Plant Physiol. (1974) 53, 114–117

Phytochrome and Seed Germination

VI. PHYTOCHROME AND TEMPERATURE INTERACTION IN THE CONTROL OF CUCUMBER SEED GERMINATION[1]

Received for publication June 6, 1973 and in revised form August 22, 1973

FRONE A. EISENSTADT[2] AND ALBERTO L. MANCINELLI
Department of Biological Sciences, Columbia University, New York, New York 10027

Downloaded from https://academic.oup.com/plphys/article/53/1/114/6073945 by guest on 16 August 2022

ABSTRACT

Phytochrome control of cucumber seed germination is temperature-dependent. A prolonged exposure to radiation from broad spectrum far red sources (Pfr/P = 0.05 to 0.07) prevents germination at temperatures below 20 C. Above 20 C there is no inhibition and it appears as if there is an escape from phytochrome control. However, radiation from a monochromatic, narrow band 730 nanometer source (Pfr/P < 0.02) inhibits germination at temperatures above 20 C. This result supports the idea that, even at high temperatures, Pfr is responsible for the activation of germination. After 4 days of exposure to far red, a short red irradiation is quite effective in promoting germination if temperatures during the dark incubation periods are maintained below 20 C; red becomes effective at temperatures above 20 C. Promotion of germination will take place at a temperature of 25 C or higher without red irradiation. Again, we have an apparent escape from phytochrome control at high temperatures. However, if higher temperatures are used for only short periods, 2 to 6 hours, in combination with short red irradiation, one can demonstrate that activation of germination at high temperatures is still dependent on phytochrome. Phytochrome is probably destroyed during prolonged exposure to far red. Thus, the subsequent short red irradiation establishes levels of Pfr which may not be sufficient to promote germination at low temperatures but are probably adequate at high temperatures.

Cucumber seeds are dark-germinating, light-inhibited seeds. Inhibition of germination requires prolonged exposure to light (6, 9) and can be brought about by blue, FR,[3] various combinations of R and FR, and white light from incandescent lamps (1). Seeds exposed to prolonged irradiation do not germinate during a dark incubation period following the light treatments, unless they are exposed to a short R irradiation or to increased temperatures (1, 6). Intermittent FR is almost as effective as continuous FR in preventing germination of cucumber and other seeds (4, 5–7). Germination is not inhibited if each short FR in an intermittent FR light treatment is immediately followed by a short R, proving that the action of prolonged FR is mediated through the phytochrome system. Prolonged exposure to FR is necessary to inhibit germination effectively because Pfr is continuously reappearing in the system. Its reappearance may be the result of *de novo* synthesis, slow rehydration of Pfr present in the unimbibed seed, or an inverse dark reversion of Pr to Pfr (2, 4, 5, 8). Nonimbibed seeds of cucumber contain phytochrome in the Pfr form (8). Reappearance of Pfr following FR irradiation has been measured spectrophotometrically in cucumber seeds (8).

The germination of cucumber seeds exposed for 4 or more days to an inhibitory light treatment is not promoted by a short red irradiation at temperatures below 20 C (1, 6). At 25 C, germination is promoted without exposure to R (1, 6). These high temperature results seem to show that phytochrome is no longer the factor responsible for the activation of germination and that at high temperatures there is an escape from phytochrome control. The purpose of the research reported here is to establish the relationship between phytochrome and temperature upon the activation of germination in cucumber seeds.

MATERIALS AND METHODS

Cucumber (*Cucumis sativus* L., cv Pixie) seeds were used in all experiments. The seeds were placed in glass Petri dishes which contained one disc of filter paper (Eaton-Dikeman Grade 923) and 10 cc of distilled water. At least four replicates of 40 seeds each were used for every treatment, and dark controls were included in all experiments. Germination was counted at the end of a prolonged light treatment, at the end of a subsequent dark incubation period, or both. Results are expressed as germination percentages. The average standard error was 5%. For dark incubation, the dishes were put into black bags made of double layers of heavy, darkroom-type satin cloth and kept in incubators at the indicated temperatures.

