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Participation of labile and stabile phytochrome in the control of chlorophyll accumulation during the deetiolation of oat seedlings

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Abstract

The effect of the phytochrome system on the accumulation rate of chlorophyll-a and b in 96-hour-old oat seedlings during the first 3 hours of white light action was investigated. It was established that initial irradiation with red light stimulated the accumulation rate of both forms of chlorophyll. This effect depended on the level of the P_{FR} form of phytochrome obtained during the initial irradiation and it reached the treshold value in the case of about 20% of P_{FR} in relation to P_R in etiolated seedlings. Far red light stimulated only the accumulation of chlorophyll-a. The effect of red light was reversible if far red light was applied directly after red light. The reversibility diminished gradually together with the extension of the dark period between red and far red light, disappearing completely after 6 hours. The results suggest the participation of two pools of phytochrome – a labile and a stabile one – in the reaction stimulating chlorophyll accumulation. A model of labile phytochrome action through the destruction products of phytochrome is proposed.

Key words: *phytochrome pools, phytochrome destruction, chlorophyll accumulation, oat seedlings*

INTRODUCTION

The process of deetiolation is connected with two physiological phenomena – the change of the growth pattern (Muir and Chang 1974, Schaer et al. 1983) and the beginning of chlorophyll accumulation (Kasemir 1983, Apel

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Abbreviations: R – red light, FR – far red light, P_R – phytochrome form absorbing red light, P_{FR} – phytochrome form absorbing far red light, Chl-a – chlorophyll-a, Chl-b – chlorophyll-b, Chl-(a+b) – sum of chlorophyll-a and chlorophyll-b, pChl – protochlorophyll, ALA – 5-aminoleavulinic acid.

et al. 1984, Gardner and Gorton 1985). The latter process is especially characteristic of angiospermous plants which are unable to accumulate chlorophyll in darkness (Apel et al. 1984).

In the coleoptiles of etiolated oat seedlings there is an extremely hight level of phytochrome in the biologically inactive P_R form. The irradiation of a seedling with red or white light results in the photoconversion of phytochrome into the active P_{FR} form, which undergoes destruction, with a half-life of about two hours, to the level of a few per cent of its initial content (Kopcewicz and Cymerski 1983). Advances in the development of immunological methods and their application in studies on phytochrome led to the discovery that, apart from labile pool of phytochrome P_{FR}, a second, stabile pool, with a several times longer half-life also exists (Jabben 1980).

Both these phytochrome populations show immunological dissimilarity and this indicates that the protein parts of the pigment differ (Shimazaki and Pratt 1985).

According to Kasemir (1983) the formation of chlorophyll in the seedlings of higher plants is dependent on two photoreactions – the formation of P_{FR}, and the reduction of protochlorophyll. It has been pointed out that the phytochrome system activates a complex process of chlorophyll accumulation on continuous white light (Kasemir 1983). However, there are no reports on the participation of both pools of phytochrome – a labile and a stabile one – in this process.

The aim of the present work was to investigate the role of phytochrome in the process of chlorophyll accumulation during the deetiolation of oat seedlings in relation to the possible participation of both pools of phytochrome.

MATERIAL AND METHODS

PLANT MATERIAL

Dehusked caryopses of *Avena sativa* L. variety Diadem were soaked in distilled water for two hours and afterwards sown into moist sterilized sawdust and grown in darkness at 26°C. After 24, 48 or 72 hours, darkness was interrupted with monochromatic light. During the 96th hour of incubation one part of the seedlings was irradiated once again. The dose of light and the wavelenght were different, depending on the experiment devised. Seedlings treated in such a way were harvested and the content of chlorophyll-a and b, as well as protochlorophyll, was determined. The remaining seedlings were subjected for 3 hours to the action of white light at 26°C and afterwards the content of chlorophyll and protochlorophyll was determined.

