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Phytochrome involvement in regulation of the photosynthetic apparatus and plant adaptation*

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Plants grown in high, as compared to low, population densities receive a higher ratio of far-red (FR) relative to red (R) light because more FR is reflected from competing leaves. The FR/R ratio acts through the phytochrome system within the plant to sense competition and to regulate development of the photosynthetic apparatus and the partitioning of photosynthate among plant components. Plants that developed with a relatively high FR/R ratio had longer stems; and leaves that were thinner, had chloroplasts with more but small grana, and had a higher chlorophyll *a*/*b* ratio. Leaves that developed with a higher FR/R ratio fixed more CO_2 on leaf mass and total chlorophyll bases with a given amount of light.

Additional key words – Chloroplast structure, chlorophyll *a*/*b* ratio, photosynthate partitioning.

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Résumé. *Les plantes cultivées en forte densité de population, par rapport aux faibles densités, reçoivent une plus grande proportion de lumière rouge sombre (RS) relativement à la lumière rouge clair (RC) parce qu'une plus grande quantité de lumière RS est réfléchie par les feuilles environnantes. Le rapport RS/RC agit par l'intermédiaire du système phytochromique à l'intérieur de la plante pour percevoir la compétition et réguler le développement de l'appareil photosynthétique et la répartition des photosynthétats parmi les constituants de la plante. Les plantes qui se développent en présence de rapports RS/RC relativement élevés présentent des tiges plus longues, des feuilles plus minces, des chloroplastes avec plus de granums, mais plus petits, et un rapport chlorophylle* a/b *plus élevé. Les feuilles qui se développent en présence d'un rapport RS/RC plus élevé fixent, pour une même quantité de lumière, plus de* CO_2 *par unité de masse foliaire ou de chlorophylles totales.* Mots clés additionnels : structure du chloroplaste, rapport chlorophylle *a*/*b*, répartition des assimilats.

Abbreviations. Chl, chlorophyll; FR, far-red light; R, red light; P_{fr}, far-red absorbing form of phytochrome; P_r, red absorbing form of phytochrome.

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INTRODUCTION

Many investigations have been conducted on photosynthetic rates in developed leaves, but few have considered the influence of light spectral distribution (during leaf development) on the photosynthetic apparatus. In nature, the strategy of a plant is to adapt to its environment and survive long enough to produce the next generation. Therefore, the plant must be able to adapt its photosynthetic apparatus and its assimilate partitioning according to growth environment and the plant characteristics that favor survival in that environment. Since light is a major factor in photosynthesis and survival, the plant must be able to sense (measure) and adapt to light changes, such as those associated with amount of competition from other plants.

Recent studies have shown that a plant can sense competition from other plants by measuring the relative amount of far-red light (FR) reflected from competing plants (Kasperbauer, 1987). Green leaves absorb most of the red light (R) and reflect much of the FR. Therefore, a plant surrounded by many green leaves receives more reflected FR and a higher FR/R ratio than is received by an isolated plant. The phytochrome system within the plant responds to the ratio of FR relative to R and regulates a number of developmental aspects that can influence survival of the plant. Rapid adaptation is very important for survival of seedlings.

The objectives of the present report were (*a*) to compare light absorption by, reflection from, and transmission through soybean leaves of different ages, (*b*) to compare responsiveness of *Glycine max* and *Nicotiana tabacum* to R and FR acting through the phytochrome system within the plant, and (*c*) to relate FR/R ratio during leaf development to leaf characteristics and photosynthetic efficiency.

MATERIALS AND METHODS

Plant material. Soybean [*Glycine max* (L.) Merr cv. Coker 338] and tobacco (*Nicotiana tabacum* L. cv. Burley 21) plants were used. All were started from seed, grown in potting soil, and watered as needed with half-strength Hoagland (1950) nutrient solution during the starting, conditioning and treatment periods.

