PHOTOREVERSIBILITY OF LEAF AND HYPOCOTYL ELONGATION OF DARK GROWN RED KIDNEY BEAN SEEDLINGS[1,2]

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Many of the light-controlled responses of plants are known to be most effectively regulated by radiation in the red region of the spectrum. Among these are such diverse phenomena as initiation of flowers in photoperiodically sensitive plants (7), germination of light-sensitive seeds (3), pigmentation of the tomato fruit cuticle (9), and growth of parts of dark-grown seedlings (1, 5, 6, 8).

In 1952 Borthwick et al (3) found that far-red radiation near 7350 Å given after a treatment with red reversed the promotive action of the red on germination of light-sensitive lettuce seeds and thus left the seeds in a non-germinating condition. Far-red was also found to reverse the action of red in the control of flower initiation in short-day plants (2, 5) and pigmentation of the tomato fruit cuticle (9). More recently Liverman et al (6) showed that the red reaction inducing expansion of bean leaf discs was reversed by far-red, and Withrow (11) demonstrated the far-red reversal of the red reaction that leads to the straightening of the plumular hook of excised bean hypocotyls.

The photoreaction has been shown to be repeatedly reversible. A succession of alternate red and far-red irradiations on light-sensitive seeds (3, 4) showed that germination depended upon the quality of the energy last received. That is, when the seeds received red energy last, they germinated, but when they received far-red energy last, they did not germinate. The red action for the control of pigmentation of the tomato fruit cuticle (9), flower initiation of cocklebur (5), and straightening of the plumular hook of excised bean hypocotyls (11) has also been shown to be repeatedly reversible by far-red. Such results indicate that the actions of red and of far-red are the reversal of a single reaction.

The purpose here is to demonstrate the existence of the same photoreversible reaction in controlling elongation of leaves and hypocotyl of intact dark-grown seedlings of Red Kidney beans and to investigate some of the details of the response.

Methods and Results

Dark Red Kidney beans were treated with Arasan and planted in well-drained redwood flats filled with white quartz sand moistened with a nutrient solution. Two parallel grooves of constant depth were made with a template in the sand in all flats. Individually weighed beans, weighing 0.4 to 0.6 gm, were placed about 2 cm apart in the grooves with their long axes vertical and raphes lowermost. The beans were then carefully covered with 3 cm of sand and each flat was moistened with additional nutrient solution. Immediately after planting, all flats were moved to a completely dark room where the temperature was $27 \pm 1°$ C. The illumination necessary for adding the nutrient solution was provided by a 3-cell flashlight equipped with a blue-green filter shown by tests to have no measureable effect on the seedlings. Irradiation treatments were given on the sixth, seventh, and eighth days, at which times the untreated plants were 90, 142, and 205 mm long. The plants were harvested on the tenth day after planting.

Preliminary Observations: When beans were grown in complete darkness, they exhibited the typical aspects of extreme etiolation. After 10 days of growth the hypocotyls were very long and the epicotyls very short. The petioles of the primary leaves had failed to elongate and the leaf blades had neither expanded nor unfolded. The plumular hook, which is first evident in the hypocotyl, was almost invariably located in the first internode just below the petioles of the primary leaves. The seedlings showed no visible chlorophyll. When dark-grown seedlings were exposed briefly to radiant energy from an incandescent-filament lamp on the sixth, seventh and eighth days after planting, elongation was inhibited in the hypocotyl and was promoted in the epicotyl and leaf petioles, and the primary leaves were induced to unfold and expand. The hook was in the petioles or in the leaf blades or it had disappeared entirely. The location of the hook seemed to depend upon the energy; disappearing more rapidly as the energy was increased. Elongation of the hypocotyl varied inversely with the energy and the length of the primary leaf blades increased directly with increasing energy (table I).

