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Response of Tissue with Different Phytochrome Contents to Various Initial Photostationary States[1]

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Abstract. Pretreatment of etiolated pea plants with red light and with red combined with far-red light produced morphologically similar plants having 4-fold differences in spectrophotometrically detectable phytochrome. Stem segments from the variously pretreated plants respond in the same way to different percentage conversions of phytochrome to P_{FR}. These results suggest that the P_{FR}/P_R ratio, rather than the concentration of P_{FR}, governs pea stem segment elongation. However, the ratio hypothesis does not explain contradictions between spectrophotometric and physiological assays previously obtained with this tissue, nor does it explain similar contradictions obtained in other systems. The only hypothesis consistent with the data to date is that of the existence of bulk and active phytochrome fractions, with the latter present in insufficient quantities to be spectrophotometrically detectable.

Attempts to correlate phytochrome content and state, assayed spectrophotometrically, with physiological response have led to conflicting observations. In some cases there is agreement between spectrophotometric and physiological assays (1, 2, 11). On the other hand, some tissues, such as young, intact pea roots (7), and cauliflower heads, parsnip roots, and artichoke receptacles (8) contain large amounts of spectrophotometrically detachable phytochrome yet exhibit no known light responses. A number of contradictions between spectrophotometric and physiological assays have also been reported (1, 4, 9, 12, see also 10). Such contradictions have prompted us to see whether or not differences in the amount of spectrophotometrically detectable phytochrome might be reflected in differences in phytochrome responses. The phytochrome content of etiolated seedlings is easily reduced by brief exposures to red light (3, 7, 10). However, such exposures also bring about photomorphogensis. Since differences in phytochrome responses might be expected on grounds other than phytochrome content when etiolated and de-etiolated tissues are compared, it seemed desirable to obtain morphologically similar plants which contained different amounts of phytochrome. This was made feasible by the observation that in dicot seedlings phytochrome content remains relatively constant under continuous far-red illumination, even though such illumination is highly effective in de-etiolation (5).

Materials and Methods

Seeds of *Pisum sativum* L. cultivar "Alaska" were obtained from Asgrow Seed Company, New Haven, Connecticut. They were soaked for 4 hours and then sown in either polyethylene basins (19 × 29 × 10 cm) for routine growth assays, or in cardboard ice-cream cartons (9 × 9 cm) for determining the effect of various light pretreatments on de-etiolation. Basins or cartons were nearly filled with saturated vermiculite (Zonolite No. 1), which also covered the seeds to a depth of 3 to 6 cm. A 1/10 strength solution of Hutner's medium was used for both soaking the seeds and saturating the vermiculite. Hutner's medium was prepared as previously reported (7). After planting, the seeds were allowed to develop at 26 to 27° in darkness for 6 or 7 days.

For growth assays, 10-mm segments were cut just below the apical hooks of plants with developing third internodes 12 to 48 mm in length. The segments were randomized in large petri dishes containing Na_2HPO_4-KH_2PO_4 buffer (0.02 M with respect to PO_4), pH 6.2 to 6.4. After all had been cut, lots of 15 or 20 were placed in 50 ml beakers containing 3.0 ml of a medium consisting of buffer, 3×10^{-5} M $Co(NO_3)_2$, and 2% (w/v) sucrose. Following light treatments the beakers were covered with small petri dishes, and the segments were allowed to elongate in darkness at 26 to 27° for 20 hours, after which they were measured to the nearest 0.5 mm.

Standard red light sources consisted of 2 cool white fluorescent tubes (Sylvania F15T8/CW) behind a 0.3 cm thickness of red Plexiglas (Rohm and Haas 2444). Far-red light was provided by four 15-watt and one 7 and one-half watt incandescent

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light bulbs over 8 to 10 cm of water and a 0.3 cm thickness of Rohm and Haas V-58015 "black" Plexiglas. The light sources and filters used to achieve various photostationary state levels of the far-red absorbing form of phytochrome have been described elsewhere (9); 10 minute exposures were used. All manipulations were performed under dim green safelights (9).