Treatments. One experiment with soybean and two with tobacco were conducted at different times. However, both species were grown at 25°C in controlled-environment chambers equipped with cool-white fluorescent lamps that provided about 520 µmol m^{-2} s^{-1}. Plants of both species were irradiated at the end of each day for 21 consecutive days with 5 min R, 5 min FR or 5 min FR followed immediatelly by 5 min R. After the R and FR treatments, plants were returned in darkness to the dark, 25°C chamber for the remainder of the night. The R radiation unit consisted of 2 layers of red cellophane under a bank of cool-white fluorescent lamps, and the FR unit consisted of 2 layers of red and two layers of blue cellophane under incandescent-filament lamps. Intensities of R and FR were 3.6 W m^{-2} over the wavebands of 600 to 700 and 700 to 770 nm, respectively. Soybean seedlings were started directly in the pots, thinned to two per pot and began receiving R and FR treatments in the unifoliolate stage. Tobacco seedlings were germinated in starting flats and transplanted to pots at about six weeks, and all leaves longer than one cm were removed prior to the first R and FR treatments. In this manner, all leaves that were sampled from both species developed under the indicated R and FR treatment.

Sampling and measurement. Values for transmission through and reflection from single leaves were obtained at 5-nm intervals from 400 to 850 nm using a LiCor-1800-12 integrating sphere. Values for absorption were calculated by subtracting the combined values for transmission and reflection from 100 at each measured point. Photographs of representative plants, measurements, and samples were taken after 21 days of treatment. Chlorophyll (Chl) analyses were by the method of Arnon (1949).

Photosynthetic efficiency. Tobacco plants were used to study photosynthetic efficiency of R- or FR-treated leaves. The plants were studied individually, and the order was alternated between treatments (*i. e.* R, FR, R, FR) to eliminate bias due to difference in time lapse since the last end-of-day R or FR treatment. The CO_2 assimilation rates were measured in recently-expanded attached leaves. One leaf per plant was enclosed in a transparent, double-walled chamber that was maintained at approximately 27°C by circulating water between the walls. The light source was a bank of internal-reflector incandescent-filament lamps that were partly submerged in a transparent-bottom tank of running tap water (Moss, 1964). Different light intensities within the chamber were obtained by adding or removing neutral screens between the leaf chamber and the light source. The CO_2 assimilate rates were determined by comparing CO_2 concentrations of the air at the entrance and exit ports of the chamber with an infrared CO_2 analysis system. Leaves were allowed to equilibrate at each light intensity before CO_2 assimilation rates were recorded.

RESULTS AND DISCUSSION

Leaf age

The effects of leaf age on visible and FR light absorption, reflection and transmission are shown in figure 1. All of the leaves had similar reflection spectra. But, the youngest leaves were thinnest and had the least competitive absorption of FR, which may result in their greater sensitivity to a given ratio of FR relative to R received at the leaf surface (*i. e.* seedlings may be more responsive than mature plants to changes in FR/R ratio that are associated with nearness of competing plants).

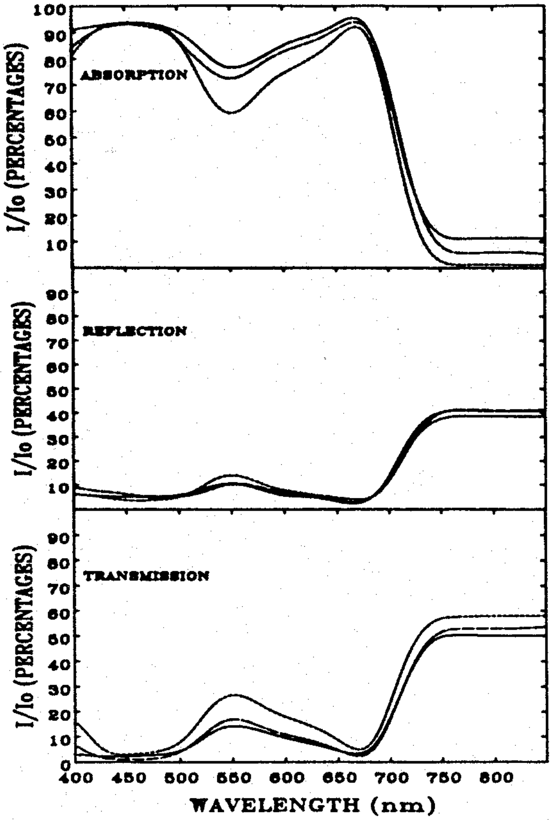

Figure 1. *Light absorption, reflection, and transmission spectra for three ages of soybean leaves.* Dots, expanding unifoliolate; dashes, expanded first trifoliolate; and solid lines, expanded trifoliolate from full-sun, field-grown plant. I/Io refers to radiation absorbed, reflected or transmitted at 5-nm intervals relative to incident radiation at the same wavelengths.

