The Reversible Inhibition by Red and Far-Red Light of Auxin-Induced Lateral Root Initiation in Isolated Pea Roots [1, 2]

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Introduction

Lateral root formation induced in cultured isolated roots by decapitation can be inhibited by a single short exposure to white light and red light inhibits lateral root formation much more effectively than blue or green light (10). With the development of knowledge about phytochrome, a photomorphogenetically active red/far-red pigment system (3, 7), it became of interest to know whether the phytochrome system acts in the light inhibition of lateral root formation. To investigate such a possibility, the present work was carried out using an assay based on IAA-induced root initiation in isolated pea root segments grown aseptically in nutrient culture.

The results obtained show that the inhibition of lateral root formation is induced by low-intensity red light, and can be reversed by subsequent treatment with far-red light; this reversibility can be repeated numerous times by successive red and far-red irradiation. Since this assay is based on the initiation of cell division in the root pericycle, the results suggest the interesting possibility that the phytochrome system may be involved in some way in auxin-induced mitotic activity in these roots.

Materials and Methods

The Lateral Root Initiation Assay. The assay was based on work with excised pea root segments described in earlier papers (11, 12). Seeds of *Pisum sativum*, var. Alaska, were surface-sterilized by soaking with 0.1 % mercuric bichloride solution for 20 minutes, and rinsed repeatedly with sterile water. Seeds imbibing water enough to grow were placed aseptically into petri dishes and kept in the dark. Sterile 10-mm root tips of 3-day-old etiolated seedlings were excised aseptically under low-intensity green light and transferred to a modified Bonner nutrient agar medium in 10 cm petri dishes with 4 tips per dish. The composition of the nutrient medium was as described earlier (12). Root tips, grown in the dark for 7 days at 25°, reached an average length of 50 to 60 mm and were ready then for use in the assay. The terminal 4-mm tip of each root was excised and then 4 additional 6-mm-long segments were cut from each root with a scalpel with replaceable blade. For convenience in description these 5 pieces were designated segments I, II, III, IV and V from tip to base along the root axis. Since it became evident that the response to light and the capacity for lateral root initiation differed along the axis of the root, only segments of 1 type were inoculated in each vial. Sterile vials (2 cm in diameter, and 5 cm in height) with cheesecloth-covered cotton plugs were used for the assay. Into each vial was pipetted aseptically 2 ml of melted Bonner medium to which had been added aseptically a freshly prepared solution of IAA to give a final concentration of 5×10^{-5} M and sufficient sterile 0.01 N NaOH to raise the final pH of the medium to pH 6.5. Ten or 12 segments were transferred to each vial, under a dim green light. Then, low-intensity red light was given to the plant materials for various periods of time. In some experiments successive light treatments were given. After the light treatment the series of vials was placed in a polyethylene bag in the dark at 25° for 1 week. Then, the segments were removed from each vial and were placed in a formalin-acetic acid-alcohol fixative solution for 2 days. The numbers of lateral root primordia in the cleared segments were determined under a dissecting microscope with transmitted light and the data were treated statistically.

The safelight used in these studies was a 15-w cool white fluorescent tube covered with 2 layers of Roscolene No. 9/40 green plastic sheets with the surface masked to allow only about a 4 square inch lamp surface irradiation which was reflected from the wall of the sterile transfer room. The low-intensity red light for all treatments except for the experiment in table I was from an incandescent source filtered with interference filters and Corning filters to give a maximum peak at 660 mμ. The far-red irradiation was a fairly pure far-red source obtained from an incandescent lamp with interference filters. Blue light (415–560 mμ) was obtained from white fluorescent lamps filtered with Roscolene blue plastic filters.