Reversibility: Red energy equivalent to about 15×10^5 ergs cm^{-2} min^{-1} at 6500 Å, the region of maximum effectiveness (10), was isolated from a bank of standard cool white fluorescent lamps (1000 fc) by a filter of two layers of red cellophane. Although the red cellophane filter was capable of transmitting far-red radiation, the emittance of the fluorescent lamps in the region between 7200 and 7600 Å was too low (less than 2 %) to measurably interfere with the consequences of the red action. This spectral region was very effective in promoting the germination of light-sensitive seeds (3, 10) and in inducing pigmentation of tomato fruit cuticles (9). Preliminary experiments with dark-grown beans showed that exposure to this radiation for 2 minutes on the sixth, the seventh, and the eighth days was sufficient to induce a 38 % inhibition of hypocotyl elongation and to cause leaf length to be 2.5 times that of the dark control. On the tenth day after planting these beans

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TABLE I

HYPOCOTYL AND LEAF LENGTHS OF DARK-GROWN RED KIDNEY BEAN SEEDLINGS EXPOSED 5 MIN TO VARIOUS ENERGIES FROM UNFILTERED INCANDESCENT-FILAMENT LAMPS

RELATIVE ENERGY	NO. OF SEEDLINGS	HYPOCOTYL LENGTH	LEAF LENGTH
fcm		*mm*	*mm*
500	18	225 ± 3 *	43 ± 0.6
250	19	225 ± 2	40 ± 1.0
125	19	232 ± 2	31 ± 1.0
60	20	239 ± 2	28 ± 0.8
30	22	247 ± 3	25 ± 0.6
15	23	249 ± 2	23 ± 0.4
7	22	257 ± 2	23 ± 0.6
0	40 **	297 ± 2	16 ± 0.2

* Standard error.
** Two lots of seedlings.

normally have hypocotyls about 100 mm long under greenhouse conditions and about 300 mm long in complete darkness. Exposures to red energy resulted in a hypocotyl length of about 225 mm, equivalent to an inhibition of about 75 mm of the possible 200 mm obtainable with natural light, or about 38 %.

Far-red energy, equivalent to about 2×10^5 ergs cm^{-2} min^{-1} at 7350 Å, the region of maximum effectiveness (10), was isolated from an incandescent-filament lamp by a filter composed of 2 layers of red and 2 layers of dark-blue DuPont cellophane. Subsequent tests with dark-grown seedlings showed that far-red isolated in this way from three 300-watt, incandescent-filament, reflector-flood lamps would reverse the effect of a brief red irradiation (fig 1). The far-red radiation repromoted hypocotyl elongation and reversed the red-induced potential elongation of the epicotyl, petioles and leaf blades. Far-red also reversed the red-induced potential straightening or disappearance of the plumular hook. An energy-response study of the far-red action was made by irradiating with various energies of far-red immediately following 2-min exposure to red. A saturation effect was obtained with exposures in excess of 4 min; additional two-fold increases in exposure time did not produce measurable increases in the response of the seedlings to far-red.

As in investigations with light-sensitive lettuce seeds (4), the questions arose as to whether the red and the far-red actions were also the reversal of a single reaction in the case of dark-grown seedlings. This was answered by the effects of several alternate exposures to red and far-red. Five lots of dark-grown beans were irradiated for 2 min with red and one lot was returned to darkness. The remaining 4 lots were immediately irradiated for 5 min with far-red and again one lot was returned to the dark. The 3 remaining lots then received another 2-min red irradiation and one lot was returned to the dark, and so on

FIG. 1 (*left*). Dark-grown Red Kidney bean seedlings after being kept in continuous darkness and after being given 2 min of red radiation, 2 min of red and 5 min of far-red, and 5 min of far-red only (from left to right).

FIG. 2 (*right*). Dark-grown Red Kidney bean seedlings that had received 8-min irradiation at the indicated wavelengths in Angstrom units, on the spectrograph immediately following a 2-min exposure to red radiation.

until all lots had been returned to darkness. A red irradiation immediately prior to returning the seedlings to darkness induced leaf elongation and inhibited hypocotyl elongation. If far-red was given just before the seedlings were returned to the dark, however, the immediate effect of previous irradiations was nullified and the seedlings retained the appearance of the dark controls (table II). There is a small but possibly real increase in red response with successive irradiations. Despite these increases, the possible reasons for which are not considered here, the reversing effects of far-red are fully apparent.