Since the FR/R ratio acts through the phytochrome system within a plant, less competition for FR within a leaf might have the effect of increasing the FR/R photon ratio available to regulate the photoequilibrium level of phytochrome and its effect on plant development. While the nearness and number of competing plants can influence the FR/R ratio of light received by a plant (Kasperbauer, 1987), the effectiveness (or efficiency) of R and FR photons that get into the plant tissue is the important factor in phytochrome regulation of adaptation to competition from other plants. Because of competitive absorption by Chl at 660 nm (the *in vitro* absorption peak for P_r), the R action peak for phytochrome responses in green leaf tissue is near 645 nm (Kasperbauer *et al.*, 1963, 1964). On the other hand, recent studies of light gradients within plant tissue by Vogelmann and Bjorn (1984) have demonstrated that scattering and internal reflection of FR within the tissue can produce local photon flux densities which are higher than the incident photon irradiance. From an ecological viewpoint, these differences may help explain why very young seedlings are so highly responsive to small changes in spectral distribution of light reflected from other seedlings. As an example, we have observed rapid stem elongation of newly-emerged, field-grown soybean seedlings when they were about 2 to 3 cm apart as compared to seedlings that were about 100 cm apart. It is of significance that such young seedlings can sense competition from nearby seedlings and adapt to the potential competition by developing longer stems, even though all of the seedlings appear to be unshaded and in direct sunlight. This observation is consistent with results of earlier experiments on the Beltsville Spectrograph in which small changes in R relative to FR resulted in appreciable alteration of P_r/P_{fr} equilibrium (Kasperbauer *et al.*, 1963, 1964), and the equilibrium level at the end of day played a major role in plant development. In addition to stem elongation, leaf characteristics are also readily affected by nearness of competing plants.

R and FR effects on morphology and the photosynthetic apparatus

In controlled environments, soybean and tobacco plants responded similarly to brief irradiations with R or FR light at the end of the day, which put phytochrome predominantly in the P_{fr} or P_r form, respectively, at the beginning of the night (*fig.* 2). Treatment with FR caused the plants to develop longer stems (*fig.* 2), and a higher shoot/root ratio as is shown in table 1. Treatment with R immediately after FR caused a reversal of the effects of FR. This photoreversibility indicates involvement of phytochrome in the sensing mechanism. A longer stem is an adaptive response to a higher FR/R ratio that could favor survival among

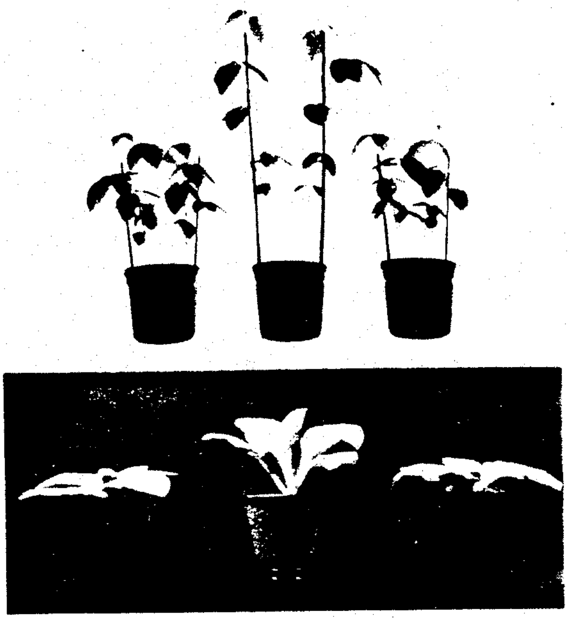

Figure 2. *Young plant phytochrome-mediated gene expression in response to 5-min end-of-day exposures to R, FR, or FR followed immediately by R (left to right), respectively.* Both *Glycine max* (top) and *Nicotiana tabacum* (bottom) seedlings were treated at the end of each day for 21 consecutive days.

competing plants by increasing the probability of keeping some leaves in sunlight.

