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DECREASE IN PHYTOCHROME PELLETABILITY INDUCED BY GREEN + FAR-RED LIGHT IN TRIFOLIUM REPENS

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SUMMARY.- The use of a simulated canopy, made by superimposing green + far-red light, decreases both pelletable phytochrome amount and percentage of pelletable Pfr in Trifolium repens seeds. This results in an enhancement in the rate of hypocotyl elongation, since membrane-linked Pfr inhibits this process. It can be postulated the existence of a "green photoreceptor" which interacts with phytochrome in order to depopulate pelletable Pfr pool.

INTRODUCTION.- The mode of action of phytochrome is often explained through a modulation of the properties of some cellular membranes (1). This hypothesis implies a true association of phytochrome with these structures. Red light enhances phytochrome pelletability *in vivo* (2) and *in vitro* (3) conditions. Roth-Bejerano and Kendrick (4) show that phytochrome association in 20,000 x g pellets, obtained from primary leaves of Hordeum vulgare, is not reversed by far-red light. In addition, Holmes et al. (5) report that photoreversion of Pfr to Pr is carried out when association achieves from soluble phytochrome and partially-purified organelles from barley leaves, but it does not result in a decrease of pelletability. Pratt (6) and Marmé (7) conclude that the *in vivo* association of phytochrome may be related to its biological action but there is no experimental evidence on this point.

In natural environments, other kinds of light can interact with red/far-red light in phytochrome photo-

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conversions. Blue light inhibits hypocotyl elongation in Sinapis alba (8) and Lactuca sativa (9) in addition to phytochrome-mediated inhibition. On the other hand, blue light induces and increase in phytochrome content of mung bean hooks (10) and Schäffer (11) reports that blue light decreases the percentage of PfrX form, considering it as the particulate active form of phytochrome. Recently (12) it has been shown that inhibition of hypocotyl lengthening in Trifolium repens, produced by a short-term far-red irradiation, is completely reversed when green light, in addition to far-red, is used. Green light is chosen to be the main component of the light filtered through natural canopies (13). We attempt to demonstrate that this action can be related to changes in phytochrome pelletability.

MATERIAL AND METHODS.- About 100 seeds of T. repens (cv. Grau) and 6.0 ml of Hoagland solution (14) were placed on filter paper in petri dishes. After 24 hours in darkness at 26°C, the seeds were irradiated for 1 hour with different kinds of light and inmediately used for pelletable phytochrome assay.

Red light was obtained from 40 W Mazda J9 fluorescent tubes filtered through one layer of No. 1 and one layer of No. 14 Cinemoid (15) with a calculated value of ζ = 13.4 (16,17). Far-red light was obtained from 150 W Philips HP 3608 incandescent lamps filtered through one layer of No. 5A and one layer No. 20 Cinemoid (15) with flowing water as protector. Calculated ζ was 0.001. Green light was obtained by filtering white light from 40 W Philips Daylength fluorescent tubes (ζ = 10.7) through a No. 61 Kodak gelatine filter. Each seed intercepted 1.32 (R), 3.0 (FR) or 2.66 (G) $nE \cdot cm^{-2} \cdot s^{-1}$.

To measure phytochrome pelletability, tissue was homogenized in ice-cold 10 mM N-morpholino-3-propanesulfonic acid buffer, pH 7.2, containing 0.4 M sucrose, 1 mM EDTA and 1 mM DTT (18). Homogenates were filtered through a nylon cloth and centrifuged at 10,000 x g for 15 minutes at 2°C. Supernatants were then centrifuged at 58,000 x g for 30 minutes at 2°C, washed with 4.0 ml of the same buffer and recovered by centrifugation. Phytochrome pelletability was estimated as the quantity of phytochrome in the pellet divided by the amount in the supernatant plus the pellet (19).

