Phytochrome-controlled level of growth substances in etiolated oat seedlings

JAN KOPCEWICZ, KAZIMIERZ MADELA

Department of Plant Physiology and Morphogenesis, Institute of Biology, N. Copernicus University,
Gagarina 9, 87-100 Toruń, Poland

(Received: April 9, 1991, Accepted: February 15, 1992)

Abstract

Irradiation with red light of coleoptiles and leaves of etiolated oat seedlings, causing photoconversion of phytochrome mainly into P_{fr} , leads to the release of free auxins and free gibberellins from conjugated forms. The effect of red light is reversible by far-red light irradiation. A correlation between the photostationary state of phytochrome and endogenous abscisic acid content was not found.

Key words: phytochrome, auxins, gibberellins, abscisic acid, oat seedlings

INTRODUCTION

It is widely accepted that the elongation of coleoptiles is controlled both by phytochrome (F u h r et al. 1980) and auxins (J a c k s o n and M c W h a 1984). It also has been postulated that two different mechanisms of phytochrome action may exist in oat seedlings (K o p c e w i c z et al. 1987a). The first would be related to the control of tissue sensitivity to IAA through the P_r form of phytochrome (K o p c e w i c z and M a d e l a 1989). The second mechanism would be involved in the regulation of the content of free and conjugated auxin in seedlings through the P_{fr} form of phytochrome and participate in the process of deetiolation and growth of seedlings under natural environmental conditions (K o p c e w i c z et al. 1987a). This mechanism could also be connected with the activation of other groups of plant hormones such as gibberellins and abscisic

acid which also take part in the control of seedling morphogenesis. Thus, the present study was undertaken to investigate the correlation between the P_{fr} content and the level of endogenous auxins, gibberellins and abscisic acid in oat seedlings, coleoptiles and leaves.

MATERIAL AND METHODS

Caryopses of oat (*Avena sativa* L. Diadem variety) were soaked in distilled water for 1 hour and afterwards sowed into moist, sterilized sawdust from deciduous trees. The germination of caryopses and cultivation of seedlings were conducted in darkness at $+26^{\circ}\text{C}$. Ninety six hours after sowing, 5 mm-long apical segments of coleoptiles and 5 mm-long apical segments of first leaves were isolated from the seedlings. The isolated coleoptiles and leaf segments were placed in Petri dishes containing 3 cm³ of buffer (0.01 M phosphate buffer of pH 7.0) and were subjected to irradiation.

A specially constructed lantern with a revolver holder of interference filters was used for monochromatic irradiations. The source of light was a 250 W halogen bulb with an optical system from a Diaprex projector. Red light — dielectric interference filters: $\lambda_{\text{max}} = 660 \text{ nm}$, $\lambda_{1/2} = 7 \text{ nm}$, $T_{\text{max}} = 60\%$, energy flux at the plant level — $\phi R = 1.08 \text{ Wm}^{-2}$. Far-red light — dielectric interference filter: $\lambda_{\text{max}} = 730 \text{ nm}$, $\lambda_{1/2} = 8 \text{ nm}$, $T_{\text{max}} = 40\%$, energy flux at the plant level — $\phi R = 0.95 \text{ Wm}^{-2}$.

Three variants of coleoptiles with different phytochrome stationary states and three variants of etiolated and irradiated leaf segments were prepared (Table 1, stages in figures).

Table 1

Stationary state of phytochrome in etiolated and irradiated coleoptiles of oat seedlings

Experimental variant	Conditions	Content of phytochrome, %		
		$P_r \pm 8$	$P_{fr} \pm 6$	$P_{\text{tot}} \pm 7$
D	96 h of darkness	100	0	100
R	96 h of darkness + 15 min of red light	20	80	100
R + FR	96 h of darkness + 15 min of red light + 20 min of far-red light	98	2	100

The phytochrome content (coleoptiles) and growth substances level (coleoptiles, leaves) in oat tissues were determined.

The phytochrome content was determined spectrophotometrically in 100 coleoptile tips on the basis of the differences in the optical density of the tissue (Δ

OD) at 660 and 730 nm according to the method described previously (Kopcewicz and Cymer ski 1983). The results of phytochrome measurements were normalized in every experiment accepting as 100 per cent the phytochrome content in coleoptiles of 96 hour-old etiolated oat seedlings.

The contents of auxins, gibberellins and abscisic acid were determined using about 20 g of isolated 5 mm-long apical segments of coleoptiles and 10 g of isolated 5 mm-long tips of first leaves of 96 hour-old etiolated oat seedlings.