Prolonged light treatments were given in growth chambers (Percival model E-57) equipped with red or far red light sources or both. The growth chambers were set to the temperature indicated in the experiments, ±0.5 C. Light sources used for brief single irradiations were on a bench at room temperature (20 C). These light sources have been described in previous papers (3).

The irradiances, at seed level, of the sources used were as follows: (*a*) growth chamber FR, 600 μw cm^{-2}; (*b*) growth chamber R, 125 μw cm^{-2}; (*c*) bench FR, 2600 μw cm^{-2}; (*d*) bench R, 126 μw cm^{-2}. These values were obtained by multiplying the values measured with an IL-150 photometer (International Light Inc.) by the half-bandwidth of the filters used in the IL-150 for the R and FR regions.

[1] Research supported in part by Grants GB-14749 and GB-35460 from the National Science Foundation.

[2] Present address: Department of Physiology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

[3] Abbreviations: FR: far red; R: red; P: phytochrome; D: dark.

Continuous and cyclic light treatments were used: FR/D (15 sec/45 sec) indicates an intermittent light treatment consisting of 15 sec of FR every minute; R/FR/D (1 min/1 min/13 min) indicates an intermittent light treatment consisting of 1 min of R immediately followed by 1 min of FR every 15 min.

Measurements of phytochrome were done with a dual wavelength photometer (ASCO Ratiospect); wavelengths of the measuring beams were 730 and 800 nm.

RESULTS

Seeds which are exposed to either continuous or intermittent FR do not germinate (Table I). Those which are exposed to a cyclic light treatment in which each short FR is followed by R (FR/R/D) germinate quite well (Table I). Seeds which receive a cyclic treatment in which each FR is immediately preceded by a short R (R/FR/D) do not germinate, and inhibition under such a treatment is stronger than under continuous or cyclic FR (Table I and II).

Seeds exposed to inhibitory light treatments for several days fail to germinate or germinate poorly when transferred to darkness unless they are exposed to R (Table II). The cyclic (R/FR/D) treatment not only has a higher inhibitory efficiency than the other two but also causes a faster rate of phytochrome destruction than cyclic FR in various systems (Table III). With the spectrophotometric instrumentation available to us, it was not possible to measure rates of phytochrome destruction in cucumber seeds maintained under the various inhibitory light treatments. From results published previously (Table III of Ref. 6), we know that the total phytochrome content is lower after 4 days of exposure to a cyclic (R/FR/D) treatment than after 4 days of cyclic FR or continuous FR, but we do not know how much of this difference is due to destruction and how much is due to an inhibition of synthesis or of rehydration.

The inhibitory efficiency of the light treatments depends upon the temperature during exposure to light (Table IV). At temperatures above 20 C, germination is about 70% or higher. At temperatures of 15 and 18 C, germination is less than 10% and repromotion of germination is more difficult than when the light treatment is applied at 12 C (Table IV). Repromotion of germination by R after exposure to inhibitory light

Table I. *Action of Various Light Treatments upon the Germination of Cucumber Seeds*

Light Treatments	Germination at:	
	17.5 C	20 C
	%	%
Dark controls	98	99
4 days continuous FR	2	17
4 days (1 min FR/14 min D)	20	82
4 days (1min FR/1 min R/13 min D)	97	98
4 days (1 min R/1 min FR/13 min D)	1	11

Table II. *Action of Light upon the Germination of Cucumber Seeds*

Seeds were exposed for 2, 3, or 4 days to the following light treatments: continuous far red (FR), FR/D (2 min FR every 30 min), or R/FR/D (2 min R-2 min FR every 30 min). The seeds were then moved to darkness for 4 days with or without further irradiations with increasing doses of red light. Temperature throughout the experiment was 17.5 C. Germination was scored at the end of the dark incubation period. Mean germination value of the dark controls was 96%.