Spectrophotometric assays of phytochrome content were performed as previously described (7, 9) on samples of segments cut for the growth assay. Each sample of 10 segments was cooled to the temperature of ice and irradiated for approximately 10 minutes with cool white fluorescent light to saturate protochlorophyll conversion in sections from plants having received light pretreatments. The low temperature prevented significant changes in phytochrome content during the illumination with fluorescent light. Segments were then cut in half and packed to a sample length of 1 cm in 6-mm diameter aluminum cuvettes. The packed cuvettes were kept on crushed ice until and during the measurement.

Results

Light pretreatments involving a number of schedules of red light, far-red light, and combinations of them were tested with the objective of optimizing differences in phytochrome content while maintaining the same state of morphological de-etiolation. For studies of this type, the seedlings in ice-cream cartons were allowed to develop in darkness at 26 to 27° for 6 days. At that time 10 seedlings per carton were selected so that the leaf scale marking initiation of the third internode was still in the apical hook region, and the remaining seedlings were cut from the carton. Replicate lots were then given various light pretreatments for different lengths of time, following which the apical buds were excised and weighed individually, and the lengths of the third internodes measured. Using stem growth inhibition and apical bud weight increase as measures of de-etiolation, it was found that a schedule (R) consisting of 3 minutes of red light every 2 hours alone, and a second schedule (RF) consisting of (R) plus continuous far-red light, caused the same degree of de-etiolation when given for 14 or 18 hours (fig 1) while maximizing the differences in phytochrome content. Pretreatment with continuous far-red light alone also caused a similar degree of de-etiolation; however, R and RF pretreated plants were used in subsequent experiments because of the similar action of these light regimes on chlorophyll synthesis, which conceivably might have modified the results of the phytochrome assays.

For a comparison of the phytochrome response of tissues subjected to R and RF light schedules, the pretreatments were administered to etiolated seedlings in basins. The R and RF pretreatments were begun when the seedlings were 6 days of age, and a total pretreatment time of 16 hours was used. The last red exposure in both R and RF pretreatments was given approximately 2 hours before the end of the pretreatment period. Those plants pretreated with the R regime received 15 minutes of far-red light at the end of the pretreatment period, to ensure that the phytochrome in both R and RF plants was in the same state. A third "pretreatment," darkness (D), was also administered to seedlings in basins. These plants were held in darkness for the duration of the pretreatment period. Sections were cut from the pretreated plants immediately following the end of the pretreatment period. The R and RF pretreatments reduced the spectrophotometrically detectable phytochrome, compared to that observed in etiolated plants of the same age, to about 20 and 80 %, respectively (table I). Thus, even though plants pretreated with R and RF light regimes were morphologically similar (fig 1), they showed a 4-fold difference in spectrophotometrically detectable phytochrome. The various P_{FR} contents brought about by photostationary state illumination of segments from D, R, and RF pretreated plants are indicated by the "relative P_{FR} units" of table I. These values are obtained by setting the phytochrome of the dark controls equal to 100, so that an assumed full conversion of this to P_{FR} would give 100 units.

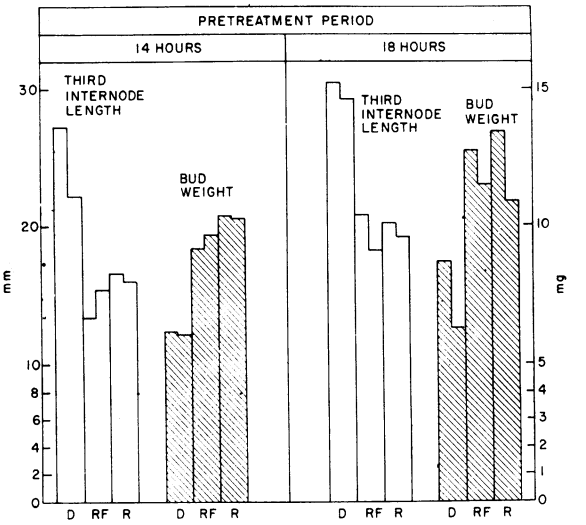

FIG. 1. De-etiolation by R and RF light schedules for 14 and 18 hours. Each half bar represents the mean of 10 plants. D) dark controls; R) 3 minutes red light every 2 hours; RF) R plus continuous far-red light.