TIME INTERVAL BETWEEN RED AND FAR-RED: Investigations into the control of flower initiation of cocklebur showed that reversibility depended upon the time interval between the red and the far-red irradiation (5). The time required for loss of far-red repromotion of flower initiation was about 30 min. The time required for loss of far-red control of leaf and hypocotyl length of dark-grown bean seedlings was determined by irradiating seedlings with red radiation and allowing various durations of darkness to intervene before application of far-red. Red irradiations resulted in seedlings with hypocotyls about 74 mm shorter and leaves more than twice as long as the controls (fig 3). A given energy of far-red applied immediately after the red resulted in the production of hypocotyls equal in length to those of the dark controls, and in leaves only a few millimeters longer than those of the dark controls. If the far-red was applied 1 hour after the red, the effects on hypocotyl and leaf length were almost as great as when it was applied immediately. When far-red was given as late as 8 hours after the red irradiation, its effectiveness in repromoting hypocotyl elongation seemed to be about 60 % as great as when it was given immediately after the red and 65 % as great in reinhibiting the potential red promotion of leaf elongation. About half of this apparent loss, however, was due to the growth made during the 8-hr periods between the red and far-red irradiations.

THE ACTION SPECTRUM: The only complete action spectrum encompassing both the red and the far-red action is the one determined for the control of germination of light-sensitive seeds (3), but enough critical points were determined for the far-red repromotion of flower initiation of cocklebur to show that the far-red actions controlling flowering and seed germination had the same general region of maximum effectiveness (2). Spectrographic studies were, therefore, instituted to extend this information and to determine whether the red and far-red actions demonstrated by filter experiments to control development of dark-grown beans have the same general region of maximum effectiveness as those controlling the germination of light-sensitive seeds and the flower initiation of cocklebur (2).

TABLE II

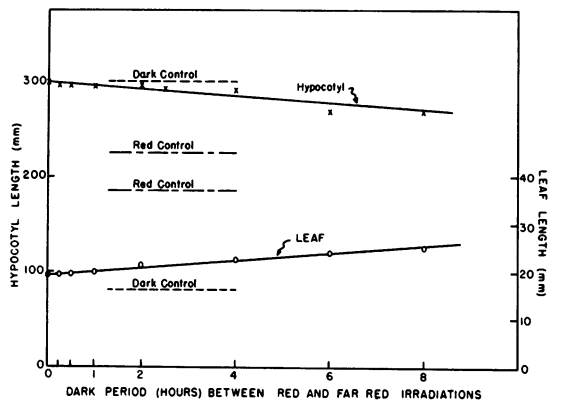
HYPOCOTYL AND LEAF LENGTHS OF DARK-GROWN RED KIDNEY BEAN SEEDLINGS EXPOSED ALTERNATELY TO 2 MIN OF RED AND 5 MIN OF FAR-RED

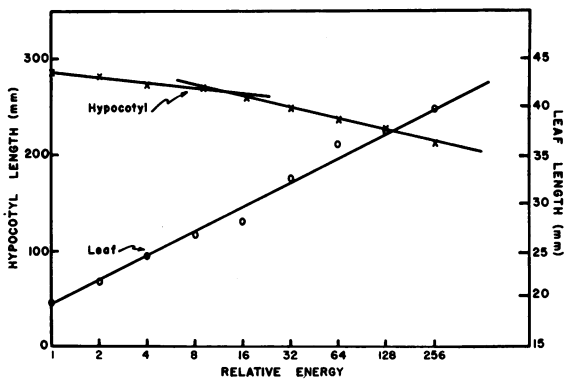
TREATMENT	NO. OF SEEDLINGS	HYPOCOTYL LENGTH	LEAF LENGTH
		mm	*mm*
Dark control	30	310 ± 2 *	17 ± 0.8
Red	29	230 ± 3	44 ± 0.8
Red, FR **	28	284 ± 2	21 ± 0.4
Red, FR, Red	28	222 ± 2	45 ± 0.2
Red, FR, Red, FR	31	287 ± 2	21 ± 0.4
Red, FR, Red, FR, Red	23	212 ± 3	46 ± 0.3

* Standard error.
** Far-red.