DETERMINATION OF CHLOROPHYLL AND PROTOCHLOROPHYLL CONTENTS

Two g of coleoptiles, cut together with the first leaf, were ground for 10 minutes on 5 cm^3 of 80% acetone with the addition of quartz sand and $CaCO_3$, in a mortar cooled to 4°C, until a homogenous pulp was obtained, and then extracted in darkness at 4°C for 30 minutes. The extract was filtered and the residue was washed with 15 cm^3 of 80% acetone. The crude extract obtained in this way was centrifuged for 10 minutes at 2000 × g. The chlorophyll content in the supernatant was determined spectrophotometrically on the basis of formulas calculated experimentally. In order to calibrate the spectrophotometer, a sample of chemically pure chlorophyll-a and b produced by Sigma Chemical Comp. was used. The following dependencies, relationships were obtained for a cuvette having a 5 cm optical path:

$$\text{Chl-a} = 1.925\ A_{665} - 0.5\ A_{648} - 0.06\ A_{626},$$

$$\text{Chl-b} = 4.92\ A_{648} - 1.23\ A_{665} - 0.07\ A_{626},$$

$$\text{pChl} = 6.0\ A_{626} - 0.8\ A_{665} - 1.4\ A_{648},$$

where A_{626}, A_{648}, A_{665} denote the extinction values for the respective wavelengths.

LIGHT

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.

Filter chracteristics. Red light – diaelectric interference filter: $\lambda_{max} = 660$ nm; $\lambda_{1/2} = 7$ nm; $T_{max} = 60\%$. The obtained flux of energy on the plant level – $\phi R = 1.08\ Wm^{-2}$. Far red light – diaelectric interference filter: $\lambda_{max} = 800$ nm; $\lambda_{1/2} = 9$ nm; $T_{max} = 40\%$. The obtained flux of energy at the plant level – $\phi_{FR} = 0.87\ Wm^{-2}$. Green light – metallized interference filter: $\lambda_{max} = 450$ nm; $\lambda_{1/2} = 12$ nm; $T_{max} = 40\%$. The obtained flux of energy on the plant level – $\phi_G = 1.4\ Wm^{-2}$.

White light. The source of white light during 3-hour-long incubation of seedlings was four 20 W fluorescent cool light type lamps with a paper filter (weakening the light intensity and giving a more uniform distribution of light). This system guaranteed an energy flux on the plant level – $\phi_W = 10\ Wm^{-2}$.

Safe light. All of the operations before cutting the seedlings were conducted in darkness because of the proved considerable influence of green light on the process of chlorophyll accumulation. The extraction of chlorophyll was carried out in dim green light.

Light measurement. Light intensity was measured with a scaled Kipp linnear Thermopile with a precision of $5 \times 10^{-2}\ Wm^{-2}$.

Measurement error. Each experiment was repeated five times. The results were estimated statistically and the LSD was calculated at a significance level of 0.01.

RESULTS

THE EFFECT OF INITIAL IRRADIATION WITH RED LIGHT ON CHLOROPHYLL AND PROTOCHLOROPHYLL ACCUMULATION

The sensitivity threshold of the reaction

Oat seedlings were initially irradiated after 24, 48 or 72 hours with red light of different duration from 10 sec. to 20 minutes, before placing them under white light. Next, the accumulation rate of chlorophyll-a and b was investigated.

Independent of seedling age at which the initial irradiation was done, and of the duration and dosage of light, the initial red light irradiation always stimulated, but with different intensity, the rate of chlorophyll accumulation under white light (Table 1). The accumulation rate increased with increasing light dose until it reached the threshold value. Further increase of the light dose did not cause any essential rise of the accumulation rate. The saturation

Table 1

Effect of various doses of red light preirradiation in 24th (A), 48th (B) and 72nd (C) hour on chlorophyll-a and chlorophyll-b levels in 99-hour-old oat seedlings after 3-hour accumulation in white light