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Table I. *Effect of Low-Intensity Red Light on Lateral Root Initiation in Segments Taken from Intact Seedling Roots (10 Segments per Treatment)*

Segment tested	Dose of red light* (min)	Average number of laterals per segment	Analysis of variance (4)
II	0	5.4	
	¼	3.8	
	½	4.6	
	1	4.6	
	2	3.9	$F = 1.34 < F_{1\%} = 2.82$ No significant difference
	4	4.8	
	8	6.4	
	16	6.0	
	32	4.8	
III	0	4.1	
	¼	2.9	
	½	4.5	
	1	2.5	
	2	4.9	$F = 2.19 < F_{1\%} = 2.80$ No significant difference
	4	4.0	
	8	5.8	
	16	4.5	
	32	5.1	

* Incident energy of red light (Sylvania F30 T8-R fluorescent tube) to the surface of the test materials was 42-kilo-ergs cm^{-2} min^{-1}.

The different light sources were generously provided through the cooperation of Bruce A. Bonner and Malcolm B. Wilkins. In light treatments, the incident energies are indicated for each of the different experiments.

Statistical methods followed Edwards (4).

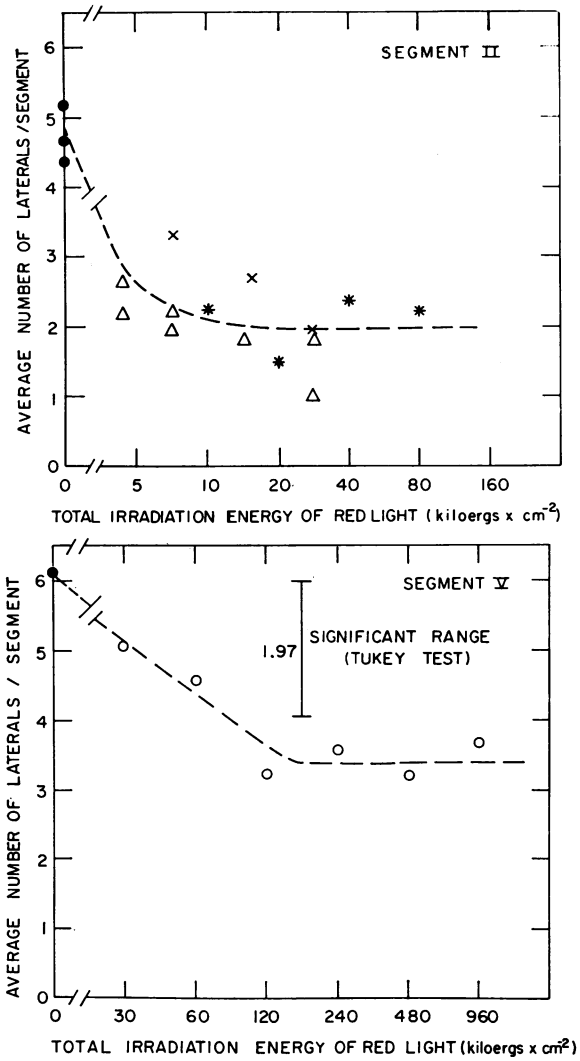
Results

Effect of Low-Intensity Red Light on Lateral Root Formation. Earlier experiments (10) showed that continuous illumination or repeated exposure to white fluorescent light was necessary to prevent lateral root initiation in roots with an attached cotyledon, while with isolated roots grown in culture, a single short exposure to such light was effective in inhibiting lateral root development. A similar relation with respect to the effect of low-intensity red light on lateral root formation was found in the present experiments between root segments taken from roots of intact seedlings and those from cultures of excised root tips.

Experiments were first performed to test the effect of low-intensity red light on lateral root initiation in segments I to IV excised from main roots of 3-day-old etiolated pea seedlings. The treatments and results obtained with segments II and III are summarized in table I; the data show no significant difference between any experimental groups in terms of the analysis of variance. Similar results were obtained with segments I and IV. There was no sign of an inhibitory effect of the red light.