Initial phytochrome conversions to P_{FR} of from 5 to 88 % were established by exposing segments under photostationary state filters for 10 minutes. The effects of such exposures on the subsequent growth of tissues from the 3 groups of pretreated plants are shown in figure 2. Each point represents the mean of 30 or 40 segments. From 5 % P_{FR} upward, elongation decreased in a linear fashion with the log of the percent P_{FR} established. These results are summarized in figure 3A, where each point represents the mean value of the points shown in figure 2.

Table I. *The Effect of Dark, R, and RF Pretreatments on the Phytochrome Content of* Pisum *Hypocotyl Tissue*

	Phytochrome content[1]		
	Dark pretreatment	RF pretreatment	R pretreatment
		$\Delta(\Delta$ OD) $\times 10^3$	
	59.0	45.0	9.5
	66.0	48.5	10.3
	55.7	43.5	12.0
	53.0	45.5	11.0
	64.0		
	58.0		
Means ± SE	59.3 ± 2.0	45.6 ± 1.0	10.7 ± 0.5
Percent	100	77	18
Relative P_{FR} units[2] for photostationary states of:			
5 %	5	4	1
20 %	20	15	4
44 %	44	34	8
88 %	88	68	16

[1] Each value for phytochrome content represents the mean of 3 determinations on the same sample. Samples of 10 segments, 10 mm in length packed to 1 cm sample thickness in 6-mm diameter aluminum cuvettes.

[2] Relative P_{FR} units obtained by setting the phytochrome content of dark controls to 100, so that an assumed full conversion of this to P_{FR} would give 100 units.

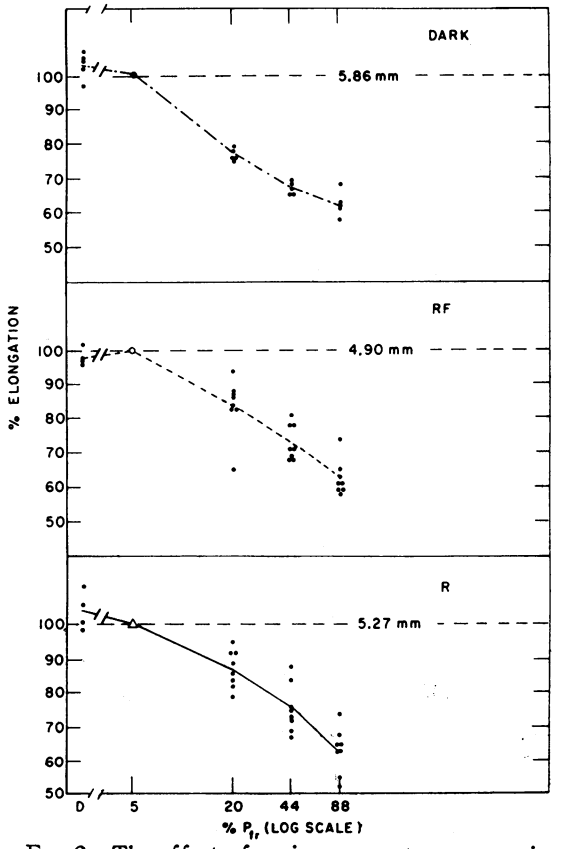

FIG. 2. The effect of various percentage conversions of phytochrome to P_{FR} on the elongation of segments from Dark, R, and RF pretreated plants. Each point represents the mean of 30 or 40 segments, initially 10 mm in length. The various % P_{FR} levels were established by 10 minutes exposure through appropriate filters (9). Elongation measured after 20 hours of growth.

In figure 3A it is clear that the segments from R and RF pretreated plants responded to various percentages of phytochrome conversion to P_{FR} in the same way, even though the RF pretreated plants contained approximately 4 times as much phyto-

