Lettuce Seed Germination: Evidence for a Reversible Light-Induced Increase in Growth Potential and for Phytochrome Mediation of the Low Temperature Effect [1]

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Photoblastic germination in lettuce has been very extensively studied (7, 17). The process is phytochrome-mediated, that is, brief irradiances with red light (R) promote subsequent germination, and the red effect is repeatedly reversible by immediate reirradiation with far-red light (F). Some lots of freshly harvested seed of the variety Grand Rapids germinate little or not at all when imbibed in the dark at 26°. Dark germination is somewhat higher at lower temperatures, and increases slowly with time during storage of the dry seeds (6, 19, 21). Promotion by low temperature is thought to be independent of phytochrome (13).

The mature lettuce achene consists of an embryo closely invested with a 2-cell-layer endosperm which is in turn surrounded by the fruit coat (2). Under ordinary conditions dark dormancy depends on the structural integrity of the endosperm alone. If this layer is removed, the radicle and, subsequently, the hypocotyl will elongate in continuous darkness, after R, and after F, and the growth rate is the same for all 3 cases (12). It has been proposed (12) that the promotive effect of R is due to a weakening of the mechanical resistance of the endosperm to embryo expansion, presumably through secretion of cytolytic enzymes by the embryo.

Dark germination is promoted by gibberellic acid (10, 14) and kinetin (e.g., 11, 18) at relatively low, and by thiourea (20) at relatively high concentrations. It has been suggested that promotion by gibberellic acid and kinetin is effected via different mechanisms, neither of which is the same as that for promotion by R (11).

The purpose of the present investigation was to reexamine the action of light and chemical germination promoters on the growth potential of the embryonic axis, and to elucidate the germination-promoting effect of low temperature.

Materials and Methods

Lettuce (*Lactuca sativa*, L.) seed of the variety Grand Rapids, Lot 1132, 1962 crop, and Lot 163R18, 1964 crop, was obtained from the Pieters-Wheeler Seed Company, Gilroy, California, and stored in the laboratory under conditions of ambient temperature, humidity, and illumination. Some experiments were performed with seed of the variety Great Lakes, R-200, Lot 257A11, 1963 crop, obtained from the same source.

The R source was 2 Sylvania 40-w warm white fluorescent tubes wrapped with 3 layers of heavy red cellulose acetate and placed at a distance of 20 cm from the illumination plane. The light from two 150-w internal reflector floodlamps at a distance of 33 cm from the illumination plane, filtered through 3 cm of water and 2 layers each of blue (Rosco #138) and red (Rosco #17) gelatine filters (obtained from the Bates Lighting Company, Hollywood, California), served as the F source. Exposure times to R and F were 10 minutes and 5 minutes respectively, representing approximately 10 times the saturation dosage for all reported effects.

Irradiations, and all dark incubations at 20°, were carried out in a darkroom maintained at 19 to 21°. Incubations at other temperatures were carried out in temperature-controlled darkrooms of the Earhart Plant Research Laboratory, where the temperature was maintained at a constant ± 0.5°.

A large part of the experiments was performed with half-seeds, containing the embryonic axis and a small portion of the cotyledons, which were obtained by slicing the dry seeds across their longitudinal axes with a Gillette thin razor blade at a point approximately 0.4 seed-lengths from the radicle end. The growth of the axes of half-seeds is in no way mechanically restricted by the endosperm. Further, elongation of the radicle proceeds at about the same rate as in the germinating intact seed, indicating little or no effect of the operation on its vigor.

Germination tests with intact seeds were carried out as follows: 100 seeds were placed in a 9-cm petri dish containing 1 disc of filter paper and 2 ml of water and removed immediately to a darkroom at either 20° or 25°. Light treatments were given 2 hours later. The sample size was 300 to 600 seeds, and the incubation time was 3 days.

