

Photomorphogenesis in *Sinningia speciosa*, cv. Queen Victoria¹ II. Stem Elongation: Interaction of a Phytochrome Controlled Process and a Red-requiring, Energy Dependent Reaction

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Abstract. When *Sinningia* plants were grown with fluorescent light of photosynthetic intensity for 8 hours each day, stems became abnormally elongated when the P_{FR} level was lowered by far red light given during the last half of several consecutive nights. However, plants were even taller if the source also emitted red light. Elongation was independent of the red/far red energy ratio if it was lower than one, but dependent upon irradiance at all values tested.

Elongation of plants irradiated by a well filtered far red source was presumed to be limited by a shortage of respiratory substrate. Enhancement by radiation shorter than 700 $m\mu$ was attributed to promotion of processes leading to increased substrate supply. Protochlorophyllide was regarded as the primary photoreceptor. Its photoreduction promoted chlorophyll synthesis which, in turn, increased photosynthetic capacity and thus substrate supply.

In a previous article (17) we described morphological changes in *Sinningia speciosa* arising when long exposures to far red light replaced the latter part of a 16 hour dark night, for several consecutive nights. Stems and petioles elongated, leaf blade expansion was inhibited, leaf position became vertical, chlorophyll per unit leaf area was reduced, and the rate of dry weight accumulation in shoots decreased. Alterations in the growth pattern were attributed to the low P_{FR} level induced by the far red light source.

In the course of our investigation, we noted the following: 1) The elongating effect of a long far red irradiation was enhanced when the source also emitted red, even though red light raised the P_{FR} level. 2) Long exposures were necessary for red light to promote stem elongation. 3) Red light also played an important role in chlorophyll metabolism.

The present study was undertaken to determine: What is the relationship between elongation and the red/far red ratio of the irradiation source? What photosystems in *Sinningia* absorb the supplementary red light and interact with phytochrome in altering chlorophyll metabolism? Do these same photosystems participate in photomorphogenesis? What is the relationship between chlorophyll metabolism and other morphological changes?

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Materials and Methods

Seedlings of *Sinningia speciosa* cv. Queen Victoria were grown as previously described (17), *i.e.* with fluorescent light for 8 hours each day, of 900 to 1150 ft-c except 600 ft-c in table I, Part A. Supplementary light treatments were given during the night; controls received dark nights.

Experimental conditions and measurement parameters were also the same (17), with these exceptions: 1) Table I: The supplementary red sources in Growth Chamber No. 2 were above the plant benches and provided uniform irradiance for all plants. The far red sources were located at one end of the plant bench as previously described. 2) Table IV: The supplementary sources were incandescent lamps mounted at one end of the plant bench in Growth Chamber No. 2 as previously described, except 2 layers of red cellophane (Dupont) replaced the far red filter. 3) The experiment described in table III was conducted in a 2 liter clear glass chamber, one-third submerged in a thermostated water bath at 26°. The light sources were a bank of Grolox and warm white (1:3) fluorescent lamps (1150 ft-c) or three 7 watt incandescent bulbs (20 ft-c). Air circulated through the chamber and through a Beckman Infra red analyzer, model IR215, in a closed system, at a rate of 2 l/min, by a diaphragm pump. The volume of the system was approximately 2.5 l. Plants from Growth Chamber No. 1, that had been irradiated by supplementary sources 16 hours each night for 17 nights, were severed at soil level and placed in small holders with

their cut stems submerged in a reservoir of water during the measurements.

Photosynthetic rates were obtained by first equilibrating the system with room air, then closing the system, and recording CO₂ uptake for a 3 to 5 minute period. The average of 3 repetitions is presented. Respiration rates were recorded for 8 minutes in the closed system in darkness, at the end of the photosynthetic period.

Results and Discussion

The Relationship Between Red/Far Red Ratio And Morphological Response. When plants were exposed to supplementary light during the last 8 hours of the night, stems and petioles were longer if the light source emitted red in addition to far red.

In table VI of (17) and in figure 1, a plant irradiated by an incandescent light source is compared to one irradiated by a filtered far red source. In another experiment, light sources were filtered to emit red light (24 $\mu\text{w cm}^{-2}$, 600–700 $\text{m}\mu$) and far red light (29 $\mu\text{w cm}^{-2}$, 700–780 $\text{m}\mu$). After 17 nightly light treatments, plants receiving only far red light were 134 % as tall as their control, but plants whose far red irradiation was supplemented by red light, were 238 % the control value.