	Time of R irradiation (s)	Dose of R light (Jm^{-2})	Chl-a		Chl-b	
			$\mu g\ g^{-1}$ f.w.	%	$\mu g\ g^{-1}$ f.w.	%
R in 24th h (A)	0	—	14.0	100	3.5	100
	300	324.0	15.4	110	3.8	109
	600	648.0	17.6	126	4.7	134
	900	972.0	19.3	138	6.7	191
	1200	1296.0	19.0	136	6.6	188
R in 48th h (B)	0	—	14.0	100	3.5	100
	60	64.8	17.4	124	5.2	149
	120	129.6	19.7	141	6.7	191
	300	324.0	23.0	164	9.5	271
	600	648.0	23.3	166	9.4	269
R in 72nd hour (C)	0	—	14.0	100	3.5	100
	10	10.8	18.1	129	5.5	157
	30	32.4	21.1	151	6.0	171
	40	43.2	25.6	182	7.7	220
	60	64.8	30.5	218	10.5	300
	120	129.6	30.1	215	10.2	291
	300	324.0	30.5	218	10.1	281
	900	972.0	30.4	217	10.3	294

of the reaction decreased with the age of an irradiated seedling. For 72-hour-old seedlings 1-minute irradiation was enough (dose of 64.8 Jm^{-2}), while 48-hour-old seedlings had to be irradiated for 5 minutes (324 Jm^{-2}), and 24-hour-old seedlings — for 15 minutes (927 Jm^{-2}) in order to reach saturation (Table 1 A, B, C).

Such doses of light account for about 20% photoconversion of phytochrome (Madela and Kopcewicz 1987).

In further experiments the following saturating doses were applied:
24-hour-old seedlings — 15 minutes R (927 Jm^{-2}),
48-hour-old seedlings — 5 minutes R (324 Jm^{-2}),
72-hour-old seedlings — 1 minute R (64.8 Jm^{-2}).

The effect of initial irradiation with red light on chlorophyll and protochlorophyll accumulation in darkness

Oat seedlings 24, 48 or 72 hours after sowing were irradiated with a saturating dose of light as above and afterwards placed once again in the

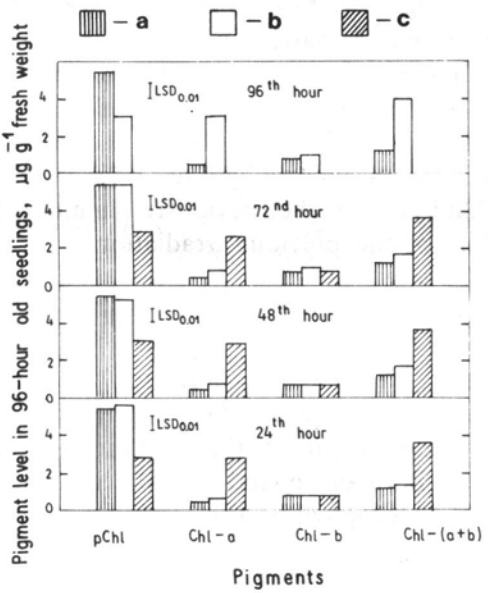

Fig. 1. Effect of red light irradiation on chlorophyll-a, chlorophyll-b, chlorophyl-(a+b) and protochlorophyll levels in 96-hour-old oat seedlings, a — nonirradiated seedlings; b — seedlings irradiated once in the 24th, 48th, 72nd and 96th hour; c — seedlings preirradiated in 24th, 48th or 72nd hour and then reirradiated in 96th hour. Doses of red light irradiation for 24-, 48-, 72-, 96-hour-old seedlings were: 927 Jm^{-2}; 324 Jm^{-2}; 65 Jm^{-2}; 927 Jm^{-2}, respectively

dark. After 96 hours, one part of the seedlings was irradiated once more with red light and then the chlorophyll-a and b and protochlorophyll levels were measured.

