Planta 136, 131–134 (1977)

Planta

Action Spectra for the Inhibition of Growth in Radish Hypocotyls

Ann M. Jose and Daphne Vince-Prue

Department of Botany, University of Reading, Whiteknights, Reading, RG6 2AS, U.K.

Abstract. In etiolated seedlings of *Raphanus sativus* L. the inhibition of hypocotyl elongation by continuous light showed a major bimodal peak of action in the red and far-red, and two minor peaks in the blue regions of the spectrum. It is argued that, under conditions of prolonged irradiation, phytochrome is the pigment controlling the inhibition of hypocotyl elongation by red and far-red light, but that its mode of action in far-red is different from that in red. A distinct pigment is postulated for blue light.

Key words: Action-spectrum – Blue-light photoreceptor – High-irradiance-response – Photomorphogenesis – Phytochrome – *Raphanus*.

Introduction

There are two basic criteria for the operation of a phytochrome induction reaction: that the effect of a brief R irradiation be reversed by a subsequent FR light pulse, and that the response exhibits reciprocity. High irradiance reactions (HIR) are most simply defined as those photoresponses for which reciprocity does not hold. Consequently, photoresponses which *require* irradiation over a long period cannot be considered as simple induction reactions.

HIR action spectra for anthocyanin accumulation show peaks in the B and over a wide range of R and FR wavelengths (see Smith, 1972). The responses of hypocotyl growth have, however, generally been ascribed to the B and FR regions, the effects of R commonly being confounded by the existence of two responses – a promotion and an inhibition (see Jose, 1977). For example, in Grand Rapids lettuce hypocotyls of a certain age, promotion by R in the apical regions almost equalled inhibition in subapical parts, giving no difference in overall length from dark controls (Häcker er al., 1964). This apparent lack of a response to R light in lettuce, resulting from the use of a single measurement of growth increment, has given rise to some confusion. Because of the excellent detail published for the action spectrum of lettuce (Hartmann, 1967b) it has become incorporated into concepts and models of phytochrome action to such an extent that considerations of effects of R light are almost completely confined to simple induction responses (e.g. Mohr, 1972; Smith, 1975).

Elongation growth of etiolated radish hypocotyls is strongly inhibited by continuous R as well as by B and FR wavebands. Phytochrome inductive effects are very small, as is the amount of apical promotion by R light compared with its inhibitory effects (Jose, 1977). Therefore a detailed study of irradiance response relationships was made in radish to compare the mode of action of light in the R, B and FR wavebands under conditions of continuous irradiation.

Materials and Methods

Raphanus sativus L. cv. Scarlet Globe (Suttons Seeds Ltd.) seedlings were raised in darkness on moist cellulose wadding (Boots, Nottingham) at 25° C for 72 h. Hypocotyl length was then measured and the seedlings were transplanted to one end of small transparent polystyrene boxes. In this way the plants were limited to a volume 2 cm × 1 cm × 6 cm throughout the experiment. There were ten seedlings per sample, and the treatments were repeated at least once. Standard errors of the mean were less than 1%. At 72 h the boxes were positioned at stations along light gradients. The plants were irradiated for 24 h at 25° C and the total growth of the hypocotyl during this period, Δmm (96–72 h), was recorded. All manipulations of the plant material were carried out in dim green safelight (Jose and Vince-Prue, 1977).

Abbreviations: B = blue; FR = far red; G = green; R = red; HIR = high irradiance reaction; P_r and P_{fr} = red and far red absorbing forms of phytochrome; R = red.

Standard slide projectors fitted with G.E.C. tungsten halogen lamps (type A1/216 24V, 150W) were used as sources (90% emission 450 to 750 nm). Al interference filters (Schott and Gen., Mainz; with approximately 20% transmission outside the nominal $^1/_2$ max band width of 20 nm) with λ max between 400 and 780 nm, were mounted immediately before the focussing lens. The λ_{max} of each optical system was measured using an ISCO spectroradiometer. Irradiances were measured with a Hewlett-Packard model 8334 A radiant flux detector in conjunction with a model 8330 A radiant flux meter. Irradiance was varied by distance only (from 0.1 to 4 Wm^{-2}, the upper limit depending on wavelength).