Red Dose	Germination at End of Dark Incubation after Indicated Light Treatments								
	2 days			3 days			4 days		
	FR	FR/D	R/FR/D	FR	FR/D	R/FR/D	FR	FR/D	R/FR/D
sec	%	%	%	%	%	%	%	%	%
0	52	61	36	20	13	8	9	19	5
5	72	89	25	20	31	16	6	22	1
10	88	93	78	31	55	22	16	33	3
20	94	91	86	62	79	50	38	58	10
40	96	96	90	73	85	52	57	52	14
80	97	94	94	92	89	53	48	53	9

Table III. *Action of Various Light Treatments upon Phytochrome Decay in Various Systems*

Light Treatments	Phytochrome Remaining					
	Avena coleoptiles	Corn coleoptiles	Corn mesocotyl	Cucumber cotyledons	Cucumber hypocotyls	Pea stem sections
	% of dark control					
3 hr continuous FR	...	...	...	82	...	...
3 hr (2 min R/2 min FR/26 min D)	...	...	...	69	...	...
4 hr (5 min FR/55 min D)	81	84	91	93	...	...
4 hr (5 min R/5 min FR/50 min D)	35	32	51	41	...	...
21 hr (5 min FR/55 min D)	...	...	...	...	81	86
21 hr (5 min R/5 min FR/50 min D)	...	...	...	...	44	40

Table IV. *Action of Temperature and Light upon the Germination of Cucumber Seeds*

Seeds were exposed for 4 days to a cyclic light treatment (LT, 18 sec FR every 60 sec) and then moved to darkness with or without further irradiations.

Temperature during LT	Germination at End of LT	Final Irradiation before 3 Days Dark	Germination at End of a 3-day Dark Incubation at Indicated Temperatures		
			15 C	17.5 C	20 C
C	%		%	%	%
12	0	None	10	80	95
12	0	2 min R	50	94	98
12	0	2 min R-2 min FR	20	78	92
12 (DC[1])	0		69	97	97
15	1	None	3	9	46
15	1	2 min R	4	69	94
15	1	2 min R-2 min FR	3	8	33
15 (DC)	77		74	96	97
18	5	None	6	7	15
18	5	2 min R	9	12	43
18	5	2 min R-2 min FR	9	7	9
18 (DC)	96		98	99	98
21	68	...	...	...	...
21 (DC)	95		...	...	...

[1] Dark control.

Downloaded from https://academic.oup.com/plphys/article/53/1/114/6073946 by guest on 16 August 2022

Table V. *Action of Light and Temperature upon Cucumber Germination*

The seeds were exposed for 2 or 4 days to continuous far red at 17.5 C, then moved to darkness for 4 days with or without additional light treatment. The temperatures during the dark period were 17.5 to 25 C. The mean germination value of the dark controls was 97%.

Light Treatments	Germination at the End of a 4-day Dark Period at the Indicated Temperatures			
	17.5 C	20 C	22.5 C	25 C
	%	%	%	%
2 days FR	63	95	98	99
2 days FR-5 min R	98	98	94	100
2 days FR-5 min R-5 min FR	51	88	98	100
4 days FR	5	12	36	97
4 days FR-5 min R	13	70	95	99
4 days FR-5 min R-5 min FR	5	8	26	96

Table VI. *Action of Broad Spectrum and Monochromatic FR Radiation upon the Germination of Cucumber Seeds*

Seeds were exposed to 2 days of continuous FR radiation. Germination was scored immediately after the light treatment.

Light Treatment	Temperature	Germination
	C	%
Broad spectrum FR source	22–23	74
Monochromatic source, 720 nm	23–25	67[1]
Monochromatic source, 730 nm	23–25	27[1]
Monochromatic source, 740 nm	23–25	72[1]
Dark controls	22–23	95

[1] Two replicates of 80 seeds each.

Table VII. *Action of Light and Short Periods at Various Temperatures upon the Germination of Cucumber Seeds*

The seeds were exposed for 4 days to continuous far red at 17.5 C, followed or not by further irradiation and/or 2 hr at various temperatures in darkness (HTD). Thereafter the seeds were kept in darkness for 4 days at 17.5 C. Germination was counted at the end of the dark incubation period. The mean germination value at the end of the 4-day light treatment was 3%, and for the dark controls, 98%.

