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Chloroplast Structure and Starch Grain Accumulation in Leaves That Received Different Red and Far-Red Levels during Development

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ABSTRACT

An important step in understanding influence of growth environment on carbon metabolism in plants is to gain a better understanding of effects of light quality on the photosynthetic system. Electron microscopy was used to study chloroplast ultrastructure in developing and fully expanded leaves of tobacco (*Nicotiana tabacum* L. cv Burley 21). Brief exposures to red or far-red light at the end of each day during growth under controlled environments influenced granum size, granum number and starch grain accumulation in chloroplasts, and the concentration of sugars in leaf lamina. Far-red-treated leaves had chloroplasts with more but smaller grana than did red-treated leaves. Red light at the end of the photosynthetic period resulted in more and larger starch grains in the chloroplasts and a lower concentration of sugars in leaves. Chloroplast ultrastructure and starch grain accumulation patterns that were initiated in the expanding leaves were also evident in the fully expanded leaves that received the treatment during development. It appears that the phytochrome system in the developing leaves sensed the light environment and initiated events which influenced chloroplast development and partitioning of photosynthate to adapt the plant for better survival under those environmental conditions.

Canopy photosynthesis has been studied in a number of plant species. Leaf age, temperature, CO_2 and light levels during photosynthetic measurements have been considered (2, 4, 16), but little attention has been given to the light conditions under which the leaves developed.

Sun and shade leaves differ in anatomy and Chl contents (11). Shaded plants often have elongated internodes and a lower percentage of dry matter in leaves. Shaded leaves in a plant canopy receive light of reduced photosynthetic photon flux density and a shift in spectral distribution because of absorption and transmission characteristics of the shading leaves (11). Because of the competitive absorption of Chl in the R[2] region of the spectrum, shaded leaves in a canopy receive more FR relative to R light than do unshaded leaves.

Under controlled environments, a few min of R or FR light at the end of the daily photosynthetic period result in leaf characteristics and Chl *a/b* ratios that are very similar to those in leaves that develop in sun or shade, respectively (11, 12). The R and FR light act through the phytochrome system to cause the plant to adapt for better survival under the existing growth environment. Leaf anatomy, photosynthetic efficiency, and photosynthate partitioning are influenced by the relative amounts of R and FR light received, especially at the end of the daily photosynthetic period during leaf development (12). Exposure to FR results in thinner leaves, a higher Chl *a/b* ratio, longer internodes, and a higher percentage of dry matter in stems relative to R-treated plants.

The objective of the present study was to examine chloroplasts in leaves that developed under a common photosynthetic environment but received 5 min of R or FR light to put phytochrome in the FR-absorbing or R-absorbing form, respectively, each day at the end of the photosynthetic period.

MATERIALS AND METHODS

Plant Materials. Tobacco (*Nicotiana tabacum* L. cv Burley 21) seedlings were started and grown to transplant size in expanded peat pellets at 28°C under 14-h photoperiods at 5 mw/cm^2 between 300 and 800 nm from cool-white fluorescent lamps. Eight-week-old seedlings were transplanted to 3-L pots containing a soil-perlite (2:1, v/v) mixture and placed in controlled environment chambers for conditioning and treatment. The seedlings were fertilized, as needed, with half-strength Hoagland (8) nutrient solution during the starting, conditioning, and treatment periods. They were conditioned to the growth chamber environments for 1 week before R and FR treatments were started. At the end of the conditioning period, all leaves longer than 5 cm were removed from each plant. Therefore, only leaves that developed during the 21-d treatment period were used in this study.

Treatments. During the conditioning and treatment periods, plants received 8-h daily illumination periods at 7.2 mw/cm^2 between 300 and 800 nm from cool-white fluorescent lamps. Day and night temperatures were 25°C. At the end of the daily illumination period, for 21 consecutive days, plants were irradiated for 5 min with R or FR light. To test reversibility, some plants received 5 min R followed by 5 min FR and others received 5 min FR followed by 5 min R light. All plants were kept in the same controlled environment chamber, except during the daily R and FR irradiations. The intensities of R and FR were 360 μw/cm^2 over the wavelength bands of 600 to 700 and 700 to 770 nm, respectively. The R radiation unit consisted of two layers of R cellophane under a bank of cool-white fluorescent lamps; whereas, the FR unit consisted of two layers of R and two of dark blue cellophane under internal-reflector, incandescent-filament lamps.

