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633.15:581.441:581.035:547.97

MEDEDELINGEN VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN, NEDERLAND **63** (14), 1-7 (1963)

# PHYTOCHROME DESTRUCTION FOLLOWING ILLUMINATION OF MESOCOTYLS OF *ZEA MAYS* L.

by

P. J. A. L. DE LINT and C. J. P. SPRUIT

*Laboratory for Plant Physiological Research, Agricultural University, Wageningen, Netherlands, 230th Communication*

*(Received 19.9.'63)*

## INTRODUCTION

In 1959, a pigment existing in two interconvertible forms and presumably responsible for the reversible red – far red effects in a large number of plant reactions was demonstrated by sensitive spectrophotometry and given the name "phytochrome" (1). It was almost immediately observed that the two forms of the pigment lost part of their interconvertibility upon irradiation (2). Apparently, upon each transition from the form absorbing maximally at 660 mμ ($P_{660}$) into the form absorbing at 730 mμ ($P_{730}$) and back again, the sum of the concentrations of the two forms diminishes.

Further data on this reduction of convertibility of phytochrome in irradiated material, are presented in this paper.

## MATERIAL AND METHODS

Mesocotyls of several culture varieties of *Zea mays* L. were used. The corn was densely sown in sand and raised at about 22 °C in the dark in fairly high humidity. Usually, mesocotyls grown to about 3/4 of their maximum size were cut out and given experimental irradiation when in horizontal position, not covering each other. For most experiments, the red radiation was obtained from fluorescent tubes and the far red from incandescent bulbs in combination with suitably transmitting filters. The apparatus used and the spectral energy distribution curves were as described by STOLWIJK (3).

The concentration of protochlorophyll in the mesocotyls is very low, and there was no interference of absorption changes due to the protochlorophyll-chlorophyll transformation with those due to phytochrome.

For the determination of phytochrome absorption, about 40 more or less straight, 2 cm long sections (2 or 3 from each mesocotyl) were densely packed

in a 1 cm wide glass box. As table 1 shows, it is allowed to use upper and basal sections of mesocotyls together, as the phytochrome is fairly uniformly distributed.

TABLE 1. Distribution of phytochrome in 6-day old seedlings of *Zea mays* L., cv. "CIV 7", grown at 20 °C.

| Part of seedling | Absorption change |
|---|---|
| coleoptile (with leaves inside) | 1.42 |
| node (ca. 1 cm) | 1.63 |
| mesocotyl (upper part) | 0.71 |
| mesocotyl (lower part) | 0.69 |

Relative concentrations of phytochrome were measured by the bichromatic method of CHANCE (4), comparing the absorption changes at 740 mμ, resulting from the transformation, with those at 792 mμ. The pigment transformations were induced by irradiating the samples in the spectrophotometer with 660 or 735 mμ, isolated from the light of a 500 Watt slide projector, with the aid of interference filters. In this paper, the term "phytochrome", will be applied only to the pigment that is detected and measured in this way.

## EXPERIMENTAL RESULTS

In dark-grown mesocotyls, only $P_{660}$ can be detected. In the isolated parts, the concentration of this form remains approximately constant during a period of about 2 days in continuous darkness at 22 °C. Increasing (small) dosages of red radiation cause a gradual conversion of $P_{660}$ into $P_{730}$, as shown in figure 1a.

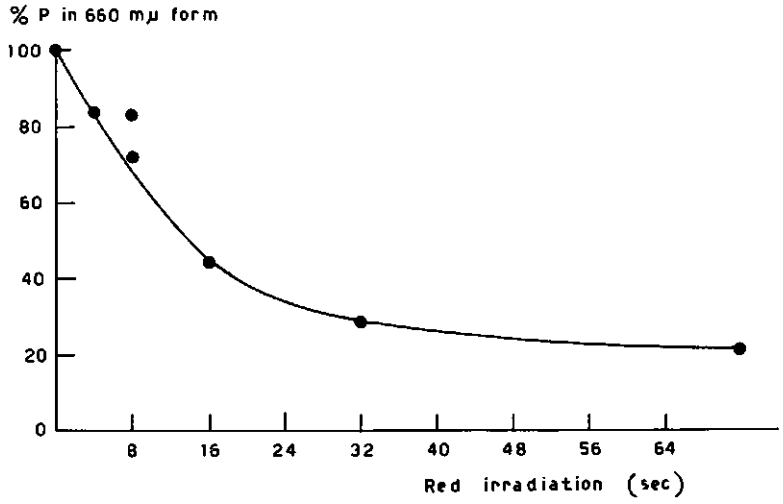


FIG. 1a. Conversion of $P_{660}$ in isolated mesocotyls by red radiation of 2500 ergs/cm² sec.

