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EFFECTS OF RED AND FAR-RED LIGHT ON GEOTROPISM IN AVENA COLEOPTILES. I. SOME PRELIMINARY INVESTIGATIONS.

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The effect of red and far-red irradiation on geotropism in *Avena stiva* L. cv Victory Svalof I was studied. Four-day old seedlings were found to be the best material for this purpose. A positive antagonism of red and far-red irradiation was observed in eliciting the geotropic curvature.

INTRODUCTION

Light is a manifestation of energy and according to the first law of photochemistry, it must be absorbed if it is to elicit a photochemical reaction. In biological systems, this may lead to profound physiological and biochemical changes. Phytochrome is one of the several plant pigments which are utilized by plants to capture the radiant energy needed to run or potentiate certain photophysiological and/or photomorphogenetic processes. Phytochrome is a photoreversible chromoprotein that can exist in two stable forms.

$$P_r \underset{\text{far red light}}{\overset{\text{red light}}{\rightleftharpoons}} P_{fr}$$

The pigment P_r has an absorption maximum near 660 mm, and P_{fr} one near 730 nm. Absorbtion of light by either form can convert it to the other form. Geotropism is a tropic response in plants which is stimulated by a directional stimulus, in this case the gravitational field. Most plant organs attain stable equilibrium at a certain angle to the gravity force vector and enforced departure therefrom will cause them to bend back into what might be called their "preferred" orientation.

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Light appears to affect both the direction and the rate of the geotropic respanse of plant organs. Blaauw (1961) reported that there was no antagonism between red and far-red light, and that far-red radiation would also induce the increased responsiveness at a dosage level about three times higher than that required with red light. In contrast, Wilkins (1965) found no inductive effect of small doses of far-red irradiation (740 nm) and that far-red irradiation would totally reverse the effect of red light (655 nm).

MATERIALS AND METHODS

Culture of Seedlings

The unhusked grains of *Avena sativa* L. (cv Svälof Victory I) were soaked in cool running tapwater for four to five hours in dark room maintained at $23 \pm 1°C$ and were later planted in vermiculite (Zonolite, industrial and chemical grade) which had previously been soaked overnight, washed thoroughly and drained to remove excess water. The grains were uniformly spread between two thick layers (3 cm and 5 cm) of moist vermiculite in a plastic container to support the uninhibited mesocotyls.

The containers were placed on trays lined with wet paper towels and arranged in a large light proof and humidified aluminum cabinet which was located in the focal curve room of the spectrograph and maintained at $23 \pm 1°C$.

Safe and Experimental Lights

A Sylvania 20-watt fluorescent lamp wrapped with six layers of green cellulose acetate (Transilwrap West. Corp.) was used as a safe light for the present experiment. The transmission spectrum of cellulose acetate and the emission spectrum of the lamp were obtained by using a Cary 14 recording spectrophotometer and an ISCO Model SR spectroradiometer respectively. As the emission spectrum of the lamp (Fig. 1) and the transmission spectrum of the cellulose acetate (Fig. 2) showed that some light was transmitted in the short and the long wavelength range, this unwanted light was removed by using a 2 percent $CuSO_4$ solution (Withrow and Withrow, 1956) which was 10 cm thick. The lamp was placed atop an aluminum cabinet and wrapped around with several layers of thick black cloth. A 7 x 4 inch area of the $CuSO_4$ filtered radiation was used as a safe-light source and the experiment carried out in the shadow of the cabinet. In this lamp position, the light intensity was undetectable by the ISCO spectroradiometer. At no time were