Groups of plants were exposed to red light of the same intensity, but far red light of varying intensity. Several experiments of this type were carried out, at different red intensities. Typical results in table I show that stem elongation, differential blade/petiole growth, and petiole angulation were independent of the red/far red ratio when it

Table I. *Elongation and Other Growth Responses of Sinningia speciosa as a Function of the Red/Far Red Energy Ratio of the Supplementary Irradiation*

Plants were grown on 8 hour photoperiods of fluorescent light at 600 ft-c for Part A and 900 ft-c for Part B. Supplementary light treatments were given during the last half of the night and were continued for 16 nights. Height data include SD. Percentage data refer to dark control.

A	Dark	Red (600–700 $\text{m}\mu$) irradiance = 65 $\mu\text{w cm}^{-2}$ Far red (700–780 $\text{m}\mu$) irradiance			
		10 $\mu\text{w cm}^{-2}$	38 $\mu\text{w cm}^{-2}$	60 $\mu\text{w cm}^{-2}$	200 $\mu\text{w cm}^{-2}$
Red/far red energy		6.5	1.7	1.1	0.3
Stem height (cm) ¹	0.67 ± 0.10	0.96 ± 0.24	2.18 ± 0.54	2.22 ± 0.60	2.49 ± 0.36
		143 %	325 %	332 %	372 %
Blade length/petiole length ²	5.5	4.2	2.6	2.7	2.7
		76 %	47 %	49 %	49 %
Petiole angle (deg) ³	17	36	61	64	60
OD 663 $\text{m}\mu$ of leaf ² pigment extract	0.51	0.62	0.53	0.52	0.45
		122 %	104 %	102 %	88 %
Shoot dry wt (mg)	159	141	108	123	105
		89 %	68 %	77 %	66 %

¹ Measured from first to fourth node.

² Third leaf pair, counted from base of stem.

³ Angle between petiole and horizontal.

B.	Dark	Red (600–700 $\text{m}\mu$) irradiance = 6 $\mu\text{w cm}^{-2}$ Far red (700–780 $\text{m}\mu$) irradiance		
		82 $\mu\text{w cm}^{-2}$	117 $\mu\text{w cm}^{-2}$	231 $\mu\text{w cm}^{-2}$
Red/far red		0.073	0.051	0.026
Stem height (cm) ¹	0.70 ± 0.06	2.11 ± 0.56	2.14 ± 0.43	2.43 ± 0.52
		302 %	306 %	347 %
Blade length/petiole length ²	7.3	3.5	3.6	3.3
		48 %	49 %	45 %
Petiole angle (deg) ³	6	54	56	53
OD 663 $\text{m}\mu$ of leaf ² pigment extract	0.54	0.34	0.37	0.36
		63 %	69 %	67 %
Shoot dry wt (mg)	239	190	211	182
		79 %	88 %	76 %

¹ Measured from first to fourth node.

² Third leaf pair, counted from base of stem.

³ Angle between petiole and horizontal.