At the low concentrations of pigment, present in the organs, light absorption by phytochrome may be assumed to be proportional to its concentration.

Therefore, the rate of conversion of $P_{660}$ by light of a wavelength, not absorbed by $P_{730}$, may be assumed to be proportional to the concentration of the absorbing substance:

$$\frac{dx}{dt} = k(l - x),$$

where 1 — x represents the remaining fraction of the pigment in its 660 mμ form Therefore,

$$\log(l - x) = -kt.$$

As explained in the discussion, it is impossible to convert the two forms of phytochrome completely into each other. In the present case, about 20% of the phytochrome remained in the 660 mμ form after saturating irradiation with red light. After subtracting this amount from the data of figure 1a, the resulting values werwe replotted according to the above equation in figure 1b.

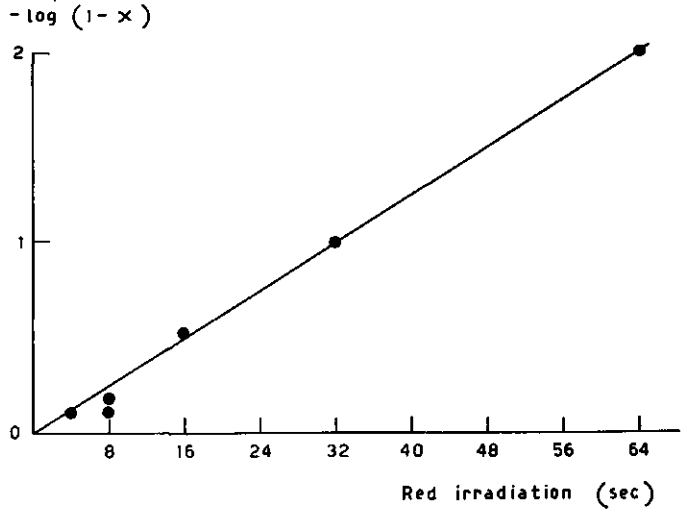


FIG. 1b. Conversion of $P_{660}$ as a function of red irradiation dosage; see text.

The equation is well satisfied, but it should be remarked that the result is very sensitive to relatively small errors in the estimation of the residual concentration of $P_{660}$ after saturating irradiation.

In a second group of experiments, mesocotyls were irradiated with red light for increasing periods, and kept in the dark subsequently for some hours, after which the total concentration of phytochrome was measured. A representative experiment is shown in figure 2.

In this type of experiment, increasing conversion of $P_{660}$ is followed by a decrease in the total concentration of both forms of phytochrome. This suggests destruction of pigment, following conversion to $P_{730}$. As, immediately after irradiation, the $P_{730}$ formed can be transformed back quantitatively into $P_{660}$ by far red light, this destruction must be due to a further conversion of $P_{730}$ in a comparatively slow dark reaction. The time course of this reaction was studied by first exposing mesocotyls to red light for 5 minutes, whereafter they were

kept in the dark for various times before total phytochrome was measured. Data of a number of experiments are combined in figure 3.