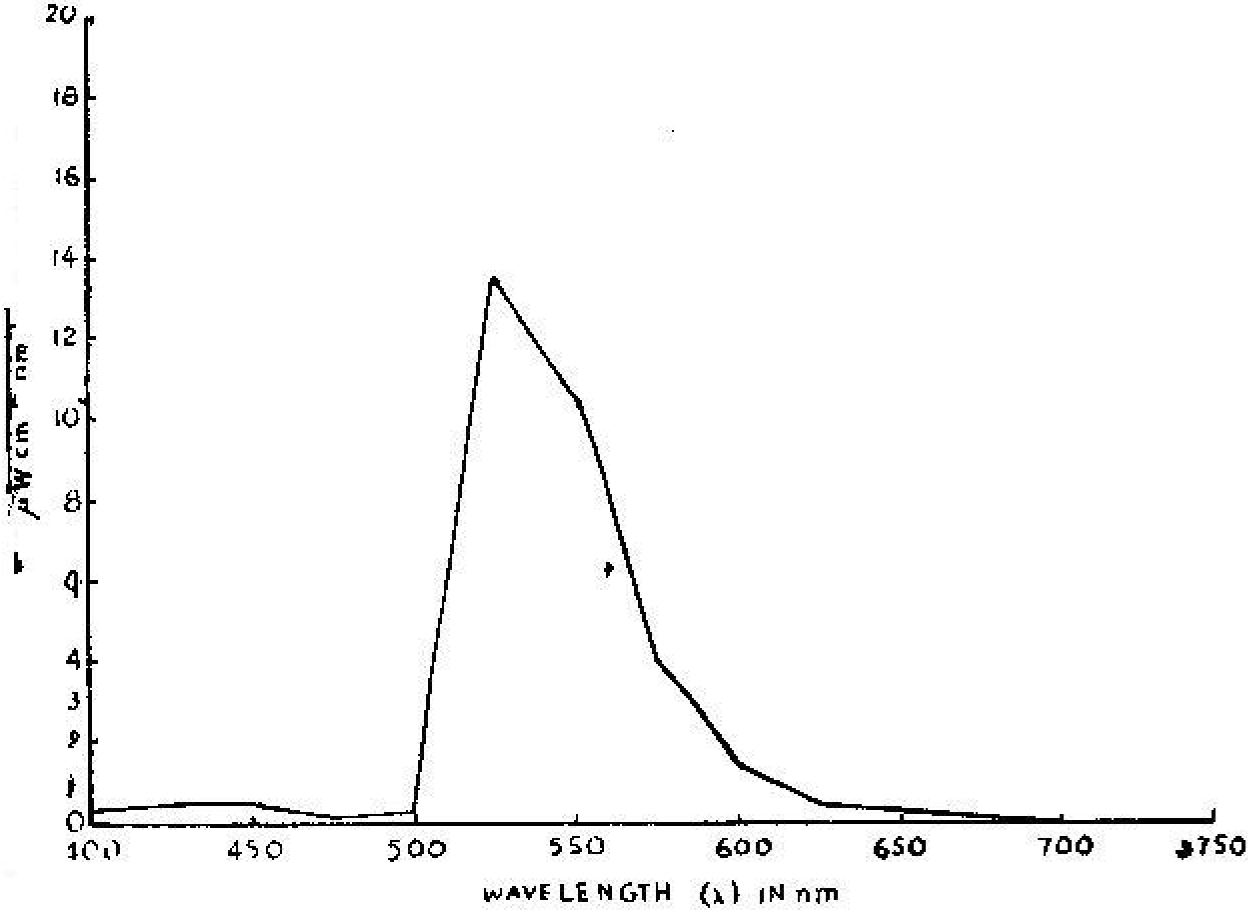

Fig 1. Emission Spectrum of Fluorescent Safe Light Lamp

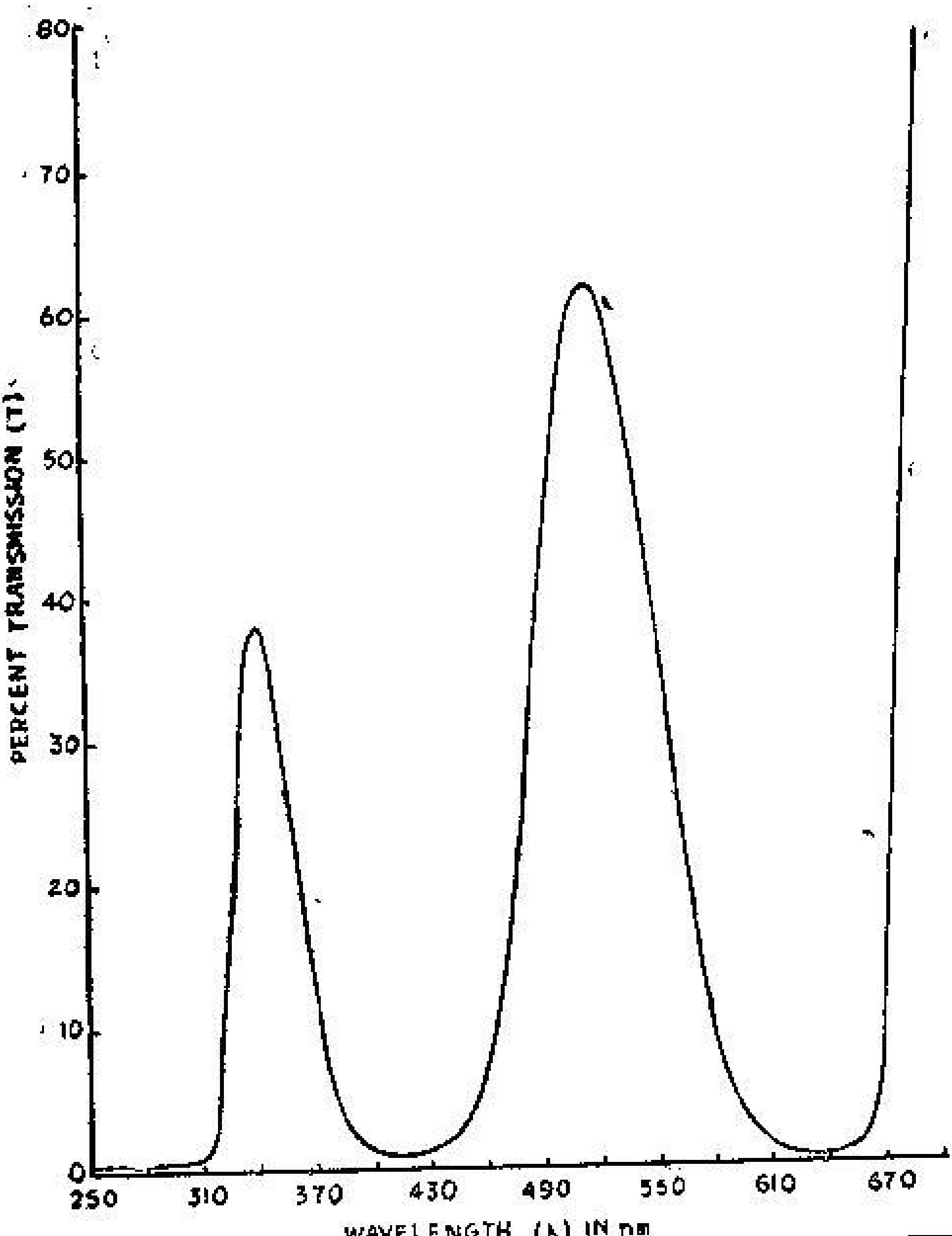

Fig. 2- Transmission Spectrum of Green Cellulose Acetate Filters.

the coleoptiles exposed for more than 20 minutes to this extremely low radiation. A large spectrograph described in detail by Balegh and Biddulph (1970) was used as a source of experimental light.

Experimental Procedure

Four day old etiolated coleoptiles were excised just above the node and the latter was located by touch. Straight coleoptiles were slipped into 1.3"x0.1" water-filled glass tubes, adjusted in place by applying a little lanolin at the junction of the coleoptile and the glass tube. Six such glass tubes were stuck into two percent solidified agar contained in a tubular plexiglass container. Care was taken not to block the lower end of the glass tube. These containers were open at one end while the other was closed by a rectangular piece of plexiglass. The tubular part of the plexiglass container had six holes at the top where the glass tubes were placed to maintain the coleoptiles in a vertical alignment.

The coleoptiles were always irradiated from above and a duplicate set of each treatment was maintained. The exposed coleoptiles were placed in a light-tight and humidified aluminum cabinet immediately after the treatment and were given a two-hour geostimulus. At the end of the geotropic exposure the coleoptiles were shadow-graphed on Kodak F-3 Velox Photographic paper by using an electronic flash gun placed at a distance so adjusted as to give a nearly collimated beam of light.

The curvatures were measured on the developed shadow-graphs by using a goniometer which was aligned with the straightest part of the coleoptile.

RESULTS AND DISCUSSION

Age and Length of the Coleoptiles

Knowledge of the growth behaviour of coleoptiles and their change in sensitivity with age, if any, is of paramount importance in a study of this type. The lengths of the coleoptiles recorded here were 2.62, 5.38, and 7.11 cm in four days, five days, and six days old, respectively (Table 1). Less than four-day-old coleoptiles did not emerge from the vermiculite surface and more than six-day-old coleoptiles were too tall to be used. It was observed that the primary leaf pierced the coleoptile after six days. Owing to the greater length, the five-and six-day old coleptiles had a tendency to lodge. Wilkins (1965) observed that the degree of sensitivity of the etiolated coleoptiles decreased on the seventh day.