Results

Irradiance response curves were constructed for hypocotyl elongation, Δmm (96 h–72 h), against log irradiance (E_e) for each λ_{max} studied (Fig. 1). The response curves varied greatly, falling into three main groups:

B/G (425–550 nm): At these wavelengths there was a steep slope in the response to irradiance with a fairly clearly defined cut-off for some wavelengths at both high and low irradiance values.

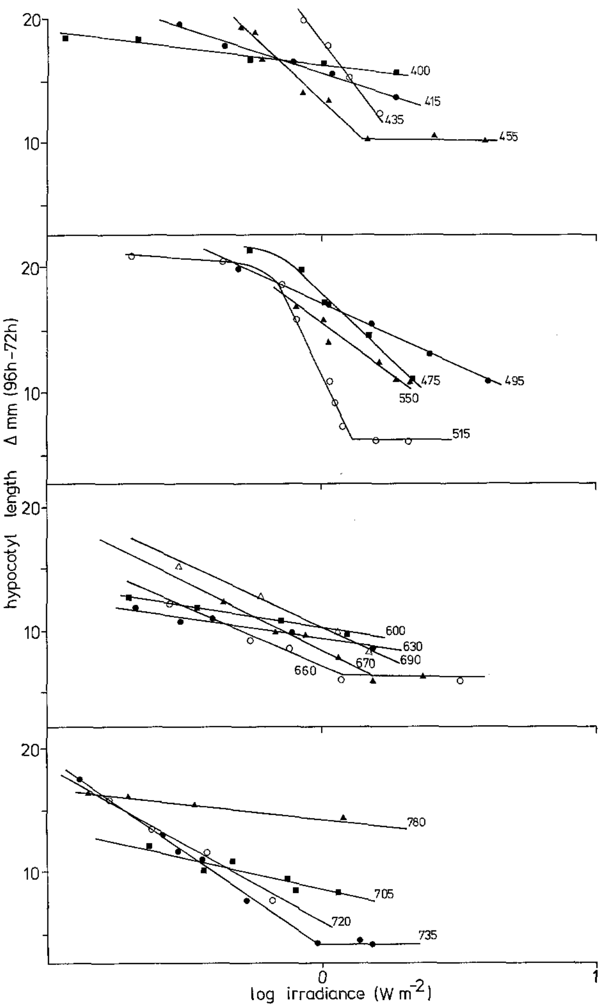

Fig. 1. Irradiance response curves of hypocotyl growth during 24 h continuous irradiation with different wavebands. Mean growth in darkness was 21.4 mm

R (645–690 nm): At these wavelengths the slope of the response to irradiance was much less steep with no evidence of a threshold irradiance as found for B/G. The saturating irradiance (660 nmm) was similar to that in B/G (515 nm and 455 nm).

FR (710–735 nm): The slope of the response to irradiance was slightly steeper than in R, but much less steep than in B/G. Saturation (735 nm) occurred at a somewhat lower irradiance than in R and B/G, and there was no evidence of a threshold irradiance within the limits of these experiments.

These regions were separated by wavebands with very flat irradiance-response slopes, characterised by high saturation irradiances which were not reached in these experiments.

For particular levels of inhibition (35% and 50%) irradiance values were noted for each λ_{max}. These were corrected for wavelength according to Planck's quantum law:

$$N\lambda \ \alpha \ E_{e\lambda}.\lambda$$

when N_λ is the total number of incident quanta. $E_{e\lambda}.\lambda$ was used as a measure of N_λ. For the two levels of inhibition the reciprocals of the $E_{e\lambda}.\lambda$ values were expressed as the fraction of the reciprocal of the lowest value $(E_{e\lambda}.\lambda)_{min}$:

$$\frac{(E_{e\lambda}\cdot\lambda)_{min}}{(E_{e\lambda}\cdot\lambda)_\lambda} = q(\lambda)\,\mathrm{rel}$$

known as the relative quantum effectiveness. These values were plotted against λ_{max} for 35% and 50% growth inhibition, giving the action spectra shown in Figure 2.