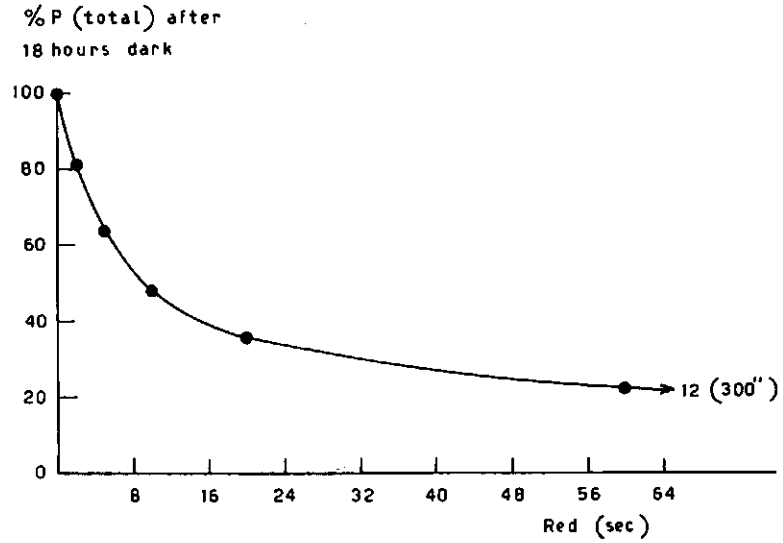


FIG. 2. Spectrophotometrically detectable phytochrome in mesocotyls, measured after 18 hours darkness at 20 °C, following irradiation with low dosages of red.

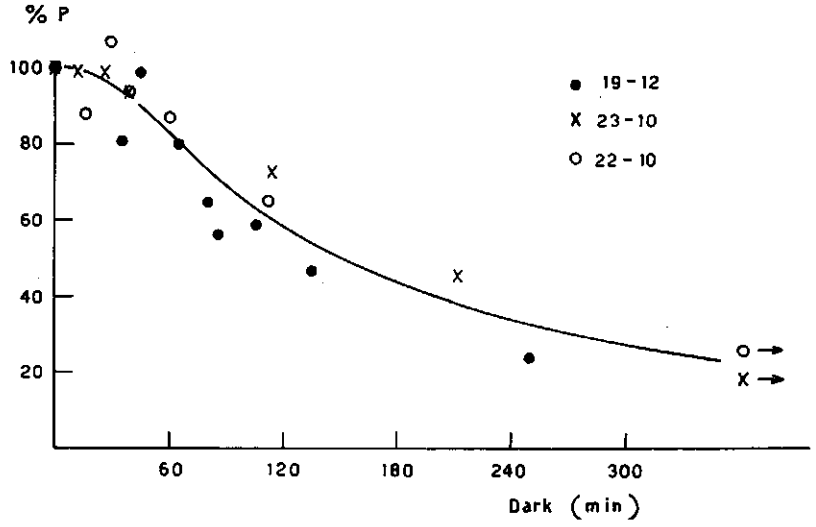


FIG. 3. Time course of total spectrophotometrically detectable phytochrome following saturating red irradiation of mesocotyls.

Obviously, this curve does not fit a first order reaction. For 30–45 minutes after irradiation, no decrease in total phytochrome can be observed. At 22 °C, the reaction is about complete when 20% of the original phytochrome remains. This fraction is in the 660 mμ form and reacts to a repeated red irradiation in

the same way as the original amount of pigment so that, after another long dark period, only a few percent of the phytochrome remains (table 2).

TABLE 2. Phytochrome reduction following saturating red irradiation. *Zea mays* L., cv. "CIV 7", grown in darkness at 22 °C.

| Red irradiation (minutes) | Dark time between red irradiation and measurement (hours) | Absorption changes |
|---|---|---|
| First 5 | 0 | 0.91 |
| | 12 | 0.22 |
| Second 5 | 0 | 0.18 |
| | 4 | 0.08 |

Within the experimental variation, both irradiations reduced phytochrome to the same percentage of the concentration present before irradiation.

Surprisingly, continued irradiation with red light during a period of 4 hours resulted in an only slightly lower concentration than 10 minutes red plus 4 hours dark (table 3). Far red radiation immediately following 10 minutes of red light to a large extent protected phytochrome against dark destruction.

TABLE 3. Phytochrome conc. in mesocotyls after various combinations of red and far red irradiations and subsequent dark periods. *Zea mays* L., cv. "Goudster", grown in darkness at 20 °C.

| Light treatment (minutes) | Dark time between red irradiation and measurement (hours) | Phytochrome conc. in rel. units |
|---|---|---|
| Dark control | 0 | 0.54/0.50 |
| Darkness | 4* | 0.74/0.60 |
| Darkness | 27* | 0.56/0.54 |
| 10 red | 0 | 0.67/–.— |
| 240 red | 0 | 0.10/0.09 |
| 10 red | 4 | 0.13/0.16 |
| 10 red immediately followed by 10 far red | 4 | 0.49/0.47 |
| 1 red | 27 | 0.17/0.19 |

* hours after isolation of mesocotyls from the seedlings.