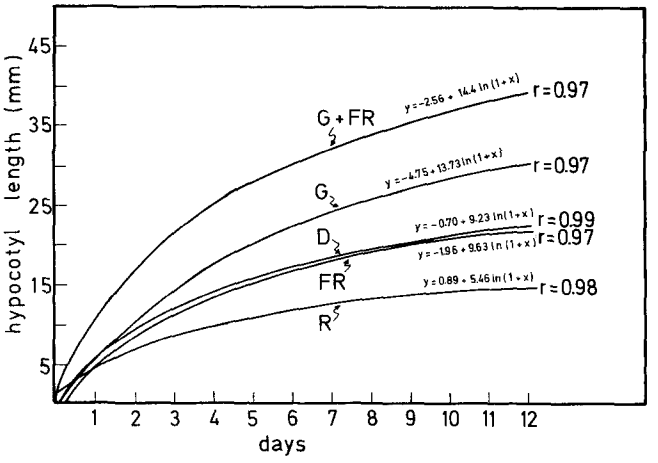

Figure 1. Time-course of hypocotyl lengthening in T. repens seedling as a function of 1 hour-initial lighting followed by continuous darkness. G = green light; R = red light; FR = far-red light; D = darkness.

Variation in apparent phytochrome content was estimated by measuring optical densities at 730 or 660 nm versus 800 nm (19) with 20 mg of $CaCO_3$ added as scattering agent (20) per ml of sample.

RESULTS AND DISCUSSION.- A model of the influence of the canopy-filtered light in simulated environments on the growth of T. repens is shown in Fig. 1. The highest rate of hypocotyl elongation is reached when T. repens seeds are irradiated for 1 hour with green + far-red light whereas a clearly inhibitory effect is shown under both red and far-red light regimes. This inhibition can be explained as a negative control of hypocotyl elongation by Pfr. Therefore, it can be postulated that green light nullifies the inhibitory effect of far-red light. By assuming that biologically-active well-orientated Pfr in the membrane is the phytochrome form which inhibits elongation, green light must depopulate PfrX pool or, by approximation, pelletable Pfr. Results in Table I show that, whereas 1 hour of red or far-red light highly

TABLE I

Effect of light treatments on pelletable phytochrome isolated from T. repens seeds.

Light treatment (1 hour after 24 h in darkness)	Pelletable phytochrome	
	Δ(ΔA)	% Pfr
None (darkness)	0.374	32.08
Red	0.362	60.22
Far-red	0.516	45.73
Green	0.237	40.50
Green + far-red	0.266	22.93
Green + far-red followed by 1 h red light	0.444	40.99

increases the percentage of pelletable Pfr isolated from the seeds, green + far-red light, as supossed, strongly decreases it as well as the amount of total pelletable phytochrome. 1 hour of red after green + far-red light reverses this effect. The highest rates of inhibition of hypocotyl elongation coincide, so that, with light treatments which induce the highest percentages of pelletable Pfr whereas the highest rates of hypocotyl elongation are induced by light treatments which promote the lowest values of pelletable Pfr. Green light, it-self, decreases the amount of total pelletable phytochrome but not pelletable Pfr.

To answer the question about the nature of green light receptor and its interaction with phytochrome or, alternatevely, if phytochrome it-self can acts as green light receptor, light treatments were carried out upon exposure of the seeds to 2 mM KI. Potassium iodide is a well-known efficient quencher of the flavin triplet excited state (21) and it is routinely used for the

TABLE II

Effect of 2 mM KI on the changes in pelletable phytochrome induced by light treatments.

Light treatment	Pelletable phytochrome	
	Δ(ΔA)	% Pfr
Red	0.423	57.68
Far-red	0.419	46.91
Green + far-red	0.405	42.46
Green + far-red followed by 1 h red in absence of KI	0.433	39.26

identification of "blue photoreceptor" like-riboflavin (22,23). As shown in Table II, KI does not modify pelletable Pfr percentage when T. repens seeds are illuminated with red of far-red light. Therefore, it can be assumed that iodide does not act as a depopulating agent of pelletable Pfr pool (22). However, when KI-treated seeds are illuminated with green + far-red light, decrease in percentage of pelletable Pfr, shown in Table I, does not appear. When the seeds, after combined lighting in the presence of iodide, are removed from the media, washed with distilled water in darkness and the irradiated with red light, values of pelletable Pfr are similar to those observed following the same treatment in absence of KI.

The absence of decrease in pelletable Pfr when KI is used in addition to light treatments which include green light indicates that a second photoreceptor, unlike phytochrome, controls the stability of pelletable Pfr pool.

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