In either of the variants, no effect of initial irradiation on the increase in the level of protochlorophyll was observed; also there were no greater changes in the level of chlorophyll, with the exception of seedlings irradiated in the 96th hour, directly before the measurement (Fig 1). This effect makes up for the dark period preceding to the measurement.

The effect of initial irradiation with red light on chlorophyll accumulation during the first 3 hours of white light action

The initial irradiation with red light always stimulated the rate of chlorophyll accumulation under white light (Table 1). When applying the saturating light dose, the highest stimulation was observed during irradiation in the 72nd hour, and the lowest – in the 24th hour. The data on Figure 2 show the level of chlorophyll-a and b after 3 hours of accumulation under white light in oat seedlings irradiated initially at different ages (24, 48, 72, 96 hours) with red light saturating the reaction. A part of the seedlings was irradiated for the second time in the 96th hour. The level of protochlorophyll after 3 hours of white light dropped to about 2 $\mu g \times g^{-1}$ of fresh weight and did not undergo any essential changes in all the variants (data not presented). Red light applied in the 96th hour, directly before placing the seedlings under the white light, did not increase much the rate of chlorophyll accumulation. Also the repeated irradiation of initially illuminated seedlings, only insignificantly increased the rate of chlorophyll accumulation. Only the repeated irradiation in the 72nd hour of the previously illuminated seedlings weakend the stimulating effect of the previous irradiation.

THE EFFECT OF GREEN LIGHT AND FAR RED LIGHT ON CHLOROPHYLL ACCUMULATION DURING THE FIRST 3 HOURS OF WHITE LIGHT

Both far red and green lights stimulated the accumulation rate of chlorophyll, but the effect of far red light was greater than that of green light and it was almost independent of the seedling age at which the initial irradiation was done. Both green and far red lights hardly influenced the accumulation rate of chlorophyll-b (Fig. 3).

THE REVERSIBILITY OF THE EFFECT

The effect of far red light applied during the 24th hour on the later accumulation of chlorophyll-a was comparable with the effect of red light, whereas when applied in the 72nd hour it was weaker than the effect of red light (Fig. 4). The stimulatory effect of red light was reversed, however, by far red light applied directly after red light both for chlorophyll-a and b