Auxins were isolated and fractionated according to the method previously described (B and ur ski and Sch ult ze 1974, Kopcewicz et al. 1987a). In the case of all three fractions (free, esterified and peptidic IAA), the final ethyl ether-ethyl acetate solutions were dried and the residues were dissolved in 2 ml of 100% methanol for chromatography. Whatmann 3 MM paper with isopropanol-ammonia-water 10:1:1 v/v as solvent was used for the initial separation. The place where IAA was localized (R_f 0.3-0.6) was eluted and rechromatographed using gas chromatography. An aliquot of the IAA fraction from paper chromatography was evaporated to dryness at +25°C in a stream of dry nitrogen. The sample was dissolved in 20 μ l of bistrimethylsilyl-trifluoroacetamide (BSTFA) and 10 μ l of acetonitrile. Silylation occurred after 15 min at +50°C gas chromatography (Chromatron GCHF 18.3) with 3% SP-2401 absorbed on 100/120 mesh Supelcoport in a 1.5 m $\times$ 3 mm glass columns at +150°C with 40 cm³/min of nitrogen as a carrier gas was carried out. The amount of auxin was determined on the basis of recorder readings establishing the area of the IAA peak (retention time — 9 min 45 sec). The *Avena* section straight growth test (Nitsch 1956) was also used for comparative determinations.

The gibberellin content was determined according to the method described previously (Kopcewicz and Poraziński 1973, Kopcewicz et al. 1987b). The gibberellin-like substances from both acid (free) and neutral (bound) fractions were bioassayed by the lettuce hypocotyl (Frankland and Wareing 1960) and dwarf pea (McComb and Carr 1958) tests. The lettuce hypocotyl test for quantitative determination was used. The gibberellin content was read from a standard curve, drawn on the basis of the influence of several concentrations of gibberellic acid on the growth of lettuce hypocotyls. The amount of hormones characteristic for a given developmental stage is given as an equivalent of gibberellic acid in 100 g of fresh weight of the investigated tissues.

The content of abscisic acid (ABA) was determined according to the method previously described (Kulikowska-Gulewska and Kopcewicz 1985, Kopcewicz and Madala 1990). Gas chromatography (Chromatron GCHF 18.3) analyses of the methylated fractions according to Schlenk and Gellerman (1960) were carried out. Silinized glass columns (1.5 m $\times$ 3 mm i.d.) were packed with 1.5% XE-60 absorbed on Gas-Chrom Q (80-100 mesh). In all cases the nitrogen carrier gas flow rate was 60 cm³/min, the injector heaters were set at +250°C and the oven temperature was +150°C. Retention time of

ABA was 10 min 40 sec. A quantitative estimation of methylated derivatives was made by comparing the peak areas of the corresponding peaks in the investigated oat seedling fractions.

The presented results were obtained from four independent experiments. The results were statistically evaluated and the LSD was calculated at a significance level of 0.01 and 0.05.

RESULTS

During the initial experiments three variants of apical segments of coleoptiles, differing in the amount of both forms of phytochrome (Table 1) as well as three similar variants of irradiated segments of oat leaves, were prepared. The obtained results showed that etiolated coleoptiles containing the maximum amounts of the P_r form of phytochrome (Table 1) were characterized by an increased level of bound auxins (Fig. 1) and bound gibberellins (Fig.

Fig. 1. Auxins in oat coleoptiles with different P_{fr} levels

2). Fifteen minute-long irradiation with red light resulting in the photoconversion of phytochrome into the P_{fr} form (Table 1) caused the appearance of high amounts of free auxins (Fig. 1) and free gibberellins (Fig. 2). Far-red light applied after red light reversed the effects of red light. The coleoptiles again contained phytochrome in the P_r form (Table 1) and were characterized by a high amount of bound auxins (Fig. 1) and bound gibberellins (Fig. 2).

Similar results to those presented above were also obtained in the case of oat leaf segments (Fig. 3).

On contrary with auxins and gibberellins, red light did not affect the content of abscisic acid in coleoptiles and decreased its amounts in leaves (Fig. 4). Far-red light lowered the level of abscisic acid even more effectively. In darkness the leaves were characterized by an increased amount of ABA (Fig. 4).

Fig. 2. Gibberellins in oat coleoptiles with different P_{fr} levels

Fig. 3. Effect of irradiation with red and far-red light on gibberellin content in leaves of etiolated oat seedlings

Fig. 4. Effect of irradiation with red and far-red light on abscisic acid content in coleoptiles and leaves of etiolated oat seedlings

DISCUSSION

Etiolated oat seedlings are characterized by a high amount of the P_r form of phytochrome (Table 1) and very intensive elongation growth. They are also very sensitive to IAA (Kopcewicz et al. 1987a). Irradiation of etiolated seedlings with red light decreased their sensitivity to IAA, stopped intensive elongation and began the process of deetiolation. Phytochrome P_{fr} may control these processes through, among others, the decrease of tissue sensitivity to hormones and the regulation of the level of endogenous growth substances. The results obtained in this paper confirm such an assumption. Because of the red-far-red reversibility it has become evident that the endogenous level of auxins (Fig. 1) and gibberellins (Figs. 2 and 3) is controlled by phytochrome. The changes in the content of free and conjugated auxins and gibberellins in oat seedlings are correlated with the stationary state of phytochrome (Table 1). Etiolated tissues containing high amounts of the P_r form of phytochrome are characterized by an increased level of conjugated auxins (Fig. 1) and gibberellins (Figs. 2 and 3). They are, however, at that time very sensitive to even low concentrations of free IAA (Kopcewicz et al. 1987a). Saturated irradiation with active red light resulting in the conversion of phytochrome into P_{fr} (Table 1) causes a decrease in tissue sensitivity to IAA (Kopcewicz et al. 1987a) as well as an increase in the amount of