[1] Retired.
[2] Abbreviations: R, red; FR, far-red.

Sampling and Preparation. Leaf samples were taken from 'expanded' and 'expanding' leaves 2 h after the last R and FR treatments. The sampled leaves were either fully expanded or about one fifth the size of the fully expanded ones. Samples were taken from the middle of a half-leaf from each of three plants per treatment.

Leaf samples were cut into 1.5 to 2.0 mm squares, fixed in 3% glutaraldehyde in 0.05 M phosphate buffer (pH 6.8), rinsed in buffer, and postfixed in 2% phosphate buffered osmium tetroxide (5). After dehydration in an acetone series, leaf sections were embedded in Araldite-Epon[3] plastic. Blocks were cut on an ultramicrotome with a diamond-edge knife. Sections were stained with aqueous 2% uranyl acetate followed by lead citrate and observed on an electron microscope (5).

Observations. Chloroplast size, grana, and starch grain size and number per chloroplast were determined from 20 × 25 cm electron micrographs. Fifty chloroplasts were examined per treatment. Sugar concentrations were determined by the method of Kollman *et al.* (14). Samples that tested reversibility of the effects of R and FR (*i.e.* R followed by FR or FR followed immediately by R light) responded to the kind of light received last. That is, R followed by FR resulted in chloroplasts, grana, and starch grains very similar to those in the FR-treated leaves. Conversely, FR followed by R gave results very similar to those from R alone.

RESULTS AND DISCUSSION

Chloroplast size, grana, and starch grain characteristics are shown in Table I. The brief exposure to R and FR light at the end of each photosynthetic period during leaf growth and development significantly influenced chloroplast development. Representative electron micrographs are shown in Figure 1.

Chloroplast Size. Chloroplasts in the fully expanded leaves were larger than those in expanding leaves. However, there was much variation in cross-section size among chloroplasts within a given light treatment, and two-dimension chloroplast size was not consistent for R *versus* FR treatments. It is possible that differences in the third dimension may have developed in response to light quality, but these were not examined in the present study.

Grana. Chloroplasts in leaves that received FR light at the end of each photosynthetic period during development had fewer thylakoid layers per granum and more small grana spread throughout the chloroplast than did those that received R light. The same trend between R and FR treatment was observed in both expanding and expanded leaves, and the older leaves had more grana per chloroplast within each light treatment. More grana with fewer thylakoid layers per granum might be an adaptive response to a 'shade' environment in which relatively less photons would strike a leaf. In effect, this could increase the efficiency of trapping light in a low light environment. R and FR light acting through the phytochrome system are known to result in some morphological adaptations that are associated with sun and shade environments, respectively, such as short *versus* long stems and thick *versus* thin leaves (12).

Using an algae model system, Haupt (6, 7) has demonstrated that chloroplasts will change positions in response to brief exposures to R or FR light. He used *Mougeotia*, a filamentous algae having large cells with a single rectangular chloroplast which can move within the cell to adapt to light conditions. The movement is initiated by a change in the predominant form of phytochrome (7). In nature, movement in algae chloroplasts may be an adaptive adjustment for more efficient use of light in shaded or low light situations, whereas, the opposite movement may serve to protect from high light or full sun conditions.

In higher plants, influence of light on chloroplast development has been studied in etiolated seedlings (1, 3, 15). Phytochrome influenced chloroplast development and chlorophyll synthesis in etiolated cotyledons of *Brassica alba* (15). Also, reorganization of the PSII unit was studied in developing thylakoids of greening etiolated *Phaseolus vulgaris* leaves after transfer to darkness (1). Upon transfer to darkness the light-harvesting Chl protein, its 25 kD polypeptide and Chl *b* decreased while a 42 kD polypeptide increased and PSII units of smaller size formed. The reorganization occurred in thylakoids that were still in the process of development, but not in those that had already developed. The authors (1) proposed that the observed response was not turnover of the light-harvesting Chl protein complex *per se*, but environmental regulation of the ratio of light harvesting and PSII components, which influence photosynthetic rate (1).