However, when experiments were conducted with segments II, III, IV and V taken from 1-week-old cultures of excised roots, low-intensity red light was found to inhibit markedly lateral root initiation in every segment tested. Dose-response curves for segments II and V are shown in figures 1 and 2 respectively, and a similar effect of red light was observed in segments III and IV. Maximum inhibition was found to be reached at about 40 % of the dark control in segment II and about 55 % in segment V, and additional periods of exposure to low-intensity red light had no further effect in causing inhibition of the process of root initiation. It also became clear that the amount of red light energy producing a maximum effect was dependent upon the portion of root from which experimental materials were provided; that is, the younger (the closer to the tip along the axis) the segment, the less energy of red light required to cause a saturation of inhibition. For example, 10 kiloergs cm^{-2} of incident energy of red light clearly caused a significant inhibition in segment II (fig 1) but not in segment V (fig 2).

Reversal by Far-Red Light of the Inhibition Induced by Red Light. Since the low-intensity red light was found to cause a marked inhibition of lateral root formation in isolated root segments, further experiments were carried out to see whether the red light-induced inhibition was reversed by far-red light given immediately after the red light irradiation and whether far-red light itself had some effect on lateral root initiation in dark-grown root segments. The relation between dosage of far-red light and lateral root initiation in root segments III with and without prior exposure to red light at an exposure



FIGS. 1, 2. The effect of red irradiation dosage on lateral root initiation in segments II and V, respectively. Intensity of red light used in separate experiments: Figure 1: ×, 1 kiloerg cm^{-2} min^{-1}; △, 1.8; *, 5.25. Figure 2: 30 kiloergs cm^{-2} min^{-1}. Tukey test of significance according to Edwards (4).

producing maximum inhibition is shown in figure 3. It was evident that far-red light alone did not affect the present assay system if given for a limited period of time, but that far-red irradiation given immediately after the red treatment reversed the inhibition induced by red light. In the case of segment III, an irradiation for 1 minute of 120 kiloergs cm^{-2} of far-red light was enough to cause a complete reversal of the red inhibition.

Using saturation dosages of red light (100 kiloergs cm^{-2}) and of far-red light (200 kiloergs cm^{-2}) derived from the data given above, segments II, III, IV, and V were tested to see how many times such reversibility could be observed in the present assay system. The results with segment III are presented in figure 4, which shows that the reversal could be achieved by repeatedly successive red and far-red irradiations. The total amount of light energy given to the plant tissue does not appear to be at all important in controlling the response. Similar results were obtained with the other segments tested. From this reversibility by red and far-red irradiation, it is evident that the phytochrome system can mediate lateral root initiation in the present assay system.

The Effect of Blue Light on Lateral Root Formation. In previous work (10) it was reported that blue light was relatively ineffective in causing inhibition of lateral root initiation in isolated roots. This observation was confirmed in the present work, and the result is summarized in table II. When a single, brief period of illumination with blue light was given to isolated segments cut from cultured roots, there was no significant effect. But continuous illumination with blue light during the period of auxin treatment for a week largely prevented root initiation by the segments.