free forms of hormones (Figs. 1, 2 and 3). The occurrence of increased amounts of free forms of plant hormones in young seedlings seems thus to be closely correlated with an increased level of phytochrome P_{fr} . The influence of light on hormones metabolism has also been reported previously (Beevers et al. 1970, Kopcewicz et al. 1987a, b).

In contrast to auxins and gibberellins, no direct correlation between phytochrome and the content of abscisic acid was observed (Fig. 4). In darkness the oat leaves were characterized by an increased amount of ABA.

It may be assumed, based on the facts presented above, that the irradiation of seedlings with red light, which started the process of deetiolation of seedlings and at the same time the photoconversion of phytochrome into P_{fr} , also leads to the release of free auxins and gibberellins from their conjugated forms. This phenomenon, controlled by phytochrome, could be an important step in deetiolation and in establishing interorganic correlations in a seedling growing under normal environmental conditions.

REFERENCES

- Bandurski R.S., Schultze A., 1974. Concentration of indole-3-acetic acid and its esters in *Avena* and *Zea*. *Plant Physiol.* 54: 257-262.
- Beevers L., Loveys B., Pearson J.A., Wareing P.F., 1970. Phytochrome and hormonal control of expansion and greening of etiolated wheat leaves. *Planta* 90: 286-294.
- Frankland B., Wareing P.F., 1960. Effect of gibberellic acid on hypocotyl growth of lettuce seedlings. *Nature* 185: 255-256.
- Fuhr G., Bleiss W., Göring H., 1980. Phytochrome-regulated growth of excised coleoptile tips of *Triticum aestivum* induced by blue, red and far-red light. *Plant Cell Physiol.* 21: 571-580.
- Jackson D.L., Mc Wha J.A., 1984. Grain or coleoptile tips as the source of IAA in cereal shoots? *Plant Cell Environ.* 7: 15-21.
- Kopcewicz J., Cymerski M., 1983. Non-photochemical transformations of phytochrome in coleoptiles of etiolated oat seedlings. *Acta Physiol. Plant.* 5: 3-9.
- Kopcewicz J., Cymerski M., Madala K., 1987. Phytochrome and IAA in the control of oat coleoptile elongation. *Acta Physiol. Plant.* 9: 69-76.
- Kopcewicz J., Cymerski M., Madala K., 1987a. Phytochrome and endogenous gibberellin-like substances in etiolated and irradiated oat coleoptiles. *Acta Soc. Bot. Pol.* 56: 437-446.
- Kopcewicz J., Madala K., 1989. Phytochrome-regulated sensitivity of oat coleoptile tissues to light and IAA. *Acta Physiol. Plant.* 11: 193-199.
- Kopcewicz J., Madala K., 1990. Phytochrome and growth substances in the control of oat seedlings elongation. *Acta Physiol. Plant.* 12: 279-285.
- Kopcewicz J., Poraziński Z., 1973. Effect of red light irradiation on metabolism of free and bound gibberellins in Scots pine (*Pinus sylvestris* L.). *Bull. Acad. Pol. Sci.* 21: 383-387.
- Kulikowska-Gulewska H., Kopcewicz J., 1985. Abscisic acid-like inhibitor in embryonic shoots of Scots pine in relation to generative differentiation. *Acta Physiol. Plant.* 7: 163-170.

- Mc Comb A.J., Carr D.J., 1958. Evidence from a dwarf pea bioassay for naturally occurring gibberellins in the growing plants. *Nature* 181: 1548–1549.
- Nitsch J.P., 1956. The chemistry and mode of action of plant growth substances. Butterworths Sci. Publ., London.
- Schlenk H., Gellerman J.L., 1960. Esterification of fatty acids with diazomethane on a small scale. *Anal. Chem.* 32: 1412–1414.

Fitochromowa kontrola poziomu substancji wzrostowych w etiolowanych siewkach owsa

Streszczenie

Naświetlenie koleoptyli i liści etiolowanych siewek owsa światłem czerwonym, powodując fotokonwersję fitochromu głównie do P_{fr} , prowadzi jednocześnie do uwalniania wolnych auksyn i giberelin z ich form związanych. Efekty światła czerwonego są odwracane działaniem dalekiej czerwieni. Nie stwierdzono korelacji między stanem fotostacjonarnym fitochromu a zawartością kwasu abscysynowego w siewkach owsa.