Our observations with tobacco plants (Table I), grown under controlled environments with R or FR light to manipulate the phytochrome system each day at the beginning of the dark period, are consistent with this theory. That is, chloroplasts, especially those in the younger leaves, differed between the R and FR treatments. It is possible that developing chloroplasts were influenced by the phytochrome form in the cells during the dark period and that after sufficient development in darkness, the chloroplast characteristics such as number and size of grana remained different. Thus, it appears that the phytochrome system is involved in regulation of chloroplast structure for more efficient survival of plants. If this is true, shaded leaves in a plant canopy and FR-treated leaves in controlled environments should

[3] Mention of a trade name, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Table I. *Characteristics of Chloroplasts from Expanding and Expanded Tobacco Leaves that Received R or FR Light at the End of the Photosynthetic Period each Day for 21 Consecutive Days*

Samples were taken 2 h after the final treatment.

Leaf Stage	Treatment	Chloroplast Size		Starch Grain			Grana/Chloroplast[a]	Thylakoid Layers/Granum[b]
		Length	Diameter	No./chloroplast	Size			
					Length	Diameter		
		μm		*no.*	*μm*		*no.*	
Expanding	Red	1.41 ± 0.06[c]	0.73 ± 0.04	0.82 ± 0.10	0.25 ± 0.03	0.12 ± 0.01	17 ± 0.6	18 ± 1.4
	Far-red	1.24 ± 0.04	0.73 ± 0.02	0.14 ± 0.05	0.13 ± 0.02	0.06 ± 0.01	20 ± 0.7	11 ± 0.5
Expanded	Red	2.83 ± 0.11	1.00 ± 0.05	1.81 ± 0.14	0.62 ± 0.03	0.21 ± 0.01	24 ± 1.1	23 ± 1.1
	Far-red	3.10 ± 0.19	0.86 ± 0.06	1.47 ± 0.14	0.52 ± 0.03	0.17 ± 0.01	37 ± 1.6	13 ± 1.3

[a] Grana per chloroplast refer to the number with 3 or more thylakoid layers.
[b] Values for number of thylakoid layers per granum are for the largest stack per chloroplast.
[c] Values are means ± SE.