Imbibition of half-seeds in an osmoticum was carried out in 0.46 M mannitol. The imbibing medium for seeds operated in any way always contained streptomycin sulfate at a concentration of 100 mg/

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liter, to minimize bacterial contamination. The germination of intact and punctured seeds is unaffected by the antibiotic at this concentration. Half-seeds, in lots of 50, were placed to imbibe on 2 layers of 5.5-cm Whatman No. 1 filter paper pressed into a 5-cm petri dish and wetted with 2 ml of liquid. Where mannitol solutions were used, the dishes were immediately sealed in transparent plastic bags containing wet paper towelling, and removed to the darkroom. Light treatments were given 2 hours after the start of imbibition, except where stated otherwise. The appearance of a positive geotropic curvature in the radicle was used as a criterion that growth had taken place. The geotropic response was always accompanied by extrusion of the half-embryo from the surrounding layers.

Results

Germination Behavior. The relative light sensitivity of seeds of the variety Grand Rapids is known to vary widely from 1 sample to another, even in freshly harvested seed. In order to facilitate comparison of the results presented in this paper with those of other authors, the germination behavior of this variety, and that of the ordinarily nonphotosensitive variety Great Lakes, is illustrated in table I. At the temperatures employed, germination is maximal at about 48 hours, and does not increase with further incubation. The germination response of Grand Rapids, Lot 1132 at 25° characterizes this seed as highly dark-dormant. For the lower temperature, the reduction in germination of F-treated seeds relative to the dark controls is highly significant, and is usually observed in seed samples possessing any amount of dark-dormancy at this temperature.

Table I. *Germination Response of 2 Varieties of Lettuce at Different Temperatures*

Germination was determined after 3 days dark incubation following the light treatment. The sample size was 300 to 600 seeds.

Variety	Temperature	Light treatment	% Germination
Grand Rapids, Lot 1132	20°	R	99
		F	2
		None	17
Lot 1132	25°	R	96
		F	1
		None	1
Grand Rapids, Lot 163R18	19°	R	99
		F	21
		None	43
Lot 163R18	25°	R	93
		F	4
		None	14
Great Lakes	20°	R	100
		F	100
		None	100
	25°	R	99
		F	100
		None	100

Experiments Using Half-Seeds. It has been proposed that light-induced germination is effected via a weakening of the mechanical resistance of the endosperm, and that radicle growth and germination are not the same phenomenon (12, 13). Evidence to the contrary has been gained in experiments using the half-seed system, and is presented below.

The results of an experiment illustrating the differential effects of R and F on the growth potential of the half-seed are depicted in figure 1. The graph shows an R-induced acceleration of growth resulting in a 2-hour gain over the F-treated material.

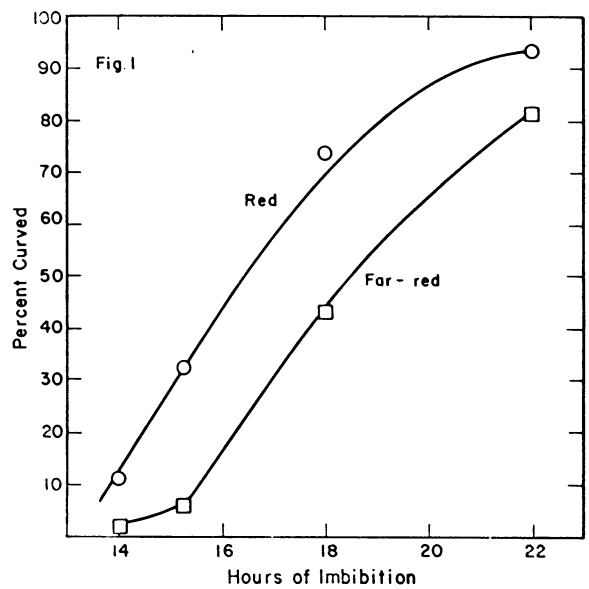

FIG. 1. Rate of appearance of curvature in Grand Rapids, lot 1132, half-seeds following R or F, at 20°. Ordinate: percentage curved. Abscissa: imbibition time. The sample size was 2 to 4 lots of 50 half-seeds.