All wavelengths tested caused considerable inhibition of elongation. There were two minor peaks of action in the B region (455 nm and 515 nm). The R/FR region showed a major bimodal peak of action

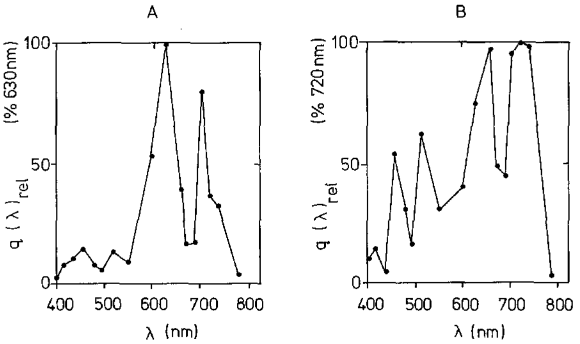

Fig. 2. Action spectra for (*A*) 35% and (*B*) 50% inhibition of hypocotyl growth during 24 h continuous irradiation from 72 h

with maxima between 630 nm and 660 nm and between 705 nm and 735 nm.

Discussion

In etiolated radish, the action spectrum for the inhibition of hypocotyl elongation by continuous irradiation resembles that for anthocyanin accumulation in both turnip (Siegelman and Hendricks, 1957; Grill and Vince, 1970) and mustard, as well as for inhibition of hypocotyl elongation in mustard (Mohr, 1957), having peaks in the B and a major bimodal peak of action in the R/FR region. In Grand Rapids lettuce the dual growth effects of R light (i.e. promotion of the apical zone and inhibition of the subapical regions) may completely cancel each other out (Häcker et al., 1964); therefore, at a certain stage of development, there is almost no apparent response to R light (Hartmann, 1967b) but strong inhibition by B and FR.

In an ideal action spectrum there are no cooperative or interfering secondary effects and the dose response curves are parallel. Therefore the action spectrum is the same for any size of response. In our experiments large variations in the slopes of the irradiance response curves were obtained. These may be a result of self screening, but this is difficult to evaluate. Alternatively, and more likely, they could from result the interaction of two or more pigment systems. The R and FR maxima (660 nm and 735 nm for 50% inhibition) have different irradiance response curves, the FR maximum having the steeper slope. A similar difference has been reported for mustard (Wagner and Mohr, 1966). Non identity of the physiological responses to broad band R and FR light has been demonstrated (Jose, 1977). The irradiance response curves for B wavebands were distinct from those for R and FR light, as is the physiological response itself (Jose, 1977). Lettuce hypocotyl hook opening showed a similar saturation irradiance difference between B and FR (Turner, 1969), although identical saturation irradiances for broad band B and FR have been reported for mustard anthocyanin accumulation (Wagner and Mohr, 1966) and lettuce hypocotyl elongation (Turner, 1969). However, there is always a problem in assessing the irradiance of physiologically active FR from a broad band source.

Mode of Action of FR

Hartmann (1966, 1967a) has presented good evidence that phytochrome is the pigment for the FR HIR in the control of lettuce hypocotyl elongation. Schäfer (1975) suggested that this operates through the first bound product of P_{fr}, $P_{fr}X$.

Mode of Action of R

There is no evidence against phytochrome mediation of the response to continuous R light. From experiments on the loss of the FR HIR in radish during de-etiolation, we have concluded that there are two modes of operation of phytochrome in R light, namely an induction reaction requiring short exposures, and a second reaction which only occurs in continuous light or with frequent short irradiations. The second reaction was thought to involve cycling of the phytochrome pigment system, perhaps the relaxation from $P_{fr}X$ to $P_{fr}X^{X'}$(Jose and Vince-Prue, 1977). It seems likely that the inhibition of elongation by continuous R light may also result from this second reaction of phytochrome and require pigment cycling.

Mode of Action of B

Kinetic studies at subsaturating irradiances of B showed that growth inhibition in lettuce and radish was transient, persisting for a period, the length of which depended on the irradiance (Turner, 1969; Jose, 1975). A possible explanation is that two separate responses are involved: a low energy response causing transient growth inhibition (probably via phytochrome) and, at irradiances above a certain threshold value, a high irradiance response inhibiting growth for the duration of the irradiation. Attempts have been made to explain B HIR in terms of phytochrome (Hendricks and Borthwick, 1959; Hartmann, 1966). The wide variation in relative effectiveness of the B and FR peaks of a number of HIR are explained better in terms of two pigment systems (Heath and Vince, 1962) and it is almost certain that the B HIR are not mediated through phytochrome.