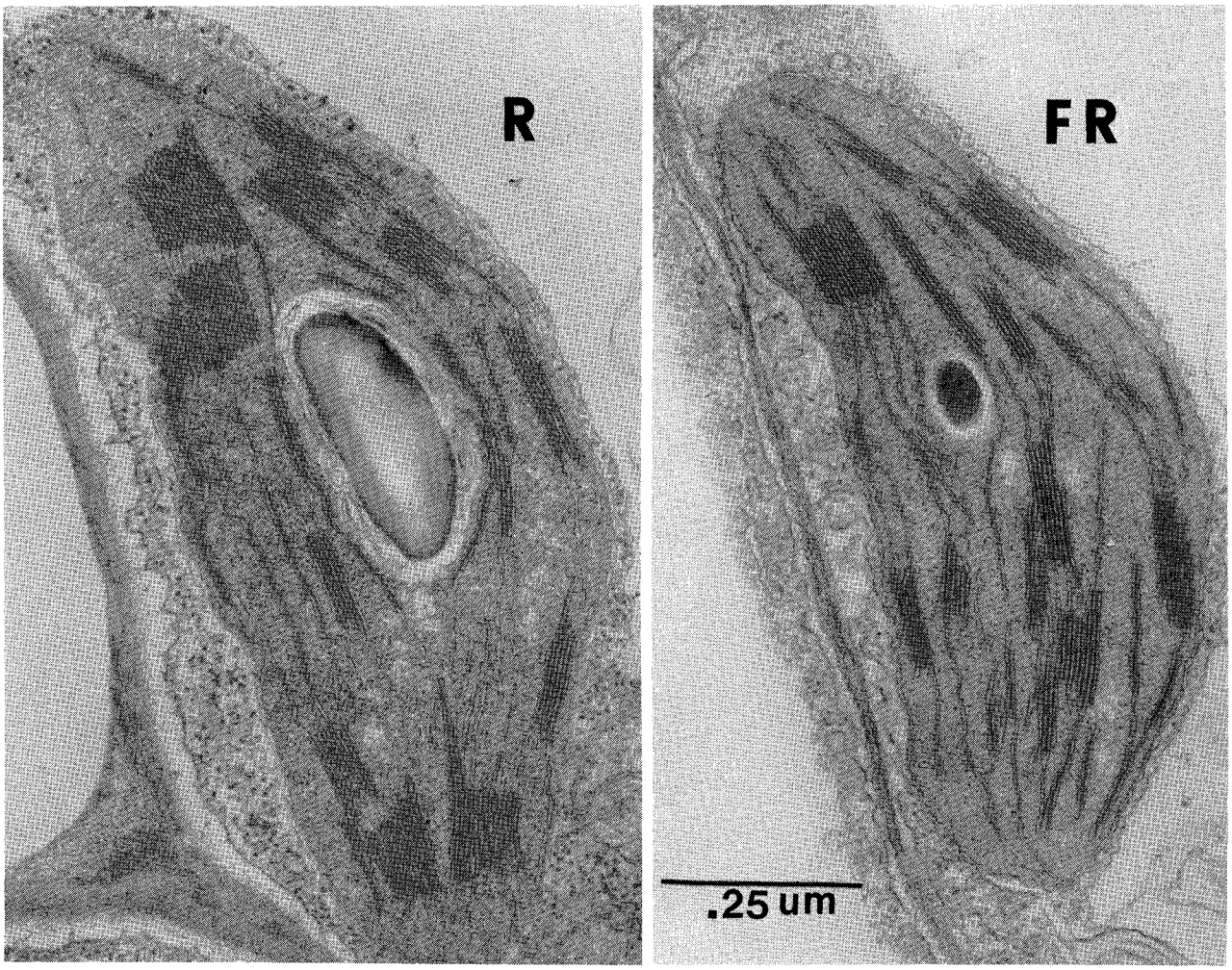

FIG. 1. Chloroplasts from expanding tobacco leaves that received brief exposures to R or FR light at the end of the photosynthetic period each day during leaf development.

be similar to each other and result in more dispersion of the grana throughout the chloroplast. This is consistent with our observations (Table I).

Starch Grains. Chloroplasts in leaves that received the R light treatment had more and larger starch grains that did those in leaves of the same age that received the FR treatment each day during development (Table I). Also, leaves that received R followed by FR were very similar to those that received FR alone, and those that received FR followed by R were very similar to those that received R. The same response pattern was evident in both the expanding and fully expanded leaves. The fact that starch grains responded to R and FR light and that the effects of R were reversed by FR, and vice versa, indicates that phytochrome was involved.

Table II. *Sugar Concentration in Expanding Tobacco Leaves that Received 5 Min R or 5 Min FR Light at the End of each Day during Development*

Time from Final Light Treatment to Sampling	Sugar Concentration in Leaves that Received	
	R	FR
h	*mg/g dry wt*	
2	21.2 b[a]	32.2 a
16	15.3 c	20.2 b

[a] Values followed by the same letter do not differ significantly at $P = 0.05$.

Previous research has shown that brief exposures to R or FR light immediately after the daily photosynthetic period influences partitioning of phytosynthate among leaves, stems, and roots (12). FR at the end of the photosynthetic period initiates events that result in relatively more photosynthate being partitioned to stems and less to leaves and roots as compared to plants that received R light. A similar pattern is often observed in plants grown in crowded plant populations. It is apparent that the phytochrome system is involved in sensing the light environment around the plant and in partitioning of photosynthate within a plant so that the plant is better able to adjust to its light environment.

Sugars. Sugar concentrations in R and FR-treated leaves are shown in Table II. Leaves that were treated with FR had more sugar than those that were treated with R light, and sugar concentrations of leaves decreased during the night. The effects of R and FR light at the end of the photosynthetic period on sugar content during the subsequent dark period were consistent with findings reported in an earlier paper (13).

In a recent report, Gifford and Evans (4) suggested that photosynthetic assimilate is partitioned between exported material and further leaf growth or temporary storage in the developing leaf. In the present study (Table I), phytochrome manipulations

influenced the amount of photosynthate present as starch grains in chloroplasts shortly after the end of the photosynthetic period. The FR treatment resulted in fewer and smaller starch grains in chloroplasts (Table I), more sugar (Table II), and more photosynthate partitioned to stems and less to leaf lamina (11). At the cellular level, Huber (9, 10) recently found that partitioning between starch and sugar was regulated by sucrose phosphate synthetase. It is not clear what regulates the enzyme system. However, it is possible that phytochrome is involved in the pathway of events leading to the enzymic regulation of partitioning within the cell and the direction of relatively more or less photosynthate to leaf lamina or to plant stem and structural material.

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