Treatment after 4 Days of Continuous FR	Germination at the End of a 4-day Dark Incubation at 17.5 C after 2 hr HTD at Indicated Temperatures				
	17.5 C	20 C	22.5 C	25 C	30 C
	%	%	%	%	%
2 hr HTD-4 days D	7	4	9	5	11
5 min R-2 hr HTD-4 days D	28	25	18	46	69
5 min R-5 min FR-2 hr HTD-4 days D	8	6	7	11	8
5 min R-2 hr HTD-5 min FR-4 days D	8	13	9	6	14
2 hr HTD-5 min R-4 days D	...	29	22	18	54
2 hr HTD-5 min R-5 min FR-4 days D	...	8	7	11	9

treatment depends upon the temperature of the light treatment (Table IV) and that used during the dark incubation periods following these treatments (Tables IV and V). When temperatures during the dark incubation period are higher than 20 C, repromotion of germination is high (Tables IV and V). If a temperature of 25 C is used during the dark incubation, a short R irradiation is not necessary to repromote germination (Table V).

When high temperatures are used, either during the exposure to the inhibitory light treatment or during the dark incubation period following it, there seems to be an escape from phytochrome control. However, if instead of the wide spectrum FR source we use a monochromatic, 730 nm source (Corion interference filter, 16 nm bandwidth), we find that there is a considerable degree of inhibition at temperatures above 20 C. As determined with a solution of oat phytochrome, the photoequilibrium ratio, Pfr/P, is about 0.05 to 0.07 for the wide spectrum FR source and less than 0.02 for the 730 nm monochromatic source. Irradiation with monochromatic sources of

Table VIII. *Action of Light and Short Periods at Various Temperatures upon the Germination of Cucumber seeds*

The seeds were exposed for 4 days to continuous far red at 17.5 C followed or not by further irradiation in combination with 6 hours at various temperatures in darkness (HTD). Thereafter the seeds were kept in darkness for 4 days at 17.5 C. Germination was scored at the end of the dark incubation period. The mean germination value at the end of the 4-day light treatment was 6%, and for the dark controls, 96%.

Treatment after 4 days of Continuous FR	Germination at the End of a 4-day Dark Incubation at 17.5 C after 6 hr HTD at Indicated Temperatures				
	17.5 C	20 C	22.5 C	25 C	30 C
	%	%	%	%	%
6 hr HTD-4 days D	8	9	9	12	78
5 min R-6 hr HTD-4 days D	28	17	25	85	94
5 min R-5 min FR-6 hr HTD-4 days D	9	7	10	26	71
5 min R-6 hr HTD-5 min FR-4 days D	8	9	11	50	77
6 hr HTD-5 min R-4 days D	...	16	13	80	98
6 hr HTD-5 min R-5 min FR-4 days D	...	6	14	8	67

Table IX. *Action of Light and Temperature upon the Germination of Cucumber Seeds*

The seeds were exposed for 4 days to continuous far red light at 17.5 C, followed by irradiation with increasing doses of red, and then moved to dark at 17.5 C with or without a 2-hr period at 30 C directly after red irradiation. The mean germination value of the dark controls was 97%.

Treatment after 4 days Continuous FR	Germination at the End of a 4-day Dark Incubation at 17.5 C after 2 hr at Indicated Temperatures	
	17.5 C	30 C
	%	%
0 sec R	10	11
1 sec R	8	12
2 sec R	9	21
4 sec R	11	29
8 sec R	12	49
16 sec R	15	47
32 sec R	19	...
64 sec R	14	51
2 min R	15	63
4 min R	15	56
8 min R	16	56

Downloaded from https://academic.oup.com/plphys/article/53/1/114/6073946 by guest on 16 August 2022

720 and 740 nm is less effective in inhibiting germination than 730 nm irradiation (Table VI).

If the temperature during the dark period following the inhibitory light treatments is high for only a short time (2–6 hr), the necessity for Pfr in promoting germination is quite evident (Tables VII, VIII, and IX). This is demonstrated by the R-FR reversibility which occurs even at temperatures as high as 25 or 30 C. The efficiency of R depends upon the energy dose used.

