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Phytochrome and Seed Germination. III. Action of Prolonged Far Red Irradiation on the Germination of Tomato and Cucumber Seeds[1]

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Summary. Prolonged irradiation with continuous or intermittent far red prevents the germination of tomato and cucumber seeds. The inhibitory efficiency of intermittent far red decreases with the lengthening of the interval between successive irradiations, and with the increase of temperature. If each far red irradiation is followed by red, germination is restored. Intermittent far red is less inhibitory than intermittent red-far red when red is given immediately before each far red. This effect is more evident when the interval between successive irradiation becomes longer.

Evidence has been presented in previous reports that the active form (P_{FR}) of phytochrome (P) is one of the factors controlling the dark germination of lettuce (3) and tomato (4) seeds. It has been demonstrated that the germination response to prolonged far red (FR) irradiation in lettuce (3) and tomato (4), as well as the response to short FR irradiation in tomato (4, 5) is phytochrome controlled. Exposure to intermittent FR radiation or to alternate intermittent red (R) and FR radiations were used to demonstrate that the response to prolonged FR was actually a low energy-requiring, phytochrome controlled response (4) and not one depending on the high energy reaction system (HER) as it has been reported for other seeds (8).

In this paper we present further data on the action of prolonged FR irradiations on the germination of tomato and cucumber seeds.

Methods and Materials

The seeds used in this research were: tomato, varieties Ace and Porte, and cucumber varieties National Pickle and Pixie. The germination tests were run in Petri dishes containing a disc of filter paper (Eaton-Dikeman, grade 923) moistened with distilled water. During the dark incubation periods the dishes were enclosed in bags made with a double layer of heavy black satin cloth, and kept in incubators. Results reported are average of 2 replicates of 4 dishes each. Light treatments were given using red (R) and far red (FR) sources contained in growth chambers (modified Percival E-57). Spectral energy distribution of these sources were measured with a Model SR Spectroradiometer (ISCO) and are reported elsewhere (6). Dark controls (DC) were included in every experiment. Germinated seeds were counted 4 days after the start of the experiments. Times of incubation longer than 4 days do not modify the response obtained during such a period.

Results

Tomato, Exposure to Intermittent Radiation. When tomato seeds are exposed to intermittent FR radiation given in cycles of 30, 60, and 120 minutes for 4 days (tables I, II), germination is completely inhibited (germination $<5\%$) at the end of the fourth day, if the temperature is maintained at 20°. If the temperature is increased to 25°, the inhibition is less pronounced and depends on the length of the dark interval between successive irradiations: the longer the cycle, the higher the germination (tables I, II). If, during each cycle, R is given alternatively with FR, at different positions in the cycles, germination depends on cycle length, temperature, and relative position of R and FR in each cycle. The longer the interval between R and FR in each cycle, the higher the germination (table I, A).

In seeds exposed to the cyclic irradiations only during the first 24 hours of imbibition, terminating with an FR irradiation, and then placed in darkness for 3 days (table I, B), germination at the end of the incubation period is very similar to that obtained at the end of 4 days of prolonged exposure (table I, A). When, after 24 hours of exposure to cyclic irradiations, the seeds are placed in darkness after receiving a terminal R irradiation, germination is repromoted (table I, C). Comparison of germination obtained at the end of 4 days of exposure to intermittent FR and to intermittent R-FR, with R applied immediately before each FR, shows that, at 25°, the

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Table I. *Action of Cyclic R and FR Irradiation on Germination of Tomato Seeds*

A) Cyclic irradiation for 4 days. B) Cyclic irradiation for 24 hours, then 1 minute of FR and 3 days in darkness. C) Cyclic irradiation for 24 hours, then 1 minute of R and 3 days in darkness. ●,▲ 1 Minute of R and/or FR in the 30 minute cycles. ●,△ 2 Minute of R and/or FR in the 60 minute cycles. Germination of the dark controls: Ace, 87 to 88 % at 20° and 25°; Porte 85 to 90 % at 20° and 25°.

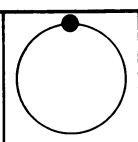
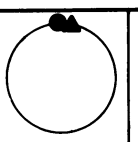
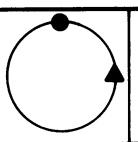
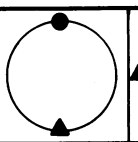
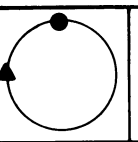
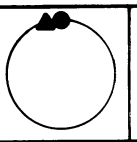
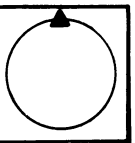
▲ RED ● FAR RED ——— DARK																
Temperature(°C)			20	25	20	25	20	25	20	25	20	25	20	25	20	25
ACE	30 min cycle	A	3	9	73	92	40	94	4	70	1	7	3	4	74	92
		B	6	14	65	89	45	84	12	63	4	11	7	9	83	87
		C	66	74	88	91	89	90	81	84	52	64	50	72	90	92
	60 min cycle	A	0	20	72	92	59	90	10	74	0	20	0	0	68	94
		B	10	18	78	86	56	85	20	74	7	22	6	10	66	87
		C	77	69	87	92	84	91	80	83	77	69	73	70	90	88
PORTE	30 min cycle	A	0	25	90	93	75	92	19	89	0	36	0	0	86	91
		B	4	38	90	93	78	82	39	88	7	51	5	37	71	92
		C	91	93	78	86	90	92	86	87	90	91	90	94	90	94
	60 min cycle	A	0	54	79	91	72	90	13	85	0	83	0	0	81	88
		B	9	56	80	83	80	88	46	86	32	82	11	15	57	92
		C	85	94	91	91	83	92	89	91	94	92	94	93	88	92

