Photoreceptive sites in the photocontrol of oat seedling growth

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Abstract

The influence of red light on the growth of coleoptiles and mesocotyles of etiolated and pre-irradiated oat seedlings was investigated. Red light (15 min.) applied on whole seedlings stimulated the elongation of coleoptiles and inhibited the growth of mesocotyles both in etiolated and in pre-irradiated plants. Irradiation with red light (2 min) of various 2-mm-long regions of etiolated and pre-irradiated oat seedlings was carried out in order to locate their light reception regions. On the basis of growth reactions after such treatment it was found that in completely etiolated seedlings the light reception sites involved in the stimulation of coleoptile elongation and inhibition of mesocotyle growth lie directly above and below the seedling node, whereas in pre-irradiated seedlings, in the top of the seedling. These results point to the existence of different growth photoregulation systems in etiolated and pre-irradiated oat seedlings. The role of phytochrome in these phenomena is discussed.

Key words: photomorphogenesis, elongation growth, oat seedlings, oat coleoptiles, oat mesocotyles, photoreceptive sites

INTRODUCTION

It is generally known that short irradiation with red light stimulates elongation of coleoptiles and inhibits elongation of mesocotyles of etiolated oat seedlings. It is also known that these processes are under the control of a phytochrome system (Gaba and Black 1983). Up to now, however, it is not

known exactly where the red light receptor region of etiolated seedling lies. An earlier attempt to locate this site did not bring unequivocal results since they indicated either the top or the node of the seedling (Goodwin 1941). Later investigations showed that the photoreception site was the region adjacent to the seedling node (Mandoli and Briggs 1982). It seems significant, however, that in such investigations green light was commonly applied and 24-hour-old seedlings were often irradiated intentionally with red light. Indiscriminate exposure to green light followed from the opinion that it is photomorphogenetically inactive. On the other hand, the intentional irradiation of young seedlings with red light was imposed by practical considerations. This light does not influence the growth of the coleoptile while at the same time inhibits the growth of the mesocotyle. Thus, such treatment made it possible to get straightened seedlings of standardized length.

Recent years, however, have brought data showing that green light may also substantially influence the growth of etiolated oat seedlings (Iino and Carr 1981). It also seems possible that the application of red light on very young seedlings may change the reaction of these plants to red light applied the next time.

It can thus be supposed that the divergence of the results of investigations concerning the location of red light reception in the photoregulation of etiolated oat seedlings growth might be, at least to a certain degree, caused by the exposure to green light during the investigations as well as by the accidental or intentional irradiation of very young seedlings with red light.

Thus, the aim of our present investigations was to compare the sites of red light reception in the photocontrol of growth of completely etiolated and pre-irradiated oat seedlings.

MATERIAL AND METHODS

Caryopses of oat (*Avena sativa* L. var. Diadem) were soaked in distilled water for 2 hours. Swollen seeds were placed embryo-up on 0.8% agar filling a 20 cm³ glass dish, one seed in the middle of each dish. Germination of caryopses and cultivation of seedlings were conducted in darkness at +26°C and relative humidity of 85%. During the 24th hour of growth, one-half of the seedlings were subjected to 30-minute-long irradiation with red light (pre-irradiation), and afterwards were cultivated further in darkness. Starting from the 48th hour of growth, samples of 100 etiolated and pre-irradiated seedlings were gathered at hour-long time intervals and the whole seedlings were exposed to red light for 15 minutes. The length of coleoptiles and mesocotyles was measured 24 hours after irradiation with an accuracy of 1 millimetre.

The investigations on the localization of red light reception in the growth processes used 66-hour-old etiolated and pre-irradiated oat seedlings. The length of coleoptiles and mesocotyles was measured 24 hours after irradiation.

Optical fibers giving an illumination field 2 mm in diameter were applied. Two optical fibers were mounted opposite each other on a light microscope instead of a movable tube to make their displacement easy by means of a tube shift screw. The examined seedling was placed on the microscope stage. This arrangement allowed irradiation of a 2-mm-long region from opposite sides in an arbitrarily selected place of the seedling (Fig. 3). The irradiation with red light of the given region through optical fibers was conducted for 2 minutes, repeating the irradiation of the same region in at least 30 morphologically identical seedlings.

The measurements of the seedling lengths were conducted 24 hours after irradiation with an accuracy of 1 millimetre. The presented results are taken from threefold repetitions of every experiment. The standard error for the groups of examined seedlings did not exceed 7 per cent.