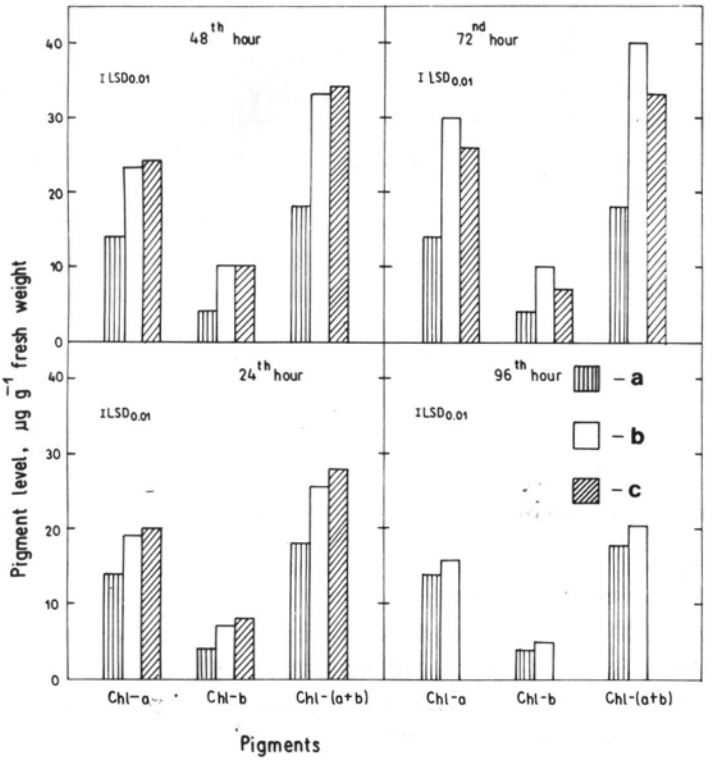

Fig. 2. Effect of red light preirradiation on chlorophyll levels in 99-hour-old oat seedlings after 3-hours accumulation in white light (10 Wm^{-2}). a – seedlings with no red light treatment; b – seedlings with one red light pretreatment in the 24th, 48th, 72nd or 96th hour; c – seedlings with double red light treatment – first in the 24th, 48th, or 72nd hour and then in the 96th hour. Doses of red light irradiation for 24-, 48-, 72- and 96-hours old seedlings were: 927 Jm^{-2}, 324 Jm^{-2}, 65 Jm^{-2}, respectively

(Fig. 4). The reversibility declined as the dark intermissions between R and FR light irradiations increased, to disappear completely following six hour long intermission. After this time the additive effect of both irradiations appeared and this can suggest the influence of red and far red light on separate stages of chlorophyll biosynthesis.

DISCUSSION

The obtained results prove that initial exposure of oat seedlings to monochromatic light always stimulates the chlorophyll accumulation rate in 96-hour old seedlings during the first three hours of white light action. The effect was observed regardless of the applied light wavelength and of the age of the irradiated seedlings. However, two types of reaction present themselves clearly:

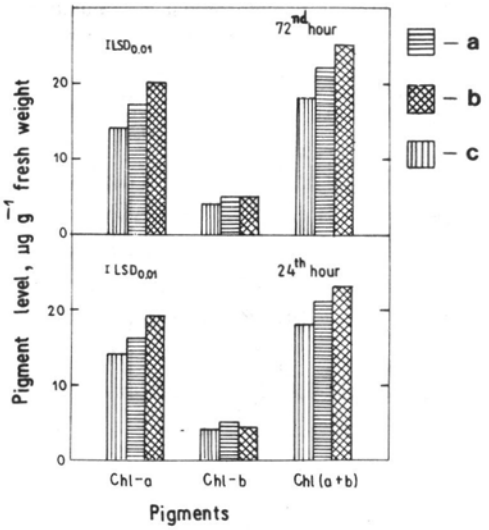

Fig. 3. Effect of green (a) and far-red (b) light preirradiation at 24th or 72nd hour on chlorophyll level in 99-hour old oat seedlings after 3-hours accumulation in white light (10 Wm^{-2}). c – seedlings with no monochromatic light pretreatment. Doses of monochromatic light irradiation; green – 1260 Jm^{-2}; far-red – 522 Jm^{-2}