Finally, it was observed that disappearance of phytochrome in the dark after red light treatment is strongly temperature dependent (table 4).

TABLE 4. Dark destruction of phytochrome at two temperatures following saturating red irradiation. *Zea mays* L., cv. "Goudster".

| Treatment | Temperature °C | Absorption changes after | |
|---|---|---|---|
| Irradiation | | 12 hours | 3 days |
| darkness | 2 | 0.63/–.— | 0.43/0.50 |
| darkness | 20 | 0.61/0.46 | 0.35/0.31 |
| 1 minute red | 2 | 0.60/0.66 | 0.45/0.36 |
| 1 minute red | 20 | 0.20/0.29 | 0. /0. * |

* almost no detectable changes left.

On the other hand, the phytochrome in non-irradiated mesocotyls, either isolated or attached to the intact plant proved stable for more than 24 hours at 20 °C in the dark.

## Discussion

The data presented lead to the conclusion that $P_{730}$ in the mesocotyls of dark-grown corn seedlings is instable and converted into a photochemically inactive form by a temperature sensitive dark process. In view of the fact that purified solutions of both forms of phytochrome, isolated from corn shoots, proved to be about equally stable in the dark, one is led to the conclusion that the intact tissue contains an enzyme, specific for the destruction of $P_{730}$. Disappearance of phytochrome in plant parts, therefore, is not an effect accompanying the reversible interconversion of $P_{660}$ and $P_{730}$, but merely a function of the presence of the latter only. This destruction reaction does not appear to be first order in corn mesocotyls at 22 °C; there seems to be a lag period between the formation of $P_{730}$ and the start of its disappearance.

We have found no evidence for a dark reconversion of $P_{730}$ into $P_{660}$ as is suggested by Hendricks *c.s.* (5, 6). It is true that, after a saturating irradiation of mesocotyls with "red" light, the total concentration of phytochrome does not reach zero even after a long dark period and that the 20 % phytochrome remaining is in the 660 mμ form. In our opinion, however, this must be due to the impossibility to completely interconvert the two forms of phytochrome. In the first place, the absorption spectra overlap to some extent, and while the absorption of one form at an intermediate wavelength decreases, that of the other increases at the same time. This effect leads to an equilibrium, and becomes more pronounced when the irradiation is not done with purely monochromatic light. For practical reasons, we had to do the initial red irradiations of the dark-grown mesocotyls with red light, obtained from fluorescent tubes in combination with a suitable glass filter (3). The contribution to this radiation of wavelengths within the absorption band of $P_{730}$ was sufficient to explain the observed level of unconverted $P_{660}$. However, it is obviously impossible to demonstrate the complete absence of any reconversion of $P_{730}$ into $P_{660}$. But if this occurs, it certainly can involve only a small fraction of the original amount of phytochrome.

## Summary

Relative total concentrations of phytochrome and of the two forms $P_{660}$ and $P_{730}$ were determined in dark-grown mesocotyls of maize, by measuring the changes in light absorption at 740 and 792 mμ. Essentially, the results of the Beltsville workers could be confirmed. However, we have not been able to demonstrate appreciable dark conversion of $P_{730}$ into $P_{660}$. On the other hand, after illumination, the total phytochrome concentration is reduced owing to instability of $P_{730}$ in the plant. About 30 minutes after $P_{730}$ is formed, destruction begins and is virtually complete in 4 hours at 22 °C.

## LITERATURE


1. BUTLER, W. L., K. H. NORRIS, H. W. SIEGELMAN and S. B. HENDRICKS: Proc. N.A.S., U.S. **45,** 1703–1708 (1959).
2. BUTLER, W. L. and R. J. DOWNS: Scientific American **203,** 56–63 (1960).
3. STOLWIJK, J. A. J.: Meded. Landbouwhogeschool Wageningen, **54,** 181–244 (1954).
4. CHANCE, B.: Rev. Sci. Instr. **22,** 634–638 (1951).
5. HENDRICKS, S. B.: Cold Spring Harbor Symp. **25,** 245–248 (1960).
6. HENDRICKS, S. B., W. L. Butler and H. W. Siegelman: J. Phys. Chem **66,** 2550–2555 (1962).