The source of red light (660 nm) for the irradiation of the whole seedlings was a Diaprex B-10 slide projector, equipped with a 250 W halogen lamp, giving an irradiation intensity of 1.08 W m^{-2} on the seedling surface. The source of red light for the irradiation through optical fibers was a low-voltage incandescent lamp with flat core filament (6 V/15 W) with an added optical system and a holder for optical fibres, producing a radiation intensity of 7.0 W m^{-2} on the seedling surface. Light monochromatization was obtained using interference filters ($\lambda = 660 \text{ nm}$, $\lambda_{1/2} = 7 \text{ nm}$, $T_{\text{max}} = 60\%$).

All the activities during the work on seedlings were carried out in the presence of physiologically inactive infra-red light (Iino and Carr 1981) with the assistance of an active red light noctovisor (night vision device). The source of infra-red light having a wavelength above 1000 nm was an incandescent lamp (25 W), which gave a radiation intensity of 0.5 W m^{-2} on the seedling surface. The radiation intensity was measured by means of a linearly scaled Kipp's thermopile with an accuracy of $5 \times 10^{-2} \text{ W m}^{-2}$.

RESULTS AND DISCUSSION

Thirty minute irradiation with red light of 24-hour-old oat seedlings (pre-irradiation) did not change the growth rate and the final length of coleoptiles, causing, however, about 40% shortening of the final length of the mesocotyles (Fig. 1). The growth reactions of etiolated and pre-irradiated oat seedlings to red light applied for 15 minutes on the whole seedlings were generally similar. Red light stimulated the growth of coleoptiles of these seedlings between the 56th and 96th hour of growth and did not influence the growth of coleoptiles of younger and older seedlings (Fig. 2). The value of

Fig. 1. The growth of coleoptiles and mesocotyles of etiolated and pre-irradiated oat seedlings. The length of coleoptiles (Δ) and mesocotyles ($\circ$) of etiolated seedlings; the length of coleoptiles ($\blacktriangle$) and mesocotyles ($\bullet$) of pre-irradiated seedlings. The mean error of the measurements did not exceed 7 per cent

photostimulation of coleoptile growth is a function of seedling age and resembles a parabola with its maximum in the 66th hour of growth, the stimulation being higher in pre-irradiated seedlings (Fig. 2). At the same time, red light inhibited the growth of the mesocotyles of these seedlings. In etiolated plants, the magnitude of photoinhibition of mesocotyle growth increased with the age of the seedlings whereas in the pre-irradiated seedlings there was a complete suspension of their growth (Fig. 2). It is generally known that growth reactions of etiolated oat as well as other cereal seedlings to red light depend on the seedling age (Gaba and Black 1983), but there have been very few detailed investigations of this phenomenon. It seems that exposure of 24-hour-old oat seedlings to 30-minute-long pre-irradiation with red light does not change the general pattern of plant growth in darkness and growth reactions to red light applied for the second time. Such irradiation increases only the light sensitivity of coleoptiles and mesocotyles (Fig. 2).

In the investigations aimed at finding the red light reception site in the photocontrol of growth, 66-hour-old seedlings which demonstrate the maximum growth reaction to red light (Fig. 2) were used. As is known, both the top and the node of a young oat seedling contain increased amounts of phytochrome (Briggs and Siegelman 1965).

Fig. 2. The influence of red light irradiation (15 min.) on the growth of coleoptiles and mesocotyles of etiolated and pre-irradiated oat seedlings of different age. The length of coleoptiles (Δ) and mesocotyles ($\circ$) of etiolated seedlings; the length of coleoptiles ($\blacktriangle$) and mesocotyles ($\bullet$) of pre-irradiated seedlings. The length of organs was measured 24 hours after irradiation. The results were presented in proportion taking the length of organs of the seedlings not subjected to irradiation as 100%. The mean error of the measurements did not exceed 7 per cent.

Growth reactions of 66-hour-old completely etiolated oat seedlings to 2-minute-long irradiation with red light of different regions of a seedling showed that irradiation of the regions directly above and below the seedling node gave maximum stimulation of elongation of the coleoptile and strongly inhibited the growth of the mesocotyle (Fig. 3). The magnitude of these reactions was similar as in the case of 15-minute-long irradiation with red light of the whole 66-hour-old etiolated oat seedling (Figs. 2 and 3). On the other hand, irradiation of the seedling top did not influence the seedling growth at all (Fig. 3). Thus, the site of red light reception in the photoregulation of growth of an etiolated oat seedling is the region directly below and above the seedling node. The obtained data confirm the results of investigations by Mandoli and Briggs (1982) carried out on 72-hour-old etiolated oat seedlings without exposure to green light.