DISCUSSION

Far red irradiation from a wide spectrum FR source effectively inhibits germination at temperatures below 20 C, but not at higher temperatures. The latter result can be interpreted as an escape from phytochrome control. However, the fact that monochromatic 730 nm radiation can effectively prevent germination at 23 to 25 C proves that phytochrome control is not lost when FR irradiations are given at temperatures above 20 C. Monochromatic, 730 nm radiation maintains a lower Pfr/Pr photoequilibrium ratio than does the wide spectrum FR source. Thus, the different inhibitory efficiency of these two treatments might be due to the different Pfr/P ratios maintained.

If the exposure to inhibitory light treatments at 17 to 18 C is prolonged for 4 days, a short R irradiation is not very effective in repromoting germination. A combination of short R irradiation and temperatures of 20 C or higher throughout the dark incubation period brings about germination. Short R and a temperature of 25 C for 6 hr at the start of the dark incubation period are also effective in repromoting germination. A temperature of 30 C for 6 hr during the dark incubation period will promote about 75 to 80% germination without the need of R irradiation. If the temperature during the inhibitory light treatment is 12 or 15 C, it is easier to repromote germination than after an inhibitory treatment at 18 C.

We submit that these results are brought about by the interdependence of the following factors: (*a*) the Pfr/P ratio maintained by the irradiation; (*b*) the rate of phytochrome destruction, which is temperature-dependent; (*c*) the rate of phytochrome-controlled reaction, which depends upon the concentration of Pfr, the concentration of the unknown substrate reacting with Pfr, and the temperature.

If less phytochrome is destroyed during an inhibitory irradiation at 12 C than at 18 C, the subsequent short R irradiation would result in a higher concentration of Pfr and a higher percentage germination after a prolonged irradiation at 12 C than after one at 18 C. The results of Table IV agree with this hypothesis.

At high temperatures the rate of the Pfr-activated reactions might be high even though low concentrations of Pfr exist. Therefore, we would expect that, given the same Pfr concentration, germination would be higher at higher temperatures; the results of Tables IV, V, VII, VIII, and IX agree with the above hypothesis.

On the basis of the results presented, we believe that the apparent escape of cucumber seed germination from phytochrome control is not real. At high temperatures the rate of the Pfr-dependent reactions is faster than at low temperatures and probably overrides the effect of phytochrome destruction. Thus high germination values occur even at low concentrations of Pfr.

LITERATURE CITED

1. EISENSTADT, F. A. 1973. Diverse aspects of phytochrome controlled seed germination. Ph.D. thesis. Columbia University, New York.
2. MALCOSTE, R., J. BOISARD, C. J. P. SPRUIT, AND P. ROLLIN. 1970. Phytochrome in seeds of some Cucurbitaceae: *in vivo* spectrophotometry. Meded. Landbouwhogesch. Wageningen 70(16): 1–16.
3. MANCINELLI, A. L. 1966. Broad-spectrum light sources, photoconversion of phytochrome and some physiological responses in tomato seed germination. Ann. Bot. 28: 675–686.
4. MANCINELLI, A. L. AND H. A. BORTHWICK. 1964. Photocontrol of germination and phytochrome reaction in dark germinating seeds of *Lactuca sativa*. Ann. Bot. 28: 9–24.
5. MANCINELLI, A. L., H. A. BORTHWICK, AND S. B. HENDRICKS. 1966. Phytochrome action in tomato seed germination. Bot. Gaz. 127: 1–5.
6. MANCINELLI, A. L. AND A. TOLKOWSKY. 1968. Phytochrome and seed germination. V. Changes of phytochrome content during the germination of cucumber seeds. Plant Physiol. 43: 489–494.
7. MANCINELLI, A. L., Z. YANIV, AND P. SMITH. 1967. Phytochrome and seed germination. I. Temperature dependence and relative Pfr levels in the germination of dark-germinating tomato seeds. Plant Physiol. 42: 333–337.
8. SPRUIT, C. J. B. AND A. L. MANCINELLI. 1969. Phytochrome in cucumber seeds. Planta 88: 303–310.
9. YANIV, Z., A. L. MANCINELLI, AND P. SMITH. 1967. Phytochrome and seed germination. III. Action of prolonged far red irradiation on the germination of tomato and cucumber seeds. Plant Physiol. 42: 1479–1482.

Downloaded from https://academic.oup.com/plphys/article/53/1/114/6073946 by guest on 16 August 2022