Therefore, under conditions of prolonged irradiation, R and FR light may both control hypocotyl elongation of etiolated seedlings through different modes of action of phytochrome, but there is a distinct pigment for B light.

This work was supported by Ministry of Agriculture, Fisheries and Food grant No. AE 6819.

References

Grill, R., Vince, D.: Photocontrol of anthocyanin synthesis in turnip seedlings. VIII Wavelength dependence. Planta (Berl.) **95**, 264–271 (1970)

Häcker, M., Hartmann, K.M., Mohr, H.: Zellteilung und Zellwachstum im Hypocotyl von *Lactuca sativa* L. unter dem Einfluß des Lichtes. Planta (Berl.) **63**, 253–268 (1964)

Hartmann, K.M.: A general hypothesis to interpret 'high energy phenomena' of photomorphogenesis on the basis of phytochrome. Photochem. Photobiol. **5**, 349–366 (1966)

Hartmann, K.M.: Photoreceptor problems in photomorphogenic responses under high-energy-conditions (UV – blue – far-red). In: Book of Abstracts, Europ. Photobiology Symp., Hvar (Jugoslavia) 29–31 (1967a)

Hartmann, K.M.: Ein Wirkungsspektrum der Photomorphogenese unter Hochenergiebedingungen; seine Interpretation auf der Basis des Phytochroms (Hypokotylwachstumshemmung bei *Lactuca sativa* L.). Z. Naturf. **22**, (B), 1172–1175 (1967b)

Heath, O.V.S., Vince, D.: Some non-photosynthetic effects of light on higher plants with special reference to wavelength. Symp. Soc. exp. Biol. **16**, 110–137 (1962)

Hendricks, S.B., Borthwick, H.A.: Photocontrol of plant development by the simultaneous excitation of two interconvertible pigments. II Theory and control of anthcyanin synthesis. Bot. Gaz. **120**, 187–193 (1959)

Jose, A.M.: Light-induced changes in the photoresponses of plant stems. Ph.D. thesis, University of Reading (1975)

Jose, A.M.: Photoreception and photoresponses in the radish hypocotyl. Planta **136**, 125–129 (1977)

Jose, A.M., Vince-Prue, D.: Light induced changes in the photoresponses of plant stems; the loss of a high irradiance response to far-red light. Planta **135**, 95–100 (1977)

Mohr, H.: Der Einfluß Monochromatischer Strahlung auf das Längenwachstum des Hypocotyls und auf die Anthocyanbildung bei Keimlingen von *Sinapis alba* L. (= *Brassica alba* Boiss). Planta (Berl.) **49**, 389–405 (1957)

Mohr, H.: Lectures on photomorphogenesis. Berlin-Heidelberg-New York: Springer 1972

Schäfer, E.: A new approach to explain the „high irradiance responses“ of photomorphogenesis on the basis of phytochrome. J. Math. Biol. **2**, 41–56 (1975)

Siegelman, H.W., Hendricks, S.B.: Photocontrol of anthocyanin formation in turnip and red cabbage seedlings. Plant Physiol. **32**, 393–398 (1957)

Smith, H.: The photocontrol of flavonoid biosynthesis. In: Phytochrome pp. 433–481, Mitrokos, K., Shropshire, Jr., W., eds. New York: Academic Press 1972

Smith, H.: Phytochrome and photomorphogenesis. London: McGraw Hill 1975

Turner, M.R.: Photomorphogenesis in the lettuce seedling. Ph. D. thesis, University of Reading (1969)

Turner, M.R., Vince, D.: Photosensory mechanisms in the lettuce seedling hypocotyl. Planta (Berl.) **84**, 368–382 (1969)

Wagner, E., Mohr, H.: Kinetic studies to interpret 'high energy phenomena' of photomorphogenesis on the basis of phytochrome. Photochem. Photobiol. **5**, 397–406 (1966)

Received 8 February; accepted 4 May 1977