This relatively small but clear difference may be greatly amplified by including an osmotically active solute in the imbibition medium. D-mannitol is suitable for this purpose. The experiment of figure 1 was repeated, using 0.46 M mannitol, and including dark controls. The results are shown in figure 2. While the imposition of a water stress somewhat delays the appearance of curvature, the difference between the R and F-treated material becomes much greater. Indeed, the latter appears to attain a maximum of only about 25% curved. The position of the dark control curve between the curves for R and F-treated material points up the similarity of the half-seed response to that of the germination response in the intact seed (table I).

The results of a similar experiment employing half-seeds of the nonphotosensitive variety Great

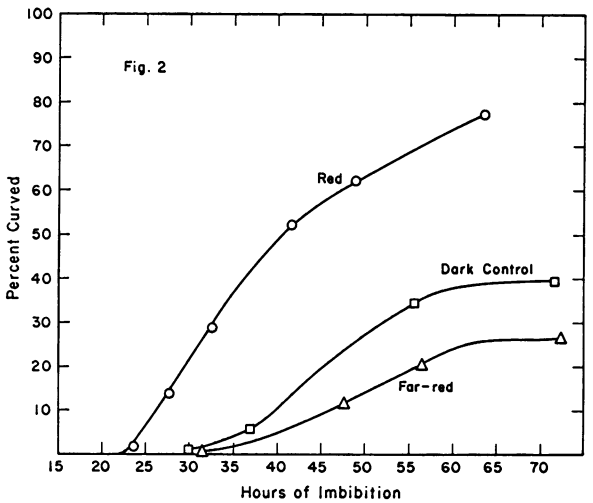

FIG. 2. Rate of appearance of curvature in Grand Rapids, lot 163R18, half-seeds in 0.46 M mannitol at 20° following R or F. The sample size was 10 lots of 50 half-seeds.

Lakes show a detectable response (fig 3). However, the response in Grand Rapids is roughly 28 times as great, by the criterion of difference in time to attain the 10 and 20 % response levels. The 2 varieties were further compared in their response to continuous R (fig 4). The ambient temperature for this experiment was 21°, but the temperature of the seeds was probably closer to 24°, owing to their proximity to the light source. A comparison of figure 3 and 4 suggests that continuous R depresses the rate of response in Grand Rapids to a single brief illumination, possibly by means of photoinduced loss of physiologically active pigment.

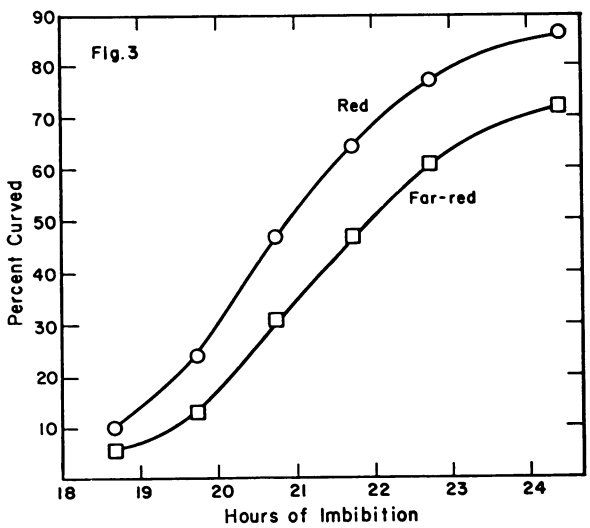

FIG. 3. Rate of appearance of curvature in Great Lakes half-seeds in 0.46 M mannitol at 20° following R or F. The sample size was 12 lots of 50 half-seeds.

