Inhibitory action of red light on the growth of the maize mesocotyl: evaluation of the auxin hypothesis

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Abstract. Brief irradiation of 3-d-old maize (Zea mays L.) seedlings with red light (R; 180 J m⁻²) inhibits elongation of the mesocotyl (70–80% inhibition in 8 h) and reduces its indole-3-acetic acid (IAA) content. The reduction in IAA content, apparent within a few hours, is the result of a reduction in the supply of IAA from the coleoptile unit (which includes the shoot apex and primary leaves). The fluence-response relationship for the inhibition of mesocotyl growth by R and far-red light closely resemble those for the reduction of the IAA supply from the coleoptile. The relationship between the concentration of IAA (1–10 μM) supplied to the cut surface of the mesocotyl of seedlings with their coleoptile removed and the growth increment of the mesocotyl, measured after 4 h, is linear. The hypothesis that R inhibits mesocotyl growth mainly by reducing the IAA supply from the coleoptile is supported. However, mesocotyl growth in seedlings from which the coleoptiles have been removed is also inhibited by R (about 25% inhibition in 8 h). This inhibition is not related to changes in the IAA level, and not relieved by applied IAA. In intact seedlings, this effect may also participate in the inhibition of mesocotyl growth by R. Inhibition of cell division by R, whose mechanism is not known, will also result in reduced mesocotyl elongation especially in the long term (e.g. 24 h).

Key words: Auxin – Light (red) and growth – Mesocotyl (growth inhibition) – Phytochrome and auxin – Zea (auxin and light).

Introduction

Growth of the mesocotyl of etiolated cereal seedlings is inhibited upon exposure to light, especially red light (R) (e.g. Johnston 1937; Goodwin 1941; Weintraub and McAlister 1942), and phytochrome has been shown to be a photoreceptor (Loercher 1966; Duke et al. 1977). More detailed studies, however, have demonstrated complexities: (1) the fluence-response curves consist of three phases, only one of which is under photoreversible phytochrome control (Blaauw et al. 1968; Mandoli and Briggs 1981); (2) the time course of growth under continuous exposure to R showed two steps of inhibition (Vanderhoef et al. 1979); (3) action spectra showed two peaks in the red region (Weintraub and Price 1947; Vanderhoef et al. 1979). It has also been shown that light inhibits both elongation and division of mesocotyl cells (Avery et al. 1937; Araki 1939; Goodwin 1941; Thomson 1954; Mer and Causton 1967); inhibition of cell division was apparent with brief R irradiation (Goodwin 1941; Mer and Causton 1967). Light inhibition of mesocotyl growth is thus complex, and probably involves several different mechanisms.

Went (1928) and van Overbeek (1936) held that light inhibits mesocotyl growth by reducing its auxin supply from the coleoptile tip, believed to be the site of auxin production in the shoot. Van Overbeek showed that illumination of oat seedlings in fact reduced the yield of diffusible auxin from the excised coleoptile tip. This was confirmed by various investigators specifically using R (see Iino 1982 for references). Kondo et al. (1969) were able to detect a decrease in diffusible auxin from the base of the coleoptile of oat seedlings following R irradiation. These effects of R on diffusible auxin yields have recently been confirmed by Iino (1982) using maize seedlings and specific physico-
chemical determinations of IAA. Iino and Carr (1982b) have further shown that the IAA in the mesocotyl comes mainly from the coleoptile, and the apical, rapidly growing region of the mesocotyl depends almost entirely on the coleoptile for its IAA. The fluence-response relationship between R or far-red light (FR) and the yield of diffusible IAA (Iino 1982) showed a resemblance to that for oat mesocotyl growth (Blaauw et al. 1968; Mandoli and Briggs 1981). Thus, reduction in IAA supply from the coleoptile is consistent with inhibition of mesocotyl elongation by R. Applied IAA has also been shown to counteract the inhibitory effect of R on mesocotyl elongation (Vanderhoef and Briggs 1978).