Table II. *Action of Intermittent R and FR Irradiations Applied for 4 Days on the Germination of Tomato Seeds, Varieties Ace and Porte, at 20° and 25°.*

Treatment	Percent germination Ace		Porte	
	20°	25°	20°	25°
30 min cycle				
1 min R	74	92	86	91
1 min FR	3	9	0	25
1 min FR — 1 min R	73	92	90	93
1 min R — 1 min FR	3	4	0	0
60 min cycle				
2 min R	68	94	81	83
2 min FR	0	20	0	54
2 min FR — 2 min R	72	92	79	91
2 min R — 2 min FR	0	0	0	0
120 min cycle				
4 min R	...	90	...	93
4 min FR	5	16	4	73
4 min FR — 4 min R	...	...	...	...
4 min R — 4 min FR	0	10	0	10

R-FR cycle has higher inhibitory effect than the FR cycle (tables I, A and II).

Cucumber, Exposure to Continuous and Intermittent Radiations. The germination response of cucumber seeds exposed to prolonged FR depends on temperature. Inhibition of germination after exposure to FR is higher at 15° than at 20°. At temperatures above 25° there is no inhibition of germination by continuous exposure to FR (table III). Continuous FR can be replaced by intermittent FR, provided the interval between successive irradiations is kept short, not more than 30 minutes. Red applied at the end of a period of exposure to continuous or intermittent FR repromotes germination during the dark period following the irradiation. Red applied after each FR in the intermittent irradiation treatments repromotes germination. Intermittent FR alone is less inhibitory than intermittent R-FR with R applied immediately before each FR (table IV).

Discussion

Germination of tomato and cucumber seeds can be inhibited by prolonged exposure to intermittent FR radiation, provided the interval between successive irradiations is not too long and the temperature not too high. In tomato seeds, at temperatures below 20° (Porte) or 25° (Ace) germination can be inhibited by a single exposure to low-energy FR radiation (4, 5), but in cucumber only prolonged exposures are effective and cycles with a shorter dark interval than those effective in tomato have to be used. The

Table III. *Action of Different Prolonged FR Irradiations on the Germination of Cucumber Seeds*

Treatments	Percent germination 15°	20°	22°	25°
DC (4dD)	80	99	100	100
1d cont FR-4dD*	24	98	...	...
1d cont FR-10mR-4dD	63	...	...	...
2d cont FR-4dD	4	84	...	...
2d cont FR-10mR-4dD	50	...	...	...
3d cont FR-4dD	1	57	...	...
3d cont FR-10mR-4dD	11	...	...	...
1d cyclic FR (1mFR-29mD)-4dD	52	...	...	...
2d cyclic FR (1mFR-29mD)-4dD	46	...	...	...
3d cyclic FR (1mFR-29mD)-4dD	21	...	...	...
1d cyclic FR (1mFR-9mD)-4dD	62	...	...	...
1d cyclic FR (1mFR-9mD)-10mR-4dD	83	...	...	...
2d cyclic FR (1mFR-9mD)-4dD	31	...	...	...
2d cyclic FR (1mFR-9mD)-10mR-4dD	87	...	...	...
3d cyclic FR (1mFR-9mD)-4dD	14	89	...	99
3d cyclic FR (1mFR-9mD)-10mR-4dD	70	...	...	...
5d cont FR	0	...	93	...
5d cyclic FR (1mFR-9mD)	0	...	...	...
5d cyclic FR-R (1mFR-1mR-8mD).	93	...	...	...

* D = dark.

Table IV. *Action of 4 Days of Intermittent FR and R-FR on the Germination of Cucumber Seeds, Varieties Pixie (CP) and National Pickle (CNP), at 17.5°*

Treatments	Percent germination CP	CNP
DC	86	95
15 min cycles		
1.5m FR-13.5mD	12	37
1.5m FR-1.5mR-12mD	91	95
1.5m R-1.5mFR-12mD	0	13
30 min cycles		
3m FR-27mD	39	69
3m R-3mFR-24mD	0	13

response to prolonged exposure to intermittent FR is phytochrome controlled. If each FR irradiation is followed by R, repromotion of germination is obtained. Under intermittent FR, increasing temperature and length of the dark interval between successive irradiation results in an increase of germination. Under intermittent, alternate R-FR irradiations, the total length of the cycle seems to be of less consequence than the temperature and the relative positions of R and FR in each cycle. In cycles of equal length, the longer the dark period between R and FR (after R), the higher the germination. For cycles with the same relative position of R and FR, the higher the temperature, the higher the germination. These results seem to indicate that the P_{FR} controlled reaction proceeds at faster rates at higher temperatures. Equal relative P_{FR} levels, operating for the same length of time, induce higher germination at higher temperatures.