Non-participation of the Endosperm. While available evidence (9, cf 16) points to the embryonic axis as locus of the light-responsive mechanism, the possibility remains that the endosperm in the region of the axis is directly or indirectly involved in photosensitive germination in some way other than, or in addition to, imposing a mechanical restriction on that process. For example, the possibility that the endosperm may secrete growth inhibitors has been advanced (22). While mechanical restriction by the endosperm has been eliminated in the half seed, the possibility remains that the light requirement depends

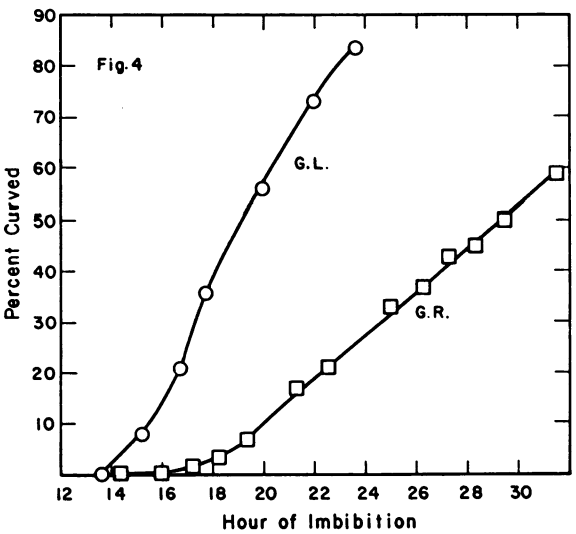

FIG. 4. Rate of appearance of curvature in half-seeds of Great Lakes and Grand Rapids, lot 1132, in continuous R, 0.46 M mannitol, at 21°. The sample size was 12 lots of 50 half-seeds.

on the presence of the endosperm. The endosperm could be involved directly in at least 2 ways: A) R could induce destruction, or prevention of synthesis or release of an inhibitor within this tissue; B) R could potentiate the ability of the embryonic axis to inactivate such an inhibitor.

To test this possibility, half-seeds were imbibed 1.5 hours in darkness in mannitol in the usual way. The half-embryo was then removed from all surrounding layers by gentle pressure on the radicle end, washed 3 times in mannitol, and transferred to another petri dish. F or R was then administered, and the dishes were returned to darkness for an additional 29.5 hours. Controls were subjected to identical treatment except for decoating and washing. The results appear in table II. It may be seen that the light response does not depend on the presence of the endosperm. Clearly, the inhibitor hypothesis broached above is untenable.

Effect of Germination Promoters. Dark germination of photosensitive lettuce seeds has been shown to be promoted by gibberellic acid and kinetin,

Table II. *Effect of Endosperm Removal on Growth of Half-Seeds of Grand Rapids, Lot 1132*

The endosperm was removed after 1.5 hours of imbibition, then light was given. The incubation time was 33.5 hours in 0.46 M mannitol, at 21°.

Treatment	No. curved half-seeds	% Curved
Endosperm absent:		
F	0, 1	1
R	47, 45	92
Endosperm present:		
F	1, 3	4
R	39, 38	77

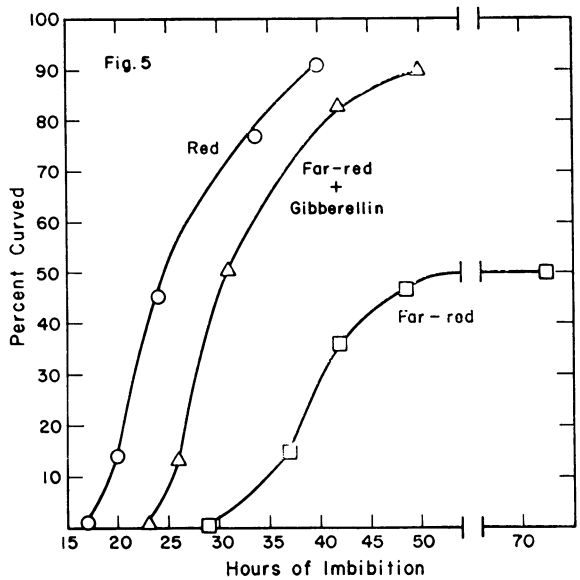

FIG. 5. Rate of appearance of curvature in Grand Rapids, lot 1132, half-seeds in 0.46 M mannitol at 20° following R and F ± gibberellic acid. The sample size was 12 lots of 50 half-seeds.