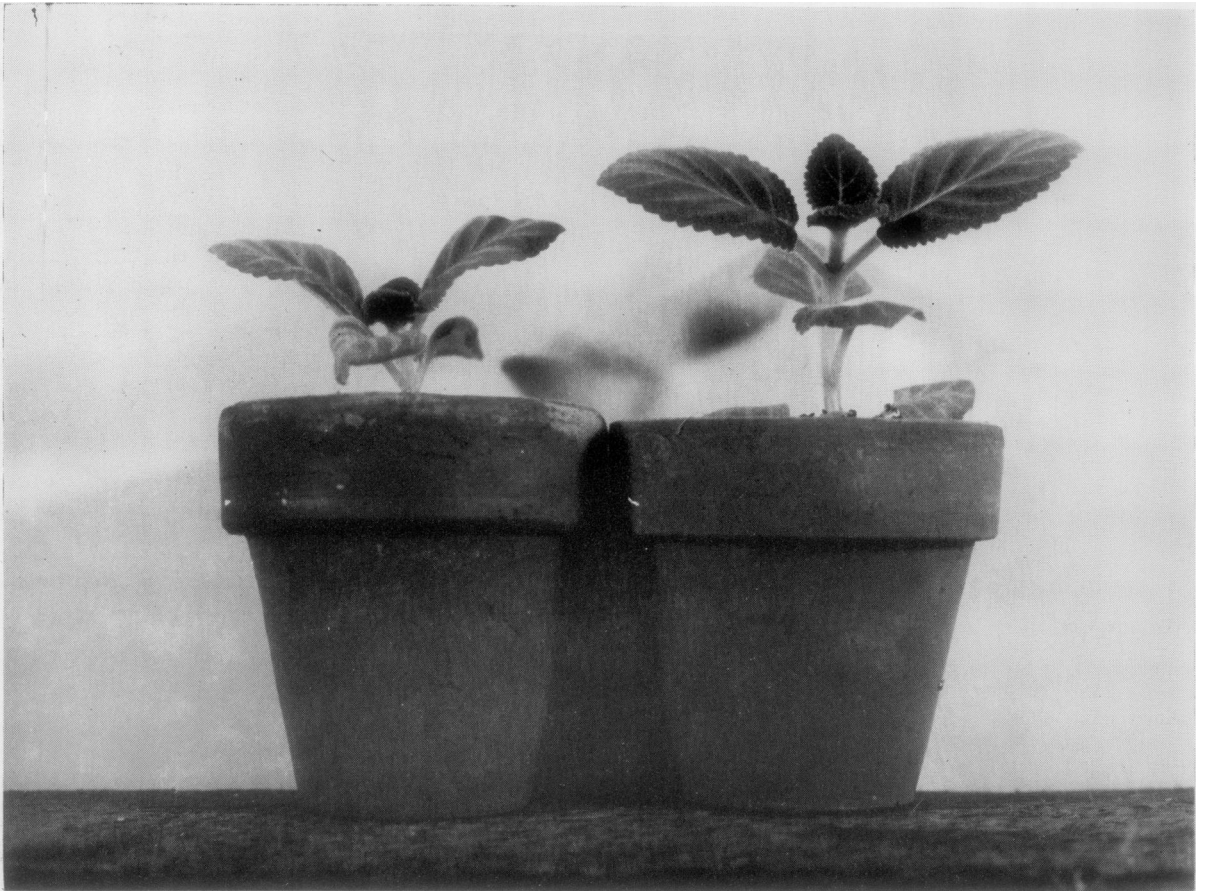


FIG. 1. A plant that was irradiated during the last half of the night for twenty consecutive nights, by a filtered far red source ($77 \mu\text{w cm}^{-2}$, 700–780 $m\mu$) is shown at the left of one irradiated during the same period by a source emitting incandescent light ($58 \mu\text{w cm}^{-2}$, 600–700 $m\mu$ and $54 \mu\text{w cm}^{-2}$, 700–780 $m\mu$).

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was lower than 1.7³. It is quite likely, however, that ratios of incident irradiation lower than 1.7 induced a very low P_{FR} level in interior cells, for microscopic examination of *Sinningia* leaf sections revealed 2 adjacent layers of palisade cells that were densely lined with chloroplasts. Since the red light sources in this experiment were above the plants, chloroplast pigments probably absorbed much of the red light. The significance of light filtration by chlorophyll for phytochrome-mediated phenomena has been discussed by others (3).

Evidence That Chlorophyll Metabolism Responds To Supplementary Light Through Another Photosystem In Addition To Phytochrome. Stem elongation, blade length/petiole length, and petiole angulation data suggest that the P_{FR} level was lower than that of controls when plants were irradiated by light with a red/far red energy ratio of 6.5 (table IA). These plants, however, had more chlorophyll per unit leaf area than their controls, even though chlorophyll was reduced by low P_{FR} levels [table IB and (17)]. Absorption of light by another photosystem was, apparently, masking the effect of phytochrome on chlorophyll metabolism.

The effect of red light on chlorophyll accumulation was examined in a series of experiments. Both etiolated and green plants were used. Biosynthetic pathways are reported to be the same in both cases (18, 25).