In addition to effects on stem elongation, treatment with FR at the end of each day resulted in thinner leaves, chloroplasts with more but smaller grana, and a higher Chl *a*/*b* ratio (*tab.* 1). Since the characteristics shown in table 1 developed on R- and FR-treated plants that received the same total light energy in the controlled environments, it is apparent that the FR/R ratio acted through the phytochrome system and initiated events that led to adaptive responses which might favor survival of a plant while competing with other plants for photosynthetically active light.

Photosynthetic efficiency

The influence of FR/R ratio during plant development extended to the photosynthetic efficiency of leaves. Tobacco leaves that received brief irradiations with FR to provide a higher FR/R ratio at the end of each day during development had higher photosynthetic rates per unit of Chl and per mass of leaf (*fig.* 3). However, photosynthetic rates did not differ significantly on a leaf area basis (even though FR-treated leaves were much thinner) between the leaves that received a low or a high FR/R ratio during development. These data indicate that FR-treatment during leaf development (analogous to a plant developing in a higher population density) acted through the phytochrome system within the plant and influenced genes for adaptation of a more efficient photosynthetic apparatus, which would favor survival of a plant that is competing with many others for light.

Although our measurements of Chl *a*/*b* ratios, chloroplast structure and photosynthetic efficiency were made on leaves that received the R and FR treatments each day for 21 consecutive days, visually detectable differences in stem length, leaf shape, and leaf color were evident in six or seven days. Subcellular changes, such as Chl *a*/*b* ratios, in response to FR/R ratio are expected to occur much more rapidly.

Table 1. Glycine max *and* Nicotiana tabacum *responses to phytochrome as regulated by 5-min end-of-day irradiations with R (low FR/R ratio) or FR (high FR/R ratio) light each day for 21 consecutive days.* All sampled leaves developed during the 21-day period.

End-of-day		Shoot/root dry mass ratio	Leaf dry mass/area (*mg cm*$^{-2}$)	Chloroplasts^a		Chl *a*/*b* ratio
Light	FR/R ratio			Grana/per chloroplast	Thylakoid layers per granum	
Glycine max						
R	Low	4.18^b	2.5	—	—	4.04
FR	High	6.00	2.3	—	—	4.53
Nicotiana tabacum						
R	Low	5.23	2.2	24	23	1.87
FR	HIgh	7.52	1.9	37	13	1.97

^{a} Data on chloroplasts are adapted from Kasperbauer and Hamilton (1984); ^{b} Within each species, each R versus FR response was statistically significant at the $P = 0.05$ level.