Hence it was thought to be of interest to examine the effectiveness of these compounds in the half-seed system. Half-seeds were imbibed in 0.46 M mannitol containing 10 mg/liter gibberellic acid (GA_3), then given F at the usual time. The clear-cut promotion by GA_3 is similar in magnitude to that induced by R, but is delayed by at least 6 hours (fig 5). This delay is probably not attributable to a suboptimal concentration of GA_3, since the germination response of punctured seeds to the hormone is saturated at about .03 mg/liter (table III). Rather, the delay may be interpreted as evidence that the gibberellin effect is mechanistically independent of the action of phytochrome.

Kinetin has been claimed to be both with and without effect on the germination of photosensitive seeds (18). Ikuma and Thimann (11) observed slight stimulation of dark germination by kinetin alone, and a strong stimulation in the presence of a suboptimal concentration of gibberellic acid. They further showed that kinetin markedly promotes expansion of the cotyledons and attributed the observed promotive effects on germination to this phenomenon.

Since the concentration of gibberellin required for promotion of dark germination may be reduced roughly 4 orders of magnitude through the simple expedient of puncturing the seed (table III), or 100-fold by injection under the endosperm (10), it was thought that the reason for conflicting reports on the effectiveness of kinetin might be difficulty of passage of this compound through the endosperm and into the embryo. A germination test using punctured seeds was performed in the following manner: dry seeds were pierced through the middle with a thin pin at a point about midway between the seed ends. One hundred punctured seeds were then sown in the usual way in water or kinetin solutions at 2°

Table III. *Effect of Puncturing the Seed on Response to GA_3*

Seed samples (180) of Grand Rapids, lot 1132, incubated 66 hours at 20°. Five minutes F given at second hour of imbibition.

(GA_3), mg/liter	% Germination Punctured	Intact
0	5	5
0.001	10	...
0.003	20	...
0.01	67	...
0.03	96	...
0.10	96	...
1	...	19
10	...	19
25	...	29
50	...	39
100	...	77
200	...	95

Table IV. *Effect of Kinetin on 60-Hour Germination of Punctured Seeds Following F*

The temperature was 20°. Seeds (300) per sample of Grand Rapids, lot 1132, were used.

Imbibition fluid	Typical germination %	Atypical germination %	Total germination %
Water	21	0	21
Kinetin, $2 \cdot 10^{-5}$ M	34	43	77
Kinetin, $5 \cdot 10^{-5}$ M	29	55	84

10^{-5} and $5 \cdot 10^{-5}$ M and placed in the dark at 20°. Two hours later, a 5-minute dose of F was administered. The plates were then returned to the dark for 58 hours. The results, presented in table IV, indicate a very substantial promotion by kinetin in the absence of any other promoting factors. Atypical germination is defined as protrusion of the embryo through the cotyledonary end of the endosperm and surrounding layers (12). Most of the promotion due to kinetin is obtained via this mode of germination (table IV).

The results of an experiment designed to detect a stimulatory action of kinetin in the half-seed system are presented in table V, and show that the only effect of kinetin here is an inhibitory one. The conclusion that the germination-promoting action of kinetin is effected through the cotyledons and not the embryonic axis (11) is thus supported.

Table V. *Rate of Appearance of Curvature in Half-Seeds of Grand Rapids Lot 1132, in 0.46 M Mannitol ± $3 \cdot 10^{-5}$ M Kinetin*

Five minutes of far-red light was given at the second hour of imbibition. The sample size was 2 lots of 50 half-seeds.

