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Short Communication

Phytochrome in Embryos of Pinus palustris[1]

Elaine M. Tobin[2] and Winslow R. Briggs[2]
Department of Biological Sciences, Stanford University, Stanford, California 94305

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Phytochrome has been shown to be involved in the germination of seeds of a number of pine species as determined by the responses of these seeds to red and far-red irradiations (4,7,9). Nyman (7) found that even before imbibition, germination of *Pinus silvestris* seeds can be promoted by red irradiation. McLemore (5) found that germination of longleaf pine seed (*P. palustris*) can be repeatedly promoted and inhibited by short exposures to red and far-red light, after imbibition. This species has relatively large embryos which can be easily isolated. Therefore, it was of interest to see whether germination of *P. palustris* seeds could be affected early during imbibition by red and far-red light, and to investigate the phytochrome spectrophotometrically.

Materials and Methods

Seeds of longleaf pine were supplied by B. F. McLemore (USDA, Pineville, Louisiana). Germination tests were conducted in a darkroom (T = 25° ± 1°, humidity = 90–92 %) on moist cellulose packing material (Kimpak 6223, Kimberly-Clark) after the seeds had been soaked for 4 and one-half hr. Generally 35 to 45 seeds were used to obtain each value in an experiment. Germinated seeds were counted after 2 weeks.

Phytochrome was measured using a Ratiospect as described by Briggs and Siegelman (1). *In vivo* spectra were made on a Biospect 61, a single beam scanning spectrophotometer adapted for taking absorption spectra of optically dense and light-scattering samples. A compensation system is used in this instrument to correct for system response and light scattering. The baseline is established for each sample by using the number of Kimwipes (Kimberly-Clark) which have approximately the same optical density as the sample. Dissection of the embryo samples was done under dim green light in the darkroom. There were usually 15 to 20 embryos/sample. Far-red illumination (2.6×10^3 erg cm^{-2} sec^{-1}) was provided by light from incandescent bulbs filtered through 3 mm of Rohm and Haas FRF far-red plexiglass. Red illumination (8.7×10^2 erg cm^{-2} sec^{-1}) was provided by light from cool-white fluorescent lights filtered through 3 mm of Rohm and Haas No. 2423 red plexiglass.

Results and Discussion

When light treatments were given during the 4 and one-half hr soaking period or when the seeds

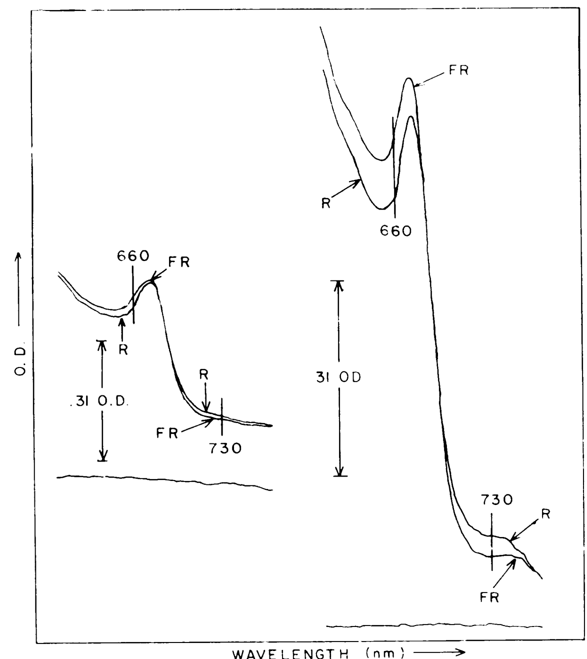

FIG. 1. *In vivo* absorbtion spectra of embryos 16 hr from start of soaking of the seeds. Left: before adding water; right: after adding water. The red (R) and far-red (FR) irradiations were 2 min saturating dosages.

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[2] Present addresses: Biological Laboratories, 16 Divinity Avenue, Harvard University, Cambridge, Massachusetts 02138.

Table I. *Effect of Light Treatment of Seeds*

Seeds received either one-half hr FR light or one-half hr FR light + one-half hr R light.

	Time (from beginning of soaking) for the light treatment	Germination FR treated	Germination FR-R treated
	hr	%	%
Dark control:	0	17 and one-half	18
38 % Germination	3	2	16
	6	7	22 and one-half
	9 and one-fourth	5	58
	12 and three-fourths	17 and one-half	41
	25	13	32 and one half
	35 and one-fourth	7	35
	50	16	62

Table II. *Rapid Appearance of Phytochrome Upon Addition of Liquid*

Sample	Liquid added	Wt of sample	$\Delta(\Delta$O.D.)/g before liquid added	$\Delta(\Delta$O.D.)/g after liquid added
		grams		
		0.29	0.031	0.110
Embryos from "dry" seeds	Water	0.28	0.050	0.129
		0.19	0.032	0.142
	0.1 mg/ml Cycloheximide	0.28	0.046	0.146
		0.26	0.050	0.161
	10^{-3} M CN^-	0.28	0.007	0.157
Lyophilized embryos	Water	0.24	None detectable	0.221
		0.19	None detectable	0.189

Table III. *Phytochrome Reversion After Red Irradiation*

Time after red light treatment	Wt of sample	No. of embryos	P_{FR}	P_{total}
min	*grams*			
0	0.32	12	0.019	0.028
27	0.32	12	0.014	0.036
57	0.33	12	0.013	0.029
89	0.32	12	0.009	0.035
136	0.32	12	0.007	0.027
208	0.32	13	0.007	0.030

were placed on the Kimpak, one-half hr far-red light always inhibited germination; one-half hr red light following the far-red treatment always reversed this inhibition. In some cases, the red light promoted germination to a level higher than that of the dark control though variability was high. The results of this experiment are shown in table I.