It is apparent from Table 1 that mesocotyl attained the maximum lenth by the fourth day while the rate of coleoptile growth became accelerated at that time and they attained their maximum on the sixth day. The leaf pierced through the coleoptile on the seventh day. The rate of mesocotyl growth was faster at first but coleoptile caught up on the fifth day and during the next 24 hours its rate of growth was faster than the mesocotyl. According to Mer (1969), the mesocotyl extends rapidly at a uniform rate during the second to fourth day, less rapidly for the next 24 hours until its final length is attained by the fifth day. He further noted that while the mesocotyl was growing quickly, the coleoptile showed slow progress, but an acceleration was noted in its rate when a slight decline occurred in the rate of the mesocotyl. However, Mer (1969) noted the maximum growth rate of the coleoptile from the fourth day onward, while its growth was over by the eighth day.

Our data also suggest that the growth rates of coleoptile and plumule were parallel to each other from the fourth to the sixth day. The present results do not agree with Mer's (1969), which may be due to the culture conditions and/or the difference in the genetic make-up of the plant material.

Effect of Red and Far-Red Irradiation

Red, far-red antagonism is a basic tenet of phytochrome physiology. Although this idea has gained wide acceptance, it remains a bone of contention between the Dutch school of Blaauw and his a associates (e.g., Blaauw et al., 1968) and Wilkins (1965) as far as geatropism is concerned

After several trials on doses, it was found that far-red light (740 nm) alone did not have any significant effect on the geotropic response of the Avena coleoptiles. I our results (Table 2) it was observed that tht geotropic response of the far-red irradiated coleoptiles (173.77 uW at 2.896 uW cm^{-2} sec^{-1}) did not differ significantly from that of the dark controls. Like Wilkins (1965 our result show a clear cut red and far-red antagonism. Failure to observe this phenomenon can invoke the possible involvement of certain other photoreceptor instead of phytochrome. Altough negative results may eliminate phytochrom as a photoreceptor, however, a positive response strengthens its cause.

TABLE 1 : *Relationship between age and length of mesocotyl and coleoptile.*

	length (cm)		
Age Days)	Mesocotyl	Coleoptile	
4	5.03 ± 0.026* (25)**	2.62 ± 0.023 (25)	+1.12±0.4 (29)
5	5.21 ± 0.029 (25)	5.38 ± 0.026 (25)	1.99±0.9 (21)
6	4.79 ± 0.024 (25)	7.11 ± 0.024 (25)	3.94±1.7 (31)
7	—	—	5.73±1.2 (28)

* Standard error of mean

** Number of Coleoptiles in sample

+ Taken from Wilkins (1965)

TABLE 1 : *Red-far red reversal of geotropic response*

Light Treatment	Curvature in Degree
Dark	31.5 ± 0.4* (10)**
1 min R	39.3 ± 0.5 (11)
2 min R	39.6 ± 0.3 (12)
1 min FR	32.1 ± 0.1 (10)
1 min R → 1 min FR	34.1 ± 0.5 (12)
2 min R → 1 min FR	34.7 ± 0.4 (11)
1 min FR → 1 min R	40.4 ± 0.3 (8)
1 min FR → 2 min R	39.6 ± 0.5 (9)

* Standard error of mean

** Number of Coleoptiles in sample

Possible reason for the differences between the results of Wilkins (1965) and Blaauw (1961) might be due to the source of far-red radiation used by the latter which possibly emitted very small amounts of red radiation. Since the change in geotropic responsiveness is induced by exceedingly small amounts of radiation in the 665 nm region of the spectrum (less than 1 erg cm^{-2}), therefore, stringent precautions need to be taken to ensure that the far-red radiation is uncontaminated with wave lengths below 700 nm.

The antagonistic effects of red and far-red radition as reported by

Wilkins (1965) and confirmed by us strongly suggest that the pigment responsible for preceiving the light stimulus is phytochrome.

We are of the opinion that increased geotropic responsiveness of Avena coleoptiles may be due to red light-induced changes in membrane permeability, which could either trigger or accelerate the downward movement of auxin and/or some other factors causing an increased geotropic curvature. Wilkins' (1965) and our results indicate that the increased geotropic responsiveness develops immediately after a fraction of a second's exposure to red light and the primary locus of red light action is possibly at the membrane level.

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