Hr from start of imbibition	% Half-seeds showing curvature	
	Mannitol alone	Mannitol + kinetin
29	0	0
36.5	25	24
42.5	52	35

Temperature-Dependent Dark Germination. Whereas the dark germination of photosensitive lettuce seed as 26° is generally close to nil, substantial germination percentages may be achieved at lower temperatures. The relative response to low temperature varies from lot to lot, and increases with increasing age of the seed. The time course of dark germination at different temperatures was investigated to determine more fully the scope of this interesting response.

Seeds were sown at room temperature, 200 per dish, in a 9-cm petri dish containing 2 layers of filter paper and 3 ml of water. The dishes were sealed in plastic bags, placed in foil-covered trays, and removed immediately to a darkroom maintained at the appropriate temperature. Five-dish samples were removed periodically and the germination percentage determined. The results are presented in figure 6. Maximum germination at all temperatures save 11° seems to be attained by the second day. The relatively high variability of the values for 15° and 19° is not due to insufficient sample sizes, but can probably be attributed to variations in rate of attainment of temperature equilibrium amongst the individual dishes, especially considering the fact that at the next higher temperature (23°) there is practically no germination. The experiment was repeated for the 15° and 19° conditions, using sample sizes of 4000 seeds and taking the precaution of sowing in temperature-equilibrated petri dishes directly in the appropriate darkroom. Germination at the third day was approximately 24 % at both temperatures, with no significant difference between the two.

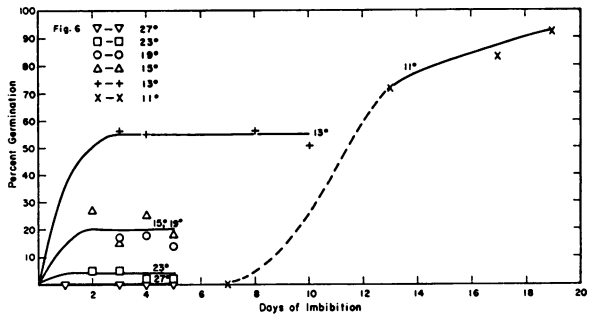

FIG. 6. Time course of dark germination of Grand Rapids, lot 1132, at different temperatures. The sample size was 1000 seeds.

Evidence for Phytochrome Mediation of the Low Temperature Effect. Dark-imbibed lettuce seeds probably always contain a certain proportion of F-absorbing phytochrome (P_f), since germination is usually reduced by exposure to F (see table I). In addition, it has been established (15) that a small but significant fraction of the phytochrome is present as P_f, even after equilibrium dosages of F of high spectral purity. It was considered that the promotive

Table VI. *Germination of Grand Rapids, Lot 1132, after 17 Days at 11° Following High Temperature and Light Treatments, as Described in the Text*

Hr at 37°	Controls	% Germination	
		Second dose F	No second dose F
0 (Dark control)	82.7	...	...
0 (Single F control)	32.3	...	...
2		56.3	2.7
4		59.3	2.1
6		36.1	0.4
8		19.6	0.7
10		11.9	0.4
20		1.9	0.0

effect of low temperature on dark germination might be in part or wholly due to retardation of dark transformation of this P_f residue.

This hypothesis was tested in the following manner. Seeds were imbibed in the usual way for 2 hours at 20°, then given a saturating dose of F to lower the ratio P_f/P_r to a constant value. The plates were set inside plastic bags in foil-covered trays and placed in a room kept at 37°. Trays were removed after 2, 4, 6, 8, 10 and 20 hours, cooled briefly in a 5° room, then removed to a darkroom where half of each set was given a second saturating dosage of F, while the other half remained in the dark. Immediately after this, the dishes were placed in an 11° darkroom for 17 days, after which germination percentages were determined. Each sample comprised 5 dishes, each of which contained 200 seeds. Dark and F-treated controls received no 37° treatment but were placed at 11° directly after illumination. The results are presented in table VI. As before, germination in the dark controls was very high, while that in the F-treated controls was lower, but still substantial. For the seeds not receiving a second dose of F following the heat treatment, a 2-hour heating seems sufficient to reduce subsequent germination to a minimum (the difference between the 2 and 4-hour samples is not statistically significant). Further reduction of germination by heat treatments exceeding 4 hours is probably a reflection of effects of high temperature other than that on P_f reversion.