Phytochrome was found to be present in embryos grown in either light or dark. If embryo samples of dry seeds were prepared and packed in the cuvette without water, low levels of phytochrome were detected [from 0.007 to 0.050 $\Delta(\Delta$O.D.) per gram]. If water was added to the sample while it remained in the cuvette on ice, additional phytochrome appeared immediately. This increase was fully apparent within the *ca.* 2 min necessary to add the water and make a measurement with the Ratiospect. There was little or no change in this level when the wet sample was left in the light for periods up to 5 hr at room temperature. The change was also visible on spectra of samples made on the Biospect (see fig 1).

Attempts were made to inhibit this rapid appearance of phytochrome by using solutions of cycloheximide (0.1 mg/ml), which has been shown to inhibit protein synthesis in plants as well as animals (6), or of cyanide (10^{-3} M) instead of water. As can be seen in table II, neither of these solutions

inhibited the increase in the amount of phytochrome upon addition of liquid. The full change was apparent as soon as it could be measured.

Finally, 2 different samples of the embryos were lyophilized. In neither case could phytochrome be detected in the completely dried sample. Addition of water, however, resulted in the usual immediate increase in phytochrome (table II).

Thus it appears that *P. palustris* embryos contain some phytochrome in a state such that it merely needs hydration to become photometrically detectable.

The phytochrome dark reactions were examined by dissecting embryos and placing them on wet Kimpak in the dark room to hydrate for 2.5 hr. Then 45 min of red light were given to all of the embryos, and samples were examined for phytochrome content after various amounts of time in the dark at 25°. The results are shown in table III. It can be seen that there was no detectable loss of total phytochrome but there was a substantial decrease in the amount of P_{FR} present, suggesting that dark reversion was occurring, and that the phytochrome of *P. palustris* is at least initially light-stable. The dark reactions and stability in light of phytochrome in other tissues is discussed extensively by Hillman (3).

The normal appearance of photoreversible phytochrome in embryos in intact seeds involves gradual hydration over a period of about 2 days when the seeds are initially soaked for 4 and three-quarter hr and are then allowed to germinate on moist Kimpak. This progressive hydration is shown in figure 2. Embryos were examined at various times from the beginning of soaking. Measurements were made of the phytochrome per embryo present before and after adding water to the sample. The amount of phytochrome present after adding water was taken as the total phytochrome present. The percent of total phytochrome detectable before addition of water was calculated; this figure was taken to be percent hydration of phytochrome. Spectra were obtained from some of the samples with the Biospect 61. In figure 1, the change in the spectrum upon addition of water to a sample obtained from seeds 16 hr from the beginning of soaking can be clearly seen. The large underlying peak in both sets of spectra is chlorophyll. The maximum difference between red- and far-red-irradiated samples is at approximately 660 nm, suggesting that pine phytochrome absorbs maximally at shorter wavelengths than oat phytochrome (2), but at longer wavelengths than that isolated from the alga *Mesotaenium* or the liverwort *Sphaerocarpos* (8). The percent of hydration of phytochrome shows a fairly steady increase with time, becoming maximal at about 36 hr after the beginning of soaking. The total phytochrome did not change significantly over this period of time.

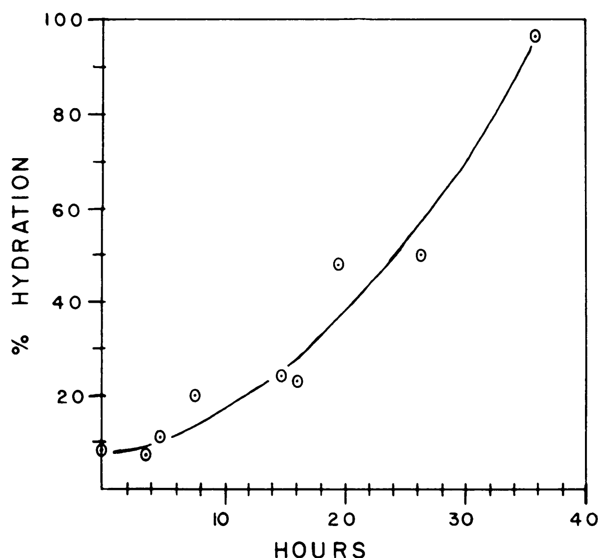

FIG. 2. Progressive hydration of phytochrome *in vivo*.

We conclude that at least some of the "dry" seeds contain some hydrated phytochrome. It is probably this phytochrome which makes the seeds sensitive to irradiation before (7) or early during imbibition. The fact that continuous light and dehydration do not lead to irreversible destruction of the phytochrome is consistent with this possibility. The rapid increase of phytochrome in embryos upon addition of water is probably hydration of pre-existing molecules rather than synthesis. It is affected neither by cycloheximide, cyanide, nor low temperature (0°).

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