Sinningia tubers were kept in the dark until sprouts appeared. Sprouts were irradiated continuously with red light, far red light, or both. After 4 days, plants that had received red light with or without far red had green leaves and stems, while those irradiated with far red alone had no green pigment. Dark controls also lacked chlorophyll. This is in agreement with the generally accepted scheme for chlorophyll synthesis, *i.e.* radiation shorter than 700 $m\mu$ is absorbed by protochlorophyllide, which is subsequently converted to chlorophyll (12, 27).

In another experiment, plants that received fluorescent light of 950 ft-c for 8 hours each day were exposed to red light for 13 consecutive nights. Chlorophyll accumulation varied linearly with irradiance of the supplementary light. Plants irradiated with 1 $\mu w\text{ cm}^{-2}$ were indistinguishable from controls receiving dark nights, while plants that received 53 $\mu w\text{ cm}^{-2}$ (600–700 $m\mu$) had 130% as much chlorophyll per unit leaf area.

When plants were exposed to low intensity fluorescent light for part of the night, chlorophyll accumulation was most efficient if the exposure took

Table II. *The Effect of Supplementary Cool White Fluorescent Light Emitted during the First Half, Second Half or Whole Night, on the Chlorophyll Content of Sinningia speciosa*

Plants were grown on 8 hour photoperiods of fluorescent light at 1150 ft-c. Supplementary light treatments were continued for 10 nights, at illuminance levels of 75 ft-c (16 hr irradiation) and 150 ft-c (8 hr irradiation). Percentage data refer to dark control.

	Dark	Fluor (8 hr) Dark (8 hr)	Dark (8 hr) Fluor (8 hr)	Fluor (16 hr)
OD 663 $m\mu$ of leaf pigment extract ¹	0.49	0.59 120%	0.69 141%	0.65 133%

¹ Third leaf pair, counted from base of stem.

place during the second half of the night (table II). Chlorophyll synthesis in some species shows a rhythmic sensitivity to light (1, 2, 15, 22). Our data suggest this might be true in *Sinningia*, too. These data also show the strong promotion of chlorophyll accumulation by low supplementary irradiance. Supplementary energy, only 13% that of the photosynthetic period, raised the chlorophyll level by 41%.

We interpret these several *Sinningia* experiments as follows: red light emitted by our supplementary sources was absorbed by protochlorophyllide, whose photoreduction was a rate limiting step in chlorophyll synthesis. Thus chlorophyll concentration was significantly increased by supplementary red light. Protochlorophyllide appears to be the other photoreceptor that mediates the effect of red light on chlorophyll metabolism.

Relatively low light intensities have proved optional for accumulation of chlorophyll in *Euglena* (23).

Interaction Of Phytochrome And Other Photosystems. Chlorophyll accumulation was inhibited when the P_{FR} level was low during the last part of the night [table IB and (17)]. In (17) we suggested that phytochrome control of chlorophyll was indirect and that chlorosis was a consequence of the

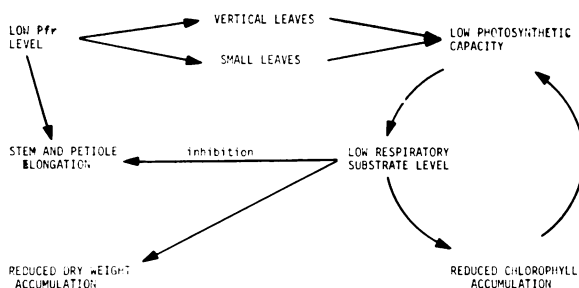


FIG. 2. Postulated interactions resulting from the repeated far red irradiation of *Sinningia* plants during the night.

³ Measured ratios required to saturate these processes depend upon the geometrical arrangement of the light sources, as discussed in the Materials and Methods section of (17).

low substrate levels (7, 10, 26, 27) of far red irradiated plants. We believe that the autocatalytic low chlorophyll-low photosynthesis cycle, depicted in the diagram below (fig 2), caused substrate deficiencies that inhibited all assimilatory processes including stem and petiole elongation.

Inter-relationships are summarized in figure 2. The effectiveness of red light in reversing this trend is dramatic. Absorption by protochlorophyllide leads to new chlorophyll synthesis, which, in turn, enhances photosynthetic efficiency and thus respiratory substrate levels. Additional photosynthesis during the period of supplementary irradiation is also a contributory factor, albeit a minor one (table III). The increased rates of photosynthesis and respiration, of plants exposed to incandescent rather than filtered far red light, are consistent with this interpretation (table III). The intensity dependence of all assimilatory responses also supports our hypothesis (table IV).

