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Phytochrome and endogenous gibberellin-like substances in etiolated and irradiated oat seedlings

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Abstract

The level of gibberellin-like substances was investigated in oat coleoptiles with different stationary states of phytochrome and in leaf segments which had been etiolated and irradiated with red light. Etiolated coleoptiles and leaves containing maximum amounts of the P_R form of phytochrome were characterized by an increased level of bound gibberellins. Irradiation with red light resulting in the photoconversion of phytochrome into the P_{FR} form caused the appearance of a high content of free gibberellins. It seems that the releasing the hormones from bound forms correlated with the formation of phytochrome P_{FR} may be an important aspect of the mechanism of phytochrome action in the processes of seedling deetiolation. The interrelation between phytochrome and plant hormones in the control of photomorphogenesis of young monocotyledonous seedlings is also discussed.

Key words: *phytochrome, gibberellins, oat coleoptiles*

INTRODUCTION

It has been