Growth reactions of 66-hour-old pre-irradiated oat seedlings to 2-minute-long irradiation with red light of different regions of the plant showed that

Fig. 3. The effect of red light irradiation (2 min.) of different regions of 66-hour-old etiolated and pre-irradiated oat seedlings on the growth of coleoptiles and mesocotyles. The length of coleoptiles (△) and mesocotyles (○) of etiolated seedlings; the length of coleoptiles (▲) and mesocotyles (●) of pre-irradiated seedlings. The length of organs was measured 24 hours after irradiation. The outline of seedlings presents the mean length of a caryopsis, mesocotyle and coleoptile of an etiolated (I) and pre-irradiated seedling (II). The results were expressed in proportion taking the length of organs of the seedlings not subjected to irradiation as 100%. The mean error of measurements did not exceed 7 per cent

the irradiation of the seedling top, in contrast to completely etiolated plants gave maximum stimulation of coleoptile growth and completely inhibited the growth of the mesocotyle (Fig. 3). The magnitude of these growth reactions was similar as in the case of 15-minute-long irradiation with red light of the whole 66-hour-old pre-irradiated seedlings (Figs. 2 and 3). On the other hand, the irradiation of other regions of the seedling stimulated the growth of the

coleoptile slightly and the magnitude of the reaction decreased as the irradiation was removed from the seedling top towards other regions, while still completely inhibited the growth of the mesocotyle regardless of the site of irradiation (Fig. 3). It seems then that the site of red light reception in the process of photoregulation of the growth of pre-irradiated oat seedlings is the top of the plant. It also seems that the obtained data may explain earlier suggestions on the localization of the site of red light reception precisely in the top of the seedling (Goodwin 1941). It is possible that these results were obtained as the consequence of the investigations carried out on seedlings subjected to uncontrolled irradiation in the early period of growth.

The obtained results allow us to assume that the pre-irradiation of young etiolated oat seedlings generally does not change the pattern of seedling growth in darkness and their growth reactions to red light applied for the second time. It establishes, however, a different pathway of photocontrol of seedling growth which only superficially resembles the original path existing in etiolated seedlings. Since the coleoptile top contains great amounts of phytochrome (Briggs and Siegelman 1965), investigations on the mechanism of the photocontrol of etiolated oat seedlings growth were dealing precisely with the metabolism of phytochrome in this coleoptile region (Kopcewicz et al. 1983). The contribution of phytochrome in the photocontrol of growth of etiolated oat seedlings is established beyond any question (Gaba and Black 1983). Taking into consideration the results obtained in this paper, however, there are serious doubts whether the phytochrome localized at the top of a coleoptile can play an essential role in elongation processes. Because our present results show that a seedling node is a locus of red light reception in the photoregulation of growth of etiolated seedlings (Fig. 3) it seems likely that the phytochrome localized in this region of the seedling can play a leading role in the control of these processes. On the other hand, however, 30-minute-long irradiation with red light of 24-hour-old oat seedlings (pre-irradiation) decreases the level of phytochrome in the coleoptile top only to a slight degree (Madel and Kopcewicz 1988) and in such pre-irradiated seedlings, the site of light reception in the photoregulation of growth is the top of the plant (Fig. 3). So the role that the phytochrome localized in the coleoptile top plays in the photocontrol of the elongation growth in etiolated oat seedlings is not clear.

The physiological importance of the differentiated system of growth photoregulation of etiolated and pre-irradiated oat seedlings is difficult to define and its mechanism is not known. It may only be supposed that it is linked with the regulation of deetiolation of oat seedlings.

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Miejsca recepcji światła czerwonego w fotokontroli wzrostu siewek owsa

Streszczenie

Badano wpływ światła czerwonego na wzrost koleoptyli i mezokotyli etiolowanych i wstępnie naświetlonych siewek owsa. Światło czerwone stosowano na całe siewki bądź na dwumilimetrowe różne strefy tych siewek. Światło czerwone (15 min.) stosowane na całe siewki stymuluje wzrost koleoptyli i hamuje wzrost mezokotyli zarówno u etiolowanych siewek, jak i wstępnie naświetlonych. Reakcje wzrostowe etiolowanych i wstępnie naświetlonych siewek światłem czerwonym (2 min.) stosowanym na dwumilimetrowe, różne strefy tych siewek wskazują, że miejscem recepcji światła czerwonego w stymulacji wzrostu koleoptyla i hamowaniu wzrostu mezokotyli jest w siewce etiolowanej okolica leżąca bezpośrednio powyżej i poniżej węzła siewki, a w siewce wstępnie naświetlonej wierzchołek siewki. Uzyskane wyniki wskazują na istnienie różnych mechanizmów fotoregulacji wzrostu u etiolowanych i wstępnie naświetlonych siewek owsa. W pracy dyskutowana jest rola fitochromu w regulacji wzrostu siewek owsa.