Many potential reactions in other species are inhibited when plants are grown under conditions that limit respiratory substrate. Recorded examples include stem elongation of gherkins and tomatoes (14), peas (11) and Pinto beans (5), and flowering of *Chenopodium rubrum* (4). In these experiments inhibitions were reduced by feeding sugars.

Comparison With High Energy Reactions In Other Species. The red-requiring energy-dependent photoreaction we have described appears to act by photoreduction of protochlorophyllide. Radiation of wavelength greater than 700 m μ was completely ineffective. This photoreaction differs from most that have been described for other species under the collective title of HER (high energy reaction) (8, 9, 13, 16, 19, 20, 24).

Although these HER exhibit wide variability in action spectra, all show 1 action peak in the blue and a peak or broad shoulder in the far red region. Anthocyanin synthesis in apple skin, however, is an

Table III. *Photosynthetic (PS) and Respiratory (Resp.) Rates of Sinningia speciosa Plants That Had Been Exposed to Supplementary Far Red or Incandescent Light for 17 Nights*

Plants were grown on 8 hour photoperiods of fluorescent light at 1150 ft-c, and were irradiated for 16 hours each night by the light sources described below.

The apparent rate of photosynthesis was measured during the day when the fluorescent source was used and during the night when the low intensity incandescent source was used. Plants were darkened for the measurement of respiration rate. Photosynthetic rates given below were corrected for dark respiration.

	Dark	Far red ¹ (16 hr)	Incand ¹ (16 hr)
Stem height (cm) ²	0.5	2.1	2.8
Shoot dry wt (mg)	89	67	112
Resp rate (μ l CO ₂ /min/plant)	9.8	4.6	8.8
(μ l CO ₂ /min/gm dry wt)	110	68	79
PS rate (μ l CO ₂ /min/plant) ³	55.7	22.2	40.5
(μ l CO ₂ /min/gm dry wt)	626	333	374
PS rate (μ l CO ₂ /min/gm dry wt) ⁴	18

¹ Irradiance of the far red source = 60 μ w cm⁻² (700-780 m μ), and of the incandescent source = 64 μ w cm⁻² (600-700 m μ) and 60 μ w cm⁻² (700-780 m μ).

² Measured from first to fourth node.

³ Fluorescent source (3 cool white: 1 Gro-lux) at 1150 ft-c.

⁴ Incandescent source described above. Illuminance = 20 ft-c.

Table IV. *Responses of Sinningia speciosa to the Irradiance of Supplementary Light From a Source That Emits Red and Far Red*

Plants were grown on 8 hour photoperiods of fluorescent light at 900 ft-c. Supplementary light treatments were given during the last half of the night and were continued for 17 nights. The ratio of red (600-700 m μ) to far red (700-780 m μ) energy was 0.74. Height data include SD. Percentage data refer to dark control.

	Dark	Irradiance 600-780 μ w cm ⁻²			
		10	23	112	397
Stem height (cm) ¹	0.69 \pm 0.09	1.89 \pm 0.37	2.08 \pm 0.44	2.57 \pm 0.39	2.04 \pm 0.66
		274 %	302 %	372 %	441 %
Blade length/petiole length ²	5.5	3.8	3.4	3.0	2.8
		69 %	62 %	55 %	51 %
OD 663 m μ of leaf extract ²	0.53	0.43	0.50	0.52	0.64
		81 %	94 %	98 %	121 %
Shoot dry wt (mg)	187	172	192	201	243
		92 %	103 %	107 %	130 %

¹ Measured from first to fourth node.

² Third leaf pair, counted from base of stem.

HER that has an action peak at 650 $m\mu$ and is relatively insensitive to radiation beyond 700 $m\mu$ (6, 21). Downs *et al.* (6) indicate that anthocyanin synthesis depends upon photosynthesis, and that chlorophylls *a* and *b* are the photoreceptors for this HER. We have hypothesized that stem elongation in *Sinningia* is dependent upon chlorophyll synthesis, an energy dependent reaction with protochlorophyllide as its major photoreceptor. Thus the HER of anthocyanin production in apple skin and stem elongation in *Sinningia* are not homologous.

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