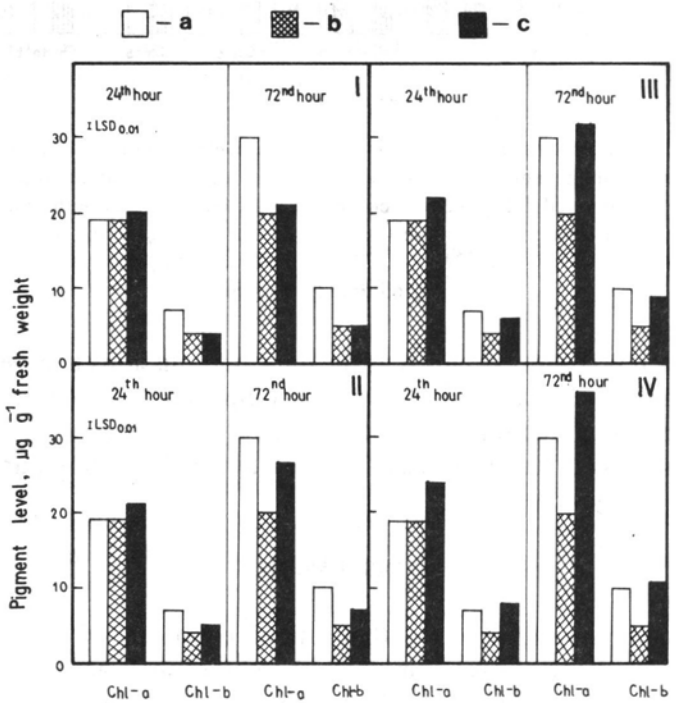

Fig. 4. Effect of far-red light on the red-light-dependent level of chlorophyll in 99-hour-old oat seedlings after 3-hours accumulation in white light (10 Wm^{-2}), a – red light; b – far-red light; c – red + far-red light. Red light was applied in the 24th hour (927 Jm^{-2}) or the 72nd hour (65 Jm^{-2}). Far-red (522 Jm^{-2}) was applied immediately after red light (I) or with 0.5 hr (II), 2 hrs (III), and 6 hrs (IV) dark intervals

1. Reaction effected by green and far red light (Fig. 3) consisting of stimulation of chlorophyll-a accumulation rate.

2. Reaction effected by red light with photoreversibility typical of phytochrome (Figs. 2 and 4) consisting of stimulation of the accumulation rate of both chlorophyll-a and chlorophyll-b.

The data in Fig. 1 preclude the possibility of direct influence of the initial irradiation on both chlorophyll and protochlorophyll levels. Ford and Kasemir (1980) proved that the synthesis of protochlorophyll precursor, 5-aminoleovulinic acid (ALA), proceeds only when the protochlorophyll level, due to reduction to chlorophyll, drops below a critical value. It may be believed that the initial irradiation, while not affecting the protochlorophyll level, does not affect ALA contents either. These facts suggest that the controlling factor produced as a result of the initial irradiation does not initially influence chlorophyll metabolism but becomes active only under continuous white light many hours later.

For the reaction induced by far red or green light irradiation (Fig. 3) such a factor could be a small level of stabile phytochrome P_{FR} form, produced by irradiation, which may survive due to its stability, to the moment of switching on the continuous white light. Because, as Masoner and Kasemir (1975) found, continuous irradiation with far red stimulates the ALA production rate under white light, it seems likely that the stable phytochrome stimulates the chlorophyll accumulation rate by stimulating ALA synthesis rate under continuous light.

However, in the case of the red light-induced reaction (Fig. 3) stable phytochrome should be excluded as the controlling factor because the reversibility of the reaction disappears within several hours (Fig. 4). This termination of reversibility proves that labile phytochrome takes part in the reaction. Moreover, saturation of the reaction with a threshold dose of red light (Table 1) indicates that a certain level of labile P_{FR} effecting a complete reaction is indispensable. However, the labile P_{FR} phytochrome is rapidly destroyed and disappears completely after about 8 hours after its formation (Kopcewicz and Cymerski 1983). So, a paradox is seen here: on one hand the rapid disappearance of reversibility points to participation of the labile phytochrome, and on the other, its quick destruction precludes it as a controlling factor in this reaction. An immediate reaction of the labile phytochrome at the moment of its formation, as suggested above, is also rather unlikely: this is further supported by the fact that the red light-induced chlorophyll accumulation rate is relatively weakest when red light is applied directly prior to white light (Fig. 3). The following facts:

1. Quick disappearance of reversibility and reaction saturation threshold (Fig. 4, Table 1).

2. Absence of the primary reaction of the red light on chlorophyll metabolism (Fig. 1).

3. Weak influence of the red light applied directly prior to the white light (Fig. 2) suggest the following properties of the hypothetical controlling factor: a) quantitative dependence of the factor on the labile phytochrome P_{FR} form, b) stability of the factor in the darkness and c) several hours delay in the formation of the factor with relation to the labile phytochrome P_{FR} form.

The above properties indicate with high probability that the controlling factor in the reaction stimulating chlorophyll accumulation by red light may be the products of destruction of the P_{FR} labile phytochrome, with the assumption as to their biological activity and stability in the darkness.