The actual effect of R on IAA content of the mesocotyl remains, however, to be studied. Duttaray and Mer (1964), measuring the content daily following a single brief irradiation, reported that the auxin content of oat mesocotyls was not reduced by R. Nevertheless, the effect of such a brief R treatment in reducing the diffusible IAA yield from the base of the coleoptile is transient, the lowest yield being found at around 5–6 h after the irradiation (Iino 1982). It is therefore necessary to investigate this R effect over much shorter periods than a whole day. Other literature data also appear to be inconsistent with the auxin hypothesis. Schneider (1941) concluded that inhibition of mesocotyl growth is direct and not through modulation of the auxin supply from the coleoptile, by showing that R inhibited elongation of excised mesocotyl segments and that this inhibition was not relieved by applied IAA. Huisinga (1964) and Vanderhoef and Briggs (1978) observed a similar inhibition of the elongation of excised mesocotyl segments by R. Although these authors concluded that the main mechanism for the inhibition of mesocotyl growth nevertheless involved auxin, there appears to be some evidence for a growth-inhibiting photomorphogenetic system which does not directly involve auxin. Also, there is no evidence that light inhibition of cell division in the mesocotyl is mediated by auxin.

The experiments described in this paper were undertaken to characterize the inhibition of maize mesocotyl growth by R further, and to re-examine the validity of the auxin hypothesis.

Material and methods

*Plant material; light sources; IAA estimation.* Etiolated seedlings of maize (*Zea mays* L. cv. GH 390: a field corn produced at the Agricultural Plant Breeding Station, Grafton, N.S.W., Australia) 3 d old, were raised on moist paper towels in seedling boxes (Iino and Carr 1981; Iino 1982). During experimental treatments to seedlings, objects were visualized, when required, in the dark using infrared radiation and an infrared-scope (Iino and Carr 1981; Iino 1982). Dim green light was used only in strictly limited instances (for the use of green light, see Iino and Carr 1981; Iino 1982).

Red and far-red light were obtained as described in Iino (1982). The content of free IAA was estimated as described in Iino and Carr (1982a), using the indole-α-pyron fluorescence method (see also Iino et al. 1980; Iino 1982).

*Measurement of mesocotyl elongation.* Elongation of the whole mesocotyl was measured as previously reported (Iino and Carr 1981). For measurements of zonal elongation, the mesocotyl was marked with India ink, applied using threads fixed at intervals on a frame. Following incubation for a given period, the marked zones were excised and their length was recorded (10-fold magnification, photographic image).

*Counting of cell numbers.* Mesocotyl segments were fixed in a mixture of formaldehyde (40%)-acetic acid-ethanol (70%) (5:5:90, by vol.) (FAA), and embedded in paraffin. Median longitudinal sections (14 μm) were stained with toluidine blue O (Polysciences, Warrington, Pa., USA). Cell numbers of the fifth row of cortical cells from the epidermis were counted. The mean cell number of each mesocotyl segment was obtained from counts made on each side of each of three sections.

**Results**

1. *Mesocotyl growth in intact seedlings; effect of R.* The mesocotyls of 3-d-old etiolated maize seedlings, raised under the conditions chosen, elongated at a constant rate (1.8 mm/h) for at least a day (the mesocotyl length 3-d after sowing was about 35 mm). When 3-d-old seedlings were irradiated with 180 J m⁻² R (10 min), which saturates spectrophotometrically measurable in-vivo *P₆₅₀*, formation at the coleoptile tip and in the apical portion of the mesocotyl (Iino 1982), the elongation rate of the mesocotyl was reduced within 4 h to about 20% of that of the dark control. The rate of elongation remained depressed from 4 to 10 h after the irradiation, but then started to increase gradually over about 6 h and reached a nearly constant rate, 50–60% of that of the dark control, which lasted for at least another 12 h.

Figure 1 shows time courses of elongation of mesocotyl zones. In non-irradiated seedlings, the apical 6-mm zone continued to elongate, becoming five times as long in 18 h. The elongation rate of this zone showed an increase during the first about 8 h. The elongation rate of the second 6-mm zone (6–12 mm) decreased gradually over a period of 18 h. The initial elongation rate of this zone, however, was about the same as that of the apical zone. When marking took place, the third 6-mm zone (12–18 mm) was about to cease elongation. Thus, the high elongation rate is confined to the apical approx. 12 mm, and below this zone the rate falls