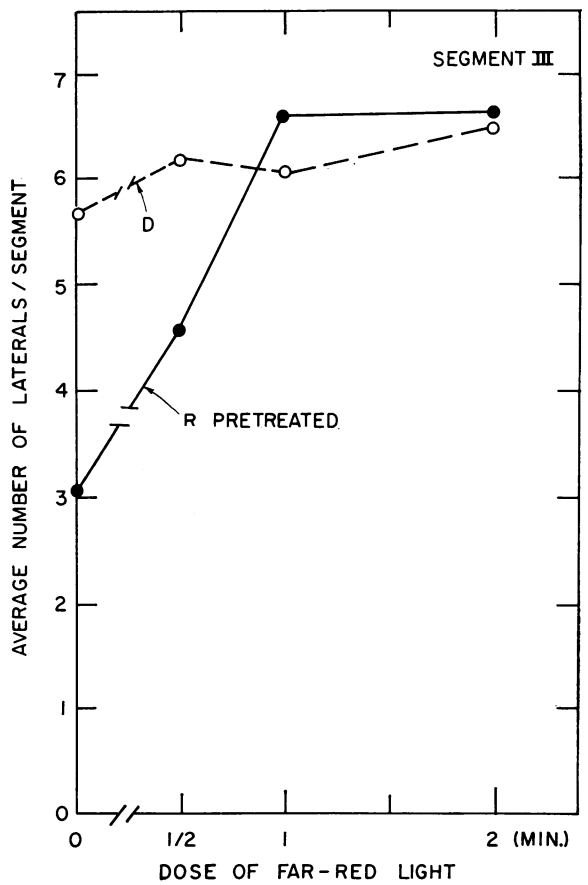


FIG. 3. The effect of far-red irradiation dosage on lateral root initiation in segment III with (R pretreated) and without (D) preirradiation with red light for 5 minutes. Intensity of red light was 42 kiloergs cm^{-2} min^{-1}; intensity of far-red light was 120 kiloergs cm^{-2} min^{-1}.

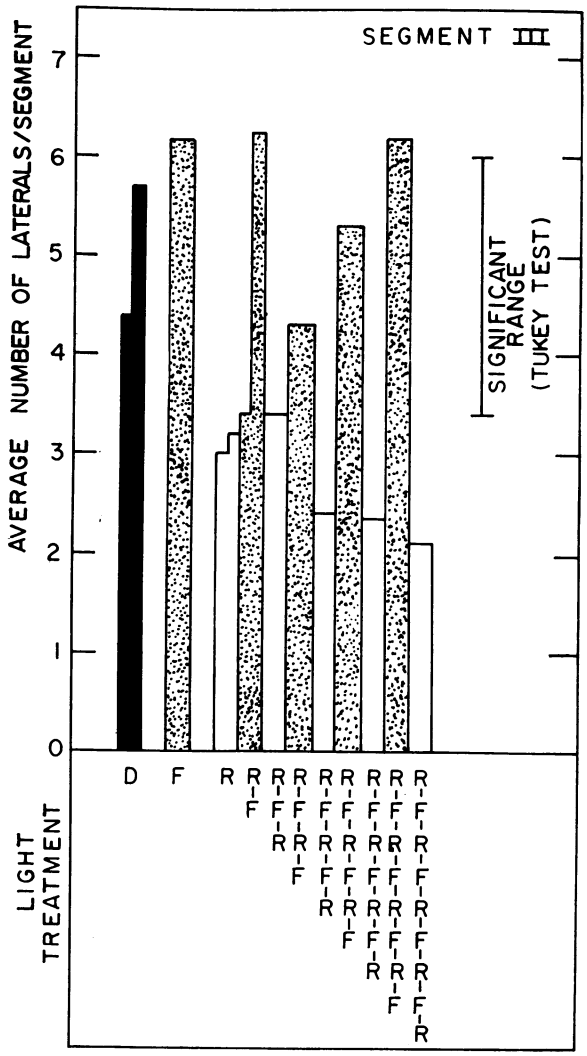


FIG. 4. Far-red reversal of red-light-induced inhibition of lateral root formation in segment III. Intensity of red light (R) was 100 kiloergs cm^{-2} min^{-1} given for 1 min; intensity of far-red light (F) was 200 kiloergs cm^{-2} min^{-1} given for 2 min. D is dark control. Tukey test according to Edwards (4). Double peaks represent data from different experiments not pooled for statistical analysis.

Discussion

The data presented here give strong evidence that under appropriate experimental conditions the phytochrome system is probably involved in some way in the process of auxin-dependent lateral root formation. A dosage range of 10 to 100 kiloergs cm^{-2} of red light to saturate completely the photoinhibition of lateral root formation appears to be comparable to that for other phytochrome-dependent phenomena in pea plants, namely, 10 kiloergs cm^{-2} (5) or 30 kiloergs cm^{-2} (8) for photoinduction of leaf growth, 50 kiloergs cm^{-2} for biosynthesis of a flavonoid complex (5), and 60 kiloergs cm^{-2} for photoinhibition of stem section growth (1).

Table II. *Effect of Blue Light on Auxin-Induced Lateral Root Formation in Isolated Segments*

Segment tested	Dose of blue light* (min)	Average number of laterals per segment
IV	0	5.8
	1	5.2
	2	7.1
	4	5.5
	8	4.6
	Continuous for 7 days	1.6
V	0	6.2
	1	5.8
	2	5.9
	4	6.4
	8	4.5
	Continuous for 7 days	1.9

* Incident energy of blue light (415–560 mμ) at the surface of the test materials was 21 kiloergs cm^{-2} min^{-1}.