The second dosage of F caused a reversal of the high temperature effect for all durations of heat treatment. The promotion by heat treatments up to 4 hours was not expected but may be due to acceleration of metabolic processes preceding germination. A less likely possibility is acceleration of the action of P_f itself. The main point is that F (which ordinarily reduces germination) promotes germination when administered under conditions in which phytochrome may be presumed to be exclusively or nearly so in the red-absorbing form.

It may be concluded that the germination-promoting effect of low temperature depends on the presence of P_f, and that this effect is most probably achieved through delay or prevention of transformation to an inactive form.

Discussion

The growth of the embryonic axis freed of mechanical restriction by the surrounding layers by various manipulations has been very thoroughly studied (12), and the axis was observed to elongate at the same rate after irradiation with light of either effective wavelength. This fact has, by default, given impetus to the notion that light-induced germination is effected via a weakening of the mechanical resistance of the endosperm, and that radicle growth and germination are not the same phenomenon (12, 13).

The contribution of P_f to germination may be envisaged as either A) an increase in embryo "push," or B) an increase in endosperm "give" (or both). The results presented here, which support the first hypothesis, constitute the only direct evidence available. While it may be argued that the acceleration obtained with R in water (fig 1) is small, or even that any acceleration observed does not necessarily mean that the intact embryo would be able to push harder against the endosperm, the situation obtaining in the osmoticum is more compelling. Here an external restraint against expansion is overcome under P_f, reminiscent of the situation in light-induced germination in the intact seed. Not only is the growth response of R-treated half-seeds greatly enhanced relative to the F-treated, but a qualitative difference is evinced in the failure of most of the latter to grow at all. The response to gibberellin is also very suggestive of that in the intact system, where the lag behind the R response has also been observed (12, unpublished data). The negative kinetin response, too, may be related to what happens in the intact seed. Most of the kinetin response of intact punctured seeds is expressed in atypical germination (table IV), which is probably a result of cotyledonary expansion and or secretion of cytolytic enzymes by the cotyledons (11).

The response to both R and GA_3 may be presumed to be restricted to the radicle, with no significant contribution from the hypocotyl, for 2 reasons. A) In normal expansion of the embryo in water, radicle extension precedes that of the hypocotyl by a very substantial time margin (12); B) the geotropic curvature observed is always positive, without even the slightest negative curvature in the region of the hypocotyl. Further, this response may be presumed to be peculiar to the in situ radicle proper, since neither R nor F elicit differential responses in the in vitro growth rate of excised roots obtained from seeds induced to germinate by R, even in the presence of mannitol (unpublished data).

The experiment of table VI has been adduced as evidenced that the low temperature effect is phytochrome-mediated; i.e., that the sole, or at least the principal, primary effect of low temperature is to delay dark reversion of P_f (and or loss of physiologically active pigment). F-irradiated seeds were used in this experiment for 2 reasons: A) to bring the P_f/P_r ratio in each seed to as constant a level as possible; B) to minimize the duration of heat treatment required for the effect. Because of the brief irradiations involved, F-treated seeds can be assumed to differ from nonirradiated seeds only in having a lower ratio of the 2 pigment forms.