With these assumptions the following mechanism of labile phytochrome action on chlorophyll accumulation may be proposed: during irradiation with saturating red light, a certain amount of labile phytochrome P_{FR} forms which, after several hours, disappears completely leaving the products of its destruction relatively stabile in the darkness. By possible binding to the cell membranes, they enhance the efficiency of the chlorophyll biosynthesis channel, hidden in the darkness and manifested in white light. In order to substantiate conclusively the above hypothesis it is necessary to determine the character of the labile phytochrome destruction products. Identification of those substances can explain the role of the labile phytochrome in photomorphogenetic phenomena.

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REFERENCES

Apel K., Motzkus M., Dehesh K., 1984. The biosynthesis of chlorophyll in greening barley (*Hordeum vulgare*). Is there a light-dependent prochlorophyllide reductase? Planta 161: 550-554.

Ford M. J., Kasemir H., 1980. Correlation between 5-aminoleavulinate accumulation and protochlorophyll photoconversion. Planta 150: 206-210.

Gardner G., Gorton H., 1985. Inhibition of phytochrome synthesis by gabaculine. Plant Physiol. 77: 540-543.

Jabben M., 1980. The phytochrome system in light grown *Zea mays* L. Planta 149: 91-96.

Kasemir H., 1983. Light control of chlorophyll accumulation in higher plants. In: Encyclopedia of plant physiology. New series. Vol. 16B. Photomorphogenesis. W. Shropshire Jr. and H. Mohr. (eds.) Springer-Verlag, Berlin-Heidelberg-New York-Tokyo, pp. 662-686.

Kopcewicz J., Cymerski M., 1983. Non-photochemical transformation of phytochrome in coleoptiles of etiolated oat seedlings. Acta Physiol. Plant. 5: 3-9

Madela K., Kopcewicz J., 1988. Effect of monochromatic and white light irradiation on the phytochrome content in the coleoptiles of oat seedlings (*Avena sativa* L. var. Diadem). Acta Physiol. Plant. (in press).

Masoner M., Kasemir H., 1975. Control of chlorophyll synthesis by phytochrome. I. The

effect of phytochrome on the formation of 5-aminoleavulinate in mustard seedlings. Planta 126: 111-117.

Muir R. M., Chen Chang R., 1974. Effect of red light on coleoptile growth. Plant Physiol. 54: 286-288.

Schaer J. A., Mandoli D. F., Briggs W. R., 1983. Phytochrome-mediated cellular photomorphogenesis. Plant Physiol. 72: 706-712.

Shimazaki Y., Pratt L. H., 1985. Immunochemical detection with rabbit polyclonal and mouse monoclonal antibodies of different pools of phytochrome from etiolated and green *Avena* shoots. Planta 164: 333-344.

Udział fitochromu labilnego i stabilnego w kontroli akumulacji chlorofilu podczas deetiolacji siewek owsa

Streszczenie

Badano wpływ układu fitochromowego na tempo akumulacji chlorofilu-a i chlorofilu-b w 96-godzinnych siewkach owsa (*Avena sativa* L. cv. Diadem), podczas 3 pierwszych godzin działania światła białego. Wykazano, że wstępne naświetlanie czerwienią stymuluje tempo akumulacji obu form chlorofilu. Efekt ten zależny jest od poziomu formy P_{FR} uzyskanej podczas wstępnego naświetlania i osiąga wartość progową przy około 20% P_{FR} w stosunku do P_R w siewce etiolowanej. Daleka czerwień stymuluje tylko akumulację chlorofilu-a. Efekt czerwieni jest odwracalny do poziomu działania dalekiej czerwieni, o ile daleką czerwień stosuje się bezpośrednio po czerwieni. Odwracalność maleje stopniowo wraz z przedłużeniem okresu ciemności między światłem czerwonym i daleką czerwienią, aby zaniknąć zupełnie po 6 godzinach. Wyniki wskazują na udział obu pul fitochromu — labilnego i stabilnego w kontroli reakcji stymulacji akumulacji chlorofilu. Proponuje się model działania fitochromu labilnego, uwzględniając znaczenie produktów jego destrukcji.