Although a study of the detection of phytochrome in vivo in pea roots was not included in the present paper, some interesting observations on this point have been made (Furuya and Hillman, unpublished[4]) and are worth noting. When root segments of intact etiolated pea seedlings were examined using differential spectrophotometry (3), the phytochrome was easily detected optically in the root, where the concentration of the pigment was highest in the tip segments and decreased along the root axis. However, when excised 10-mm root tips cultured aseptically on modified Bonner medium in the dark were examined, the reversible optical density difference of phytochrome decreased day after day during culture and, in consequence, 7-day-old isolated roots, from which segments for the present assay were obtained, did not give any optical [△ (△ OD)] value. Thus, during the period of culture in vitro there appeared to be a progressive disappearance of phytochrome in the root tissues such that, with present methods, no optically detectable phytochrome could be demonstrated in the assay segments. These facts seem not to be consistent with the physiological results presented in this paper, where segments taken from intact roots did not respond to low-intensity red light at all (table I), but those from week-old isolated root-tips were really quite sensitive to red and far-red light (fig 4). Briggs and Siegelman (2) showed that in dark-grown seedlings of several different genera, the measurable phytochrome per unit weight of tissue declined rapidly during the first 6 days of seedling development. Lane et al. (6) found that for certain species attempts to demonstrate the presence of phytochrome failed, although there was

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strong physiological evidence of presence of the pigment.

Although it has been shown that the photoinhibition of lateral root formation may be controlled by the phytochrome system, there still remains the possibility that a high-intensity light system, in addition to a low-intensity red and far-red light-sensitive system, might be involved in the process of lateral root formation. The reason for considering this possibility is that high-intensity white light gave rise in earlier work (10) to a strong inhibition (up to 90 % of the dark control) and in the present work continuous blue-light irradiation caused 72 % inhibition in segment IV and 69 % in segment V (table II). In contrast, the maximum effect of a single dose of low-intensity red light was 60 % inhibition in segment II and 44 % in segment V (fig 1, 2). These results would permit the interpretation that a high-intensity light effect (7) may swamp that produced by low-intensity light.

It has long been known that the formation of lateral branches on the main root of *Pisum* is controlled by, and dependent upon, auxin (9). The assay system in the present work is based on this auxin-dependent lateral root initiation (11, 12). However, no clear-cut evidence has so far been given which establishes a relationship between auxin action and the phytochrome system. The auxin-induced lateral root initiation observed in the present study is dependent not only on auxin but also on other chemical constituents in the nutrient medium which, under properly controlled conditions, can become limiting for root initiation (11). The system offers an opportunity to explore chemical processes associated either directly or indirectly with a phytochrome-sensitive step which leads to a specific morphological expression. Further work in this direction seems warranted.

Summary

Lateral root initiation was induced by auxin treatment of isolated segments from seedling roots of pea (*Pisum sativum*) or from roots grown in sterile culture 1 week. Low-intensity red light had no significant effect on root initiation in segments from intact seedling roots but caused marked inhibition of initiation in segments cut from isolated roots grown in culture. The sensitivity to red light inhibition decreased along the root axis from tip toward the base. A single exposure of 10 to 20 kiloergs cm^{-2} of incident energy of red light caused a maximum inhibition equal to about 40 % of the dark control in a segment 4 to 10 mm behind the tip. The red light inhibition was reversed completely by far-red irradiation (120 kiloergs cm^{-2}). The inhibition by red and reversal by far-red irradiation was achieved repeatedly with successive treatments of the same segments. Blue light at low intensity was without effect for short periods of time (up to 8 min) but continuous exposure to blue light for the duration of the test (7 days) resulted in marked inhibition of lateral root initiation. The evidence for a red/far-red light reversible control of lateral root initiation following auxin stimulation seems clearly established.

Acknowledgments

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