The proposition advanced above suggests that germination might be substantially promoted at any physiologically reasonable temperature by a relatively small P_f/P_t ratio, provided the ratio is maintained for a period of sufficient duration. Kahn (14) has presented evidence for inhibition of germination

in continuous high-intensity F, which seems to indicate that the last proposition is untrue. However, 3 points must be considered regarding his experimental conditions. The first is that the experiment was carried out at ambient temperatures of 26° or 27°, which are supraoptimal for germination after irradiation with R. The temperature of the seeds may have been even higher, considering the intensity of the light source. The second is that although the P_f/P_r ratio may have remained constant, the absolute amount of P_f present may have been substantially decreased via light-induced thermal loss of photoreversible pigment (3). The third point is that the seeds were not returned to darkness at a more equitable temperature following continuous irradiation, which might possibly have demonstrated an actual promotion by the light regime. Another result presented in the same paper (14, table VIII) indicates a slight promotion, significant at the 10 % level, over continuous darkness by 3 brief, separated irradiations with F. A highly significant promotion would quite likely have been realized had thrice-irradiated seeds been compared with seeds receiving a single dosage of F (see Kahn's table VIII, and table I of this paper).

Toole and Cathey (21) subsequently studied the effect of continuous F at 25°, using a blue fluorescent light source, and also found only inhibition, even when the illumination period was followed by a dark incubation. However, while the light source must have emitted a very low F intensity, intensity in the blue was quite high, and might conceivably have contributed to loss of physiologically active photoreversible pigment (3, 4).

Recently, Downs (5) has reported maximum germination in continuous F of light-sensitive seeds of members of the *Bromeliaceae* that exhibit typical phytochrome-controlled germination. In view of this positive result, it may be expected that lettuce seed may be induced to germinate by repeated or continuous irradiance with F, given the proper conditions.

It is commonly assumed that P_f is the physiologically active form of the pigment and that P_r is inactive. This assumption is based largely on the argument that a small proportion of P_f evokes a large physiological response in certain materials (8). However, apparently 50 % pigment conversion is required for a half-maximal germination response in lettuce seed (8). Granting this, the possibility may be entertained that in lettuce seed P_r is the active form and functions as a suppressor of germination. The very existence of decay in photosensitivity with time of imbibition (1, 10, 11, 12) could well be attributed to some positive inhibitory action of P_r, especially considering the fact that the decay rate increases with temperature (13).

The data of table VI can be invoked as evidence against this unattractive hypothesis. Firstly, under the experimental conditions, a large response is obtained to what must be a relatively small proportion of P_f. In the second place, the substantial increase in response obtained with relatively short periods at an elevated temperature (provided the second dosage of F is given), while probably attributable to processes other than phytochrome activity, certainly cannot be the result of depression of a positive inhibitory activity of P_r (by irreversible thermal inactivation, for example). The reason for this is that the effect depends on the second irradiation, which would only serve to further decrease the P_r concentration, provided dark (thermal) reversion takes place in the usual direction. Further, and for the same reasons, the effect of low temperature cannot be ascribed to greater reduction of P_r inhibitory activity relative to reduction in the rates of other metabolic processes necessary for growth and germination.

Summary

With the aid of a new technique employing the radicle half of the seed of lettuce, variety Grand Rapids, irradiation with red light and treatment with gibberellin was shown to result in an increase in the growth potential of the embryonic axis of lettuce seeds. The differential effect of red and far-red light is evident in expansion in water, but is greatly magnified if the axes are allowed to expand in 0.46 M D-mannitol.

The results with red irradiation show directly that the contribution of phytochrome (far-red-absorbing form) to germination consists in an increase of the growth potential of the embryo which enables the latter to overcome the mechanical restraint against expansion imposed by the endosperm.

The response to gibberellic acid (GA_3) is probably achieved in a different manner than that to irradiation, but both appear to be confined to the radicle. The response is not elicited by kinetin.

The light response in the half-seed does not depend on the presence of the endosperm. This eliminates the possibility that a simple chemical inhibition of growth by this tissue is responsible for the light requirement.

The promotive effect of low temperature on germination is lost if cold incubation is preceded by a short exposure to high temperature. Far-red light, normally germination-inhibiting, reverses the action of high temperature, restoring the capacity for promotion by low temperature. The promotive effect of low temperature on dark germination is most probably a result of prevention or delay of transformation of physiologically active phytochrome to an inactive form.

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