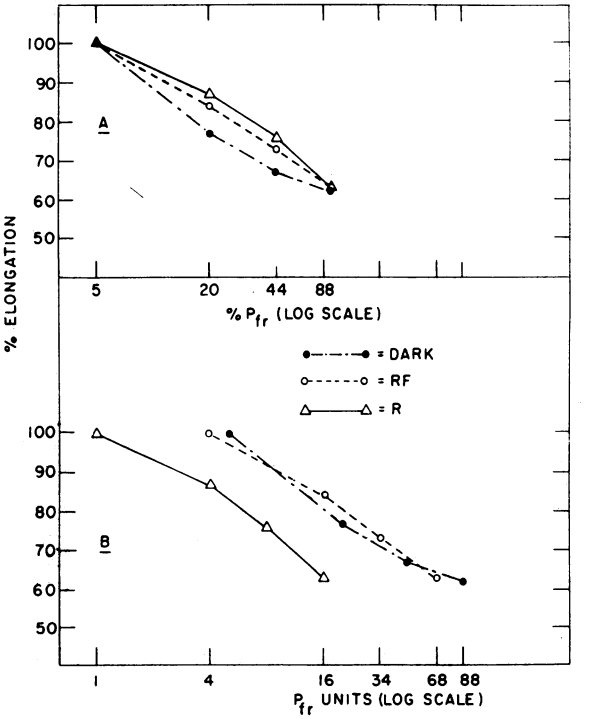

FIG. 3. The effect of various % P_{FR} levels (A) and relative P_{FR} units (B) on the elongation of segments from Dark, R, and RF pretreated plants. Each point represents the mean of the appropriate points in figure 2. For method of determining relative P_{FR} units, see text and table I.

chrome as did the R pretreated plants. However, in figure 3B the data are plotted in terms of relative P_{FR} units, as described in table I. In this plot the results for segments from R pretreated plants are now quite separate from those for the D and RF pretreated material.

Discussion

Since, for sections from any one group of pretreated plants, there appears to be a linear relationship between elongation and the log of the percentage P_{FR} established (fig 2), one might conclude that the amount of P_{FR} initially present governed the degree of growth inhibition. Such correlations have been reported previously (*e.g.*, 11, 12). However, when the growth inhibiting effects of increasing percentages of P_{FR} are compared among groups, it is clear that sections from morphologically similar (R and RF pretreated) plants responded in the same way (fig 3A), even though there was a 4-fold difference in the amount of spectrophotometrically detectable phytochrome (table I). In figure 3B the data are plotted in terms of relative P_{FR} units, so as to account for the difference in phytochrome content between R and RF pretreated plants. Here again, it appears that elongation is not regulated by the absolute amount of P_{FR} produced, but rather by the proportion of P_{FR} produced relative to the total phytochrome present. This contention is supported by the fact that the maximum inhibition observed, with an initial P_{FR} level of 88 % was the same (*ca.* 35 %) regardless of phytochrome content, and by the similarity of the slopes of the plots (fig 3A). Because of the variability in growth of pea stem segments, a large number of experiments would be required to ascertain whether or not the slight differences between segments from etiolated and de-etiolated plants are significant (fig 3A).

Apparently there is no discernible relationship between the amount of phytochrome detected spectrophotometrically and that which is physiologically active, at least in this system. These results call to mind explanations proposed for several phytochrome "paradoxes" (10). One such paradox, the *Pisum* paradox, involves pea stem segments which respond to far-red light in the absence of spectrophotometrically detectable P_{FR} (9). Another paradox, in *Zea*, involves a response to red light which is saturated by an undetectably small percentage of P_{FR}, yet is reversible by far-red filters establishing detectable amounts of P_{FR} (1, 4). The first paradox has also been observed for *Phaseolus* hypocotyl hook opening (6, 12). The concept of "bulk" and "active" phytochrome fractions has been invoked to explain these paradoxes. Bulk phytochrome represents that phytochrome which is spectrophotometrically detectable but physiologically inert, while the active fraction is present in such a small proportion of the total that no information concerning it can be obtained spectrophotometrically. To explain the present results, the only further assumption required is that the photostationary state illuminations affect both bulk and active fractions the same way.

In conclusion, an attempt to determine rigorously whether or not physiological effects are due to P_{FR} concentration, in some sense, or to its proportion of the total phytochrome present, seems to lead to the conclusion that the latter and not the former is the controlling factor. Considering these experiments alone, one would reject the widely-accepted idea that P_{FR} is the only active form of phytochrome, and propose instead a mechanism in which the P_{FR}/P_R ratio was significant because both forms are active and perhaps compete. Some such ratio mechanism, however, would still fail to explain the paradoxes discussed earlier. The bulk-and-active hypothesis remains the only one fully consistent, if uninformatively so, with all the data so far accumulated.

Acknowledgments

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