prior to imaging of fluorescence transients. Leaves 9 to 6 were exposed to 150 μmol

tem with SO_2 fumigation (22), the slow rise in developing cucumber leaves indicated the absence of the enzyme system or its incomplete function. The PS decline is due to photochemical and nonphotochemical quenching (1, 12). The slow decline of this transient in cucumber indicated an inhibition of electron flow from Q to PSI and the lack of a transthylakoid proton gradient because of the missing or incompletely developed water-splitting enzyme system. The absence of M in these fluorescence responses may indicate that carbon fixation is not occurring (24, 30).

The second step in the sequence was an increase in variable fluorescence attended by a steeper rise to P and the appearance of the remaining characteristic transients (I, D, S, M). In the final step of the sequence, the level of variable fluorescence declined, but the kinetics remained characteristic. Declines in fluorescence may be caused by fluorescence reabsorption by the increased chlorophyll content in mature leaves (4).

The development of fluorescence kinetics varies among dicot species (25, 26). In pea the time to P and M decreases as leaves complete their expansion (26), while in cucumber the time to P increased (Fig. 3). This occurred as the DP rise and variable fluorescence increases. An increased time to M has been reported in cucumber (25), but did not occur in leaves of this study (Fig. 3). The quenching of P in young pea leaves initially increases and then declines as they age (26), whereas in developing sunflower (25) and cucumber leaves (Figs. 2 and 3), the rate of quenching after P continuously increases. The variation among species indicates that there are differences in the synthesis of individual components, their assembly into functional photochemical units, and/or interrelations between photochemical and assimilatory elements.

The ordered changes in the kinetics of fluorescence transients occurred among and within leaves, with one or more stages in the transition being present in a single leaf. Thus, photochemical activity was not acquired uniformly within these dicot leaves as it is in monocots (1). Instead kinetics within developing leaves were heterogeneous. Since these leaves are still developing, water potential, internal CO_2 partial pressure, and capacity for stomatal function may not be uniform throughout. However, porometer measurements made on equivalent leaves of single plants (data not shown) indicated that stomatal resistance declined as the temperature decreased and light availability increased. Therefore, at least a population of stomata on the leaf was functional.

Characteristic kinetics appear first at the leaf tip, then in the middle region, and finally at the base. This progression of fluorescence response follows the basipetal course of leaf expansion and the development of air spaces (7, 15) and stomata (18), and parallels the cessation of carbon import to the leaf (11, 28).

Gas exchange measurements cannot be made with the spatial resolution equivalent to dynamic imaging, but the progressive increases in assimilation follow the same developmental pattern as the attainment of characteristic fluorescence transients. However, until the spatial pattern of competent stomata is known, we cannot know if the availability of CO_2 or the photochemical machinery is limiting carbon fixation. Based on the presence of the S to M to T transients, the majority of assimilation was due to the leaf tip alone when leaves are 2% of full expansion (leaf 9 of Figs. 3 and 4) and due to the middle and tip region until the leaf was more than 30% expanded (leaf 7 of Figs. 3 and 4).

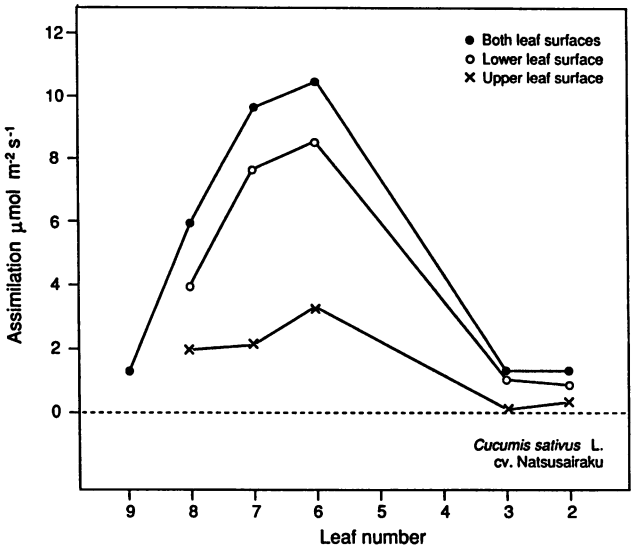

Figure 4. Carbon assimilation measurements in a cucumber plant with 9 leaves, equivalent to the plant imaged in Figure 3. Data for both surfaces of each leaf are presented with the exception of the smallest leaf for which a single value represents total assimilation from both surfaces. Photon flux density and air temperature were the same as for fluorescence measurements; leaf numbering as in Figure 3.

These results demonstrate that the appearance of photochemical function in dicots is fundamentally different from monocot leaves. In dicot leaves, cells are not photochemically competent when first exposed to light nor is photochemical activity homogeneous within a leaf until late in its expansion. Dynamic imaging of fluorescence kinetics showed that photochemical activity develops sequentially over time and progressively within defined leaf areas. The attainment of characteristic Chl *a* fluorescence transients and the increased assimilation of carbon dioxide in cucumber parallels the directional expansion of the leaf and the growth of spongy parenchyma cells and the presence of air spaces. However, the directional extension of the leaf is superimposed over a complex, mosaic pattern of cell division and growth within the blade. Thus, detailed analyses of fluorescence images may show that photochemical function is spatially distributed to reflect this intercalary growth or position with respect to the vascular tissue.

photons m^{-2} s^{-1} of exciting light and leaf 3 was exposed to 80 µmol photons m^{-2} s^{-1} of light. The decrease in flux density for leaf 3 was necessitated by its large size and the requirement for even illumination.

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