The physiological results obtained seem to point out that something else, beside the rate of the reaction controlled by the level of P_{FR} established by irradiation, is effective in the control of the induction of germination. Considering the photoequilibrium level of P_{FR} established by FR (1, 9) and the rate of decay of P_{FR} in seedlings in dark (1, 9), short cycles of intermittent FR should maintain a relative P_{FR} level slightly higher than longer cycles. Thus, if only the relative level of P_{FR} established by FR was responsible for the activation of germination, the different length of the dark period between successive irradiations should have none or little effect. Perhaps we could expect an effect opposite to the one we found: shorter cycles could be expected to be more effective in the activation of germination than the longer cycles. We found that shorter cycles are more inhibitory than longer cycles. This result could be the consequence of a continuous, temperature dependent, input of P_{FR} into the system. If there is such a continuous input of P_{FR} into the system, we would expect higher germination under longer dark intervals between successive irradiations, since the P_{FR} coming into the system would have more time to act. The hypothesis of a continuous input of phytochrome into the system had been made before (3) to explain the necessity of prolonged FR irradiations for the inhibition of germination of certain dark-germinating seeds. The results obtained with cycles of different length seem to agree, at least on a physiological basis, with this hypothesis.

Another interesting aspect of these results is the comparison of the action of the intermittent FR (FR-D) and of the alternate R-FR, where R is applied immediately before FR in each cycle (R-FR-D). If only the relative P_{FR} levels established by irradiation were responsible for the induction

of germination, the FR-D and the R-FR-D treatments should produce the same results on germination, but they do not, at least not always. In tomato, when the seeds are exposed to such treatments only for 24 hours, terminating with a R irradiation and then returned to darkness, the same dose of red radiant energy repromotes more germination when given after the FR-D treatments than after the R-FR-D (table I, treatment C, 30 min cycle, 20, and cfr. fig 6, ref 4). If the exposure to such treatments is continued for 4 days (table II), one can appreciate the large difference between the FR-D and the R-FR-D cycles. This difference is very large only in the Porte seeds, which are less sensitive to FR than Ace seeds (4). The difference depends on temperature and on the length of the cycle. At those temperatures and length of cycles which result in a lower inhibitory efficiency of the FR-D treatments, the R-FR-D treatments still retain fully inhibitory power. The situation is very much the same in cucumber seeds (table IV). Under both treatments the same relative P_{FR} level should be maintained, that is the 1 to 4 % P_{FR} established by saturating FR (1, 9). While there is little doubt that, under both treatments, the relative P_{FR} levels are the same, it is not certain that equal relative P_{FR} levels represent equal total P_{FR} concentration under the 2 treatments. We have some preliminary proof, obtained in our laboratory, that the 2 different treatments may result in different total phytochrome levels, and consequently equal relative P_{FR} levels would represent different total P_{FR} concentrations.

In 5-day old, dark grown *Avena* seedlings, intermittent FR, 3 min of FR each hour for 4 hours, results in the loss of about 20 % of the phytochrome initially present; intermittent R results in the loss of about 80 % and intermittent R-FR causes the loss of about 60 % (Dooskin, personal communication). In cucumber seeds (7), total phytochrome content after 4 days of exposure to FR-D treatments is higher than in the seeds exposed to the R-FR-D treatments.

If we assume that the phytochrome control of germination depends on the total P_{FR} level, and not on the relative P_{FR} level only, the explanation for the different inhibitory efficiency of the FR-D and R-FR-D treatments could be based on the different total phytochrome content resulting from exposure to the 2 different treatments.

On the basis of the results presented in this paper, we might suggest that the phytochrome control of germination depend upon the P_{FR} level, the rate of phytochrome input into the system, and the rate of phytochrome decay under the different treatments. The relative importance of these 3 factors could change depending upon other factors such as temperature, different irradiation schedules, and other external and internal conditions. The P_{FR} level seems to be the only factor controlling the germination response to a single, low-energy FR irradiation. In the case of the cyclic irradiations, the 2 other factors, phytochrome input and phytochrome decay, could be as important as the P_{FR} level. There is no evidence yet for a direct physiological action due to phytochrome decay (2). In including the rate of phytochrome decay among the factors controlling seed germination, we want to indicate only that the reduction of the phytochrome level could be of some importance in modifying the germination response to light.

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