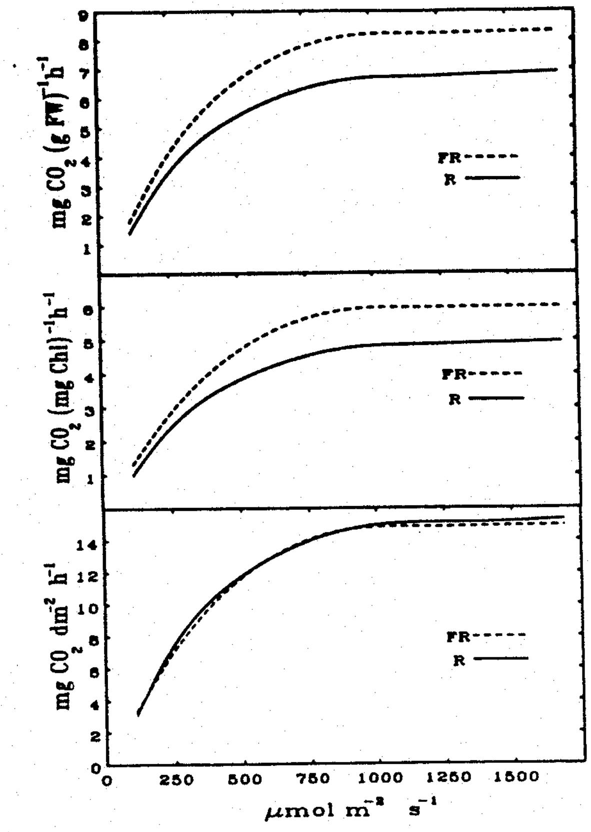

Figure 3. *Net CO_2 assimilation rates of tobacco leaves expressed on basis of (top to bottom, respectively): fresh weight of leaf lamina, total chlorophyll, and area of leaf lamina.* Studies were done with intact leaves on plants that received R (low FR/R ratio) or FR (high FR/R ratio) for 5 min at the end of each day during development (curves are drawn from Kasperbauer and Peaslee, 1973).

The action of FR/R ratio via the phytochrome system on regulation of the photosynthetic apparatus and on photosynthetic efficiency might vary among leaves on the same plant due to amount of self-shading, and even among layers within the same leaf. That is, competitive absorption of R by Chl and the amount of scattering and internal reflection of FR could result in a different effective FR/R ratio at various depths within a leaf. Differences in FR/R ratio might result in local differences in photosynthetic efficiency within a given leaf. Investigation of direct or indirect effects on FR/R ratio via phytochrome on enzymes such as ribulose bisphosphate carboxylase might add significantly to understanding of photosynthetic efficiency of leaves that develop under various light conditions.

CONCLUSIONS

The data and examples discussed in this paper support the concept that leaves of the same genotype can have a modified photosynthetic apparatus and an altered photosynthetic efficiency because of differences in light environment during plant development. Leaves of plants in high population densities receive more reflected FR and a higher FR/R ratio. The FR/R ratio acts through the phytochrome system within the plant and influences the photosynthetic apparatus and photosynthate partitioning. A higher FR/R ratio results in longer stems, thinner leaves, chloroplasts with more but smaller grana, an increased Chl *a*/*b* ratio, and more CO_2 fixed per mass of leaf and Chl with a given amount of photosynthetic light. Better understanding of the influence of spectral distribution of light on development of the photosynthetic apparatus and on photosynthetic efficiency is important in plant-soil-water-light management systems that influence crop yield and quality.

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REFERENCES

Arnon D. I., 1949. — Copper enzymes in isolated chloroplasts. *Plant Physiol.*, **24**, 1-15.

Hoagland D. R. and Arnon D. I., 1950. — The water-culture method for growing plants without soil. *Calif. Agri. Exp. Sta. Cir.*, 347.

Kasperbauer M. J., 1987. — Far-red reflection from green leaves and effects on phytochrome-mediated assimilate partitioning under field conditions. *Plant Physiol.*, **85**, 350-354.

Kasperbauer M. J., Borthwick H. A. and Hendricks S. B., 1963. — Inhibition of flowering of *Chenopodium rubrum* by prolonged far-red radiation. *Bot. Gaz.*, **124**, 444-451.

Kasperbauer M. J., Borthwick H. A. and Hendricks S. B., 1964. — Reversion of phytochrome 730 (P_{fr}) to P660 (P_r) assayed by flowering in *Chenopodium rubrum*. *Bot. Gaz.*, **125**, 75-80.

Kasperbauer M. J. and Hamilton J. L., 1984. — Chloroplast structure and starch grain accumulation in leaves that received different red and far-red levels during development. *Plant Physiol.*, **74**, 967-970.

Kasperbauer M. J. and Peaslee D. E., 1973. — Morphology and photosynthetic efficiency of tobacco leaves that received end-of-day red or far-red light during development. *Plant Physiol.*, **52**, 440-442.

Moss D. N., 1964. — Optimum lighting of leaves. *Crop Sci.*, **4**, 131-136.

Vogelmann T. C. and Bjorn L. O., 1984. — Measurement of light gradients and spectral regime in plant tissue with a fiber optic probe. *Physiol. Plantarum*, **60**, 361-368.