The spectrographic procedures were similar to those previously described by Parker et al (8) and Borthwick et al (1). Pertinent details are summarized here for immediate reference. The energy source of the spectrograph is the crater of a direct-current, high-intensity carbon arc lamp operated at 150 amperes and 100 volts. The slit of the spectrograph, which has an effective width of 50 Å at 5000 Å, is placed in a beam of radiant flux converging from condenser lenses and at one of the conjugate foci of the front-surfaced, concave mirror used as a focusing element. Two large glass prisms, set for a minimum deviation at 5780 Å are located in the beam converging from the concave mirror. The spectrum emerging from the prisms is reflected from a front-surfaced plane mirror that is adjustable in both horizontal and vertical direction. The spectrum produced in the focal plane of the instrument is about 10 cm broad with a dispersion of 16 Å/cm at 5000 Å and 75 Å/cm at 7500 Å. The visible part of the spectrum is spread over about 1.7 meters and contains not over 0.05 % stray flux. The variation in energy with wavelength was measured with a ten-junction thermopile calibrated with a National Bureau of Standards certified lamp. The exact positions of various wavelengths were determined from the emission spectra of barium and strontium.

Experimental technique already developed (1, 8) required that the spectrographic data be transformed by means of a reference slope or energy-response curve. This slope was obtained by measuring the response to various energies at several different wavelength regions. The slope, based on results at 6500 Å, was made linear by a plot of the response to the log of the relative incident energy (fig 4). Borthwick et al (1) found that the slope of the function was independent of wavelength but that it varied with the type of response. Therefore, separate curves had to be drawn for the hypocotyl and the leaf-length responses. Semi-log plots of these responses (fig 4) show a single linear response for leaf length and two linear functions for hypocotyl length over the range of energies used. It should be noted that such two-slope curves have been found also for leaf elongation of dark-grown peas (8) and for the elongation of the second internode of dark-grown barley (1). A two-slope curve is also obtained for leaf expansion of





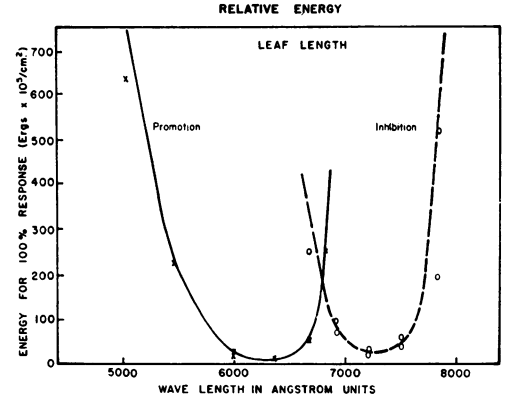


FIG. 3 (*top*). Apparent effect of various periods of darkness between red and far-red irradiations on the elongation of hypocotyl and leaf. Values for controls: hypocotyl length—dark 298 mm, red 224 mm; leaf length—dark 16 mm, red 37 mm.

FIG. 4 (*middle*). Semi-log plot of the promotion of leaf elongation and inhibition of hypocotyl elongation as a function of energy.

FIG. 5 (*bottom*). Action spectra for the promotion and reversal of promotion of leaf length of dark-grown bean seedlings.

beans when area or width is used for measurement of the response. Curves of this type are often indicative of two populations with different sensitivities to irradiation. The responses of leaf and hypocotyl to various energies of far-red result in curves of similar slope but in the opposite direction to their respective red responses.

To obtain data for construction of an energy-response curve, flats of dark-grown Red Kidney beans were placed across the spectrum from 4145 to 7100 Å and the responses to several irradiances were measured. Stations were 16.5 cm broad so that half of each flat constituted a station. The results, though preliminary, showed that the most effective region of the spectrum was in the red near 6500 Å. In order to define more precisely the portion of the red region that was most effective, the flats were divided into sections by cardboard partitions. This procedure kept the seedlings confined in a smaller spectral region and was of convenience in defining the wavelength stations. Flats of seedlings prepared in this manner were placed in the spectrum between 5830 and 7100 Å. In order to test for any promotive effect of the far-red, others were placed in the spectrum between 6497 and 8350 Å. Different lengths of hypocotyl and leaf were obtained at each station, each value resulting from a different energy. The energy required at each station to give the same length at all stations was then calculated by making use of response-log energy curve and the energy and leaf measurements for each station. The mathematical manipulations required for transformation of such data have been adequately described by Parker et al (8).

Investigations of the far-red effect were conducted along similar lines as those of the red effect. Flats of seedlings were again divided by cardboard partitions, but before they were placed across the spectrum they were irradiated with red of sufficient energy to induce more than a 100 % increase in leaf length. The flats were then placed across the spectrum between 6497 and 8350 Å (fig 2) for various periods. Leaf length values, transformed in the described manner (8), are presented as curves showing the promotive effects of radiation between 5000 and 6850 Å and the reversal effect between 6600 and 8000 Å (fig 5). On a relative energy scale the hypocotyl-elongation results would essentially be superimposed over those of leaf length. On an absolute energy scale hypocotyl and leaf length results would produce curves of similar shape with the same regions of maximum effectiveness. They would not be superimposed, however, because twice as much energy in the red region was required for the hypocotyl response as for the leaf length response. In the far-red the absolute energies necessary to produce the arbitrarily selected response limits for leaf and hypocotyl lengths were essentially the same.

DISCUSSION

Irradiation with red sets in motion a chain of reactions that, if not stopped by far-red or other means, will terminate in expression of the particular response investigated. Irradiation with far-red, if applied soon after the red, reverses the action of red in such a way that little or no measurable progress is made toward exhibition of the red response. Separation of red and far-red irradiations by various time intervals yields

evidence of the rate of decay of the products of the red treatment; that is, one can find the minimum period of time that permits the action to escape reversible control by far-red. For responses previously studied these times range from about 30 min for the complete loss of repromotion of flowering of cocklebur (5) to more than 8 hrs for a 50 % loss of regulation of cuticle color of tomato fruits (9) and germination of light-sensitive seed (4). Control of lengths of hypocotyl and leaves of dark-grown Red Kidney bean seedlings have time constants approximately the same as that for regulation of tomato cuticle color and for control of seed germination. In the light of our present knowledge the most logical explanation for the loss of far-red reversibility seems to be that a reaction subsequent to the red irradiation removes something from the system that is essential for the operation of the reversing action of far-red. If that substance were present in limited quantity and if replenishment of the supply were slow, its removal would cause a failure of reversal of the photoreaction, as illustrated by the 30-min time constant for repromotion of flower initiation in cocklebur. However, if the concentration of this essential substance were high or if it could be replenished as fast as it were removed, enough would remain available to allow at least partial control with far-red. This seems to be the case in the loss of far-red control of seed germination, tomato fruit cuticle coloration, and elongation of dark-grown Red Kidney bean seedlings.

At any rate, the close similarity of action spectra and the repeatedly photoreversible nature of the actions indicate that all of the responses tested to date are controlled by a common basic mechanism. The reaction controls flower initiation in photoperiodically sensitive plants, pigmentation of the cuticle of tomato fruits, germination of light-sensitive seed, and growth of parts of dark-grown seedlings. Since hypocotyl and leaf elongation are universally photo-controlled expressions of plant growth, it seems probable that this reversible photoreaction is of general occurrence.

Summary

1. Dark Red Kidney beans grown in complete darkness except for brief irradiations on the sixth, seventh and eighth days were harvested on the tenth day. Short exposures to radiant energy from unfiltered incandescent-filament lamps or to red radiation inhibited elongation of the hypocotyl, induced elongation of the leaves, and caused the plumular hook to straighten. When the red radiation was followed immediately by a short exposure to far-red radiation, the effects of the red were nullified and the seedlings retained the appearance of the dark controls.

2. Action of the red energy was repeatedly reversible by far-red, illustrating in part that the red and the far-red action were the reversal of a single reaction.

3. Separation of red and far-red irradiations by various time intervals gave a measure of the rate of decay of the products of the red treatment. The ability to repromote flower initiation in Xanthium with far-red was completely lost if the far-red radiation was not applied within 30 minutes after the red interruption of the inductive dark period. The apparent loss of far-red control of hypocotyl and leaf elongation of dark-grown bean seedlings was quite slow in comparison; about 40 % after an 8-hour time lapse. The real loss, however, was only about 20 % since half of the apparent loss was due to the growth that took place during the interval between the red and far-red irradiations.

4. An action spectrum was determined for both the red and the far-red action. The regions of maximum effectiveness for regulation of length of both hypocotyl and leaf were about 6400 Å for the red action and 7300 Å for the opposing far-red action. These wavelength regions of maximum effectiveness are thus essentially the same as those previously determined for the control of flower initiation and germination of light-sensitive seed.

5. Similarity of action spectra for several plant responses and the reversible features of the photoreaction are evidence of a common basic mechanism controlling these responses. The fact that such universal responses as hypocotyl and leaf elongation are controlled by this same photoreversible reaction suggests that the basic mechanism is of rather general occurrence.

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INTERACTION OF TEMPERATURE AND LIGHT IN GERMINATION OF SEEDS[1]

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Fully imbibed, viable seeds of many plants may fail to germinate. One limitation on germination may be a light requirement which can be removed by the correct poising of a reversible photoreaction (1, 2, 8). This reaction which can be repeatedly reversed by alternate exposures to red and far-red radiation also controls photoperiodic flowering responses, etiolation, bulbing, and a number of other growth responses (5). That the photocontrol is ubiquitous in all higher plants is shown by the etiolation response of seedlings (6).

The temperature, too, must be in a favorable range for seed germination which, for many kinds of seeds, is well below that best suited for subsequent growth of the seedling. A change in temperature is often effective in promoting germination. For limited periods, the temperature may greatly exceed the favorable constant range. A response to a change in temperature is also evident in vernalization, tillering, and dormancies of plant buds (9) as well as in diapause of animals. It is a phenomenon with broad significance.

Germination requirements are examined here with reference to the results of a few simple physiological experiments involving the effects of light and temperature conditions and to some extent the effects of nitrate solutions. The results suggest a pattern for the control of the germination process as well as for the growth of all organisms that must follow the temperature of the environment.

MATERIALS AND METHODS

The experiments reported here were chiefly with seeds of *Lepidium virginicum* L. collected from plants grown in the greenhouse. Other seeds were obtained from various sources and all were held in dry storage at − 18° C or + 5° C. The seeds were placed in Petri dishes on two layers of standard germination blotters saturated with water or 0.2 % KNO_3 solution. The dishes were immediately covered with two or more layers of black cloth throughout all tests except when irradiations were in progress. That the cloth excluded visible light was indicated by failure of exceedingly light-sensitive seeds to respond to visible radiant energy when so protected. Durations of germination experiments varied with the kinds of seeds and with the purposes of the experiments. They ranged mainly from 2 to 5 days after the final experimental variable was introduced. Seeds with emerged radicles were counted as germinated.

Radiation sources were incandescent and standard cool white fluorescent lamps. The incandescent-filament lamp, with a filter of two layers of red and two layers of dark-blue cellophane, either with or without a supplemental water filter, was an effective source of far-red radiant energy in the region of wavelengths greater than 7000 Å for inhibiting germination. When provided with a two-layer red cellophane filter to eliminate the blue part of the visible spectrum the fluorescent lamp, because of its relatively low emission between 7000 and 8500 Å, was an adequate source of red radiant energy in the region of 5800 to 7000 Å for promotion of germination. The sources used each gave about 0.3 milliwatts/cm^2 power in the wavelength regions of maximum effectiveness. Blue cellophane filters have been found to fade when subjected to the full intensity of 300-watt flood lamps at a distance of a foot or 18 inches, but only after several hundred hours of use. Care was accordingly exercised to replace them before significant change in transmission occurred.

During germination tests in seed germination cabinets, temperatures were usually controlled within ± 1° C. Some of the experiments involved several different constant temperatures; others made use of daily temperature alternations, an 8-hour period at one temperature being followed by 16 hours at a lower temperature. In still other experiments, a single change was made from the temperature employed during imbibition to one used for a short period or during the remainder of the germination period. Exposures to radiant energy and in some cases applications of KNO_3 or other solutions were made concurrently with, before, or after the temperature change.

In all experiments with *Lepidium virginicum* L. and *Nicotiana tabacum* L. four lots of 100 seeds each were used for each treatment. Results for *Fragaria virginiana* Duchesne and *Verbascum thapsus* L. in table II were based on single lots of 100 seeds and those used for other species on two lots each. Control lots of seeds were held in darkness in all experiments and under special conditions in some experiments as required by their particular designs. All seeds of *L. virginicum* in the dark control lots almost invariably failed to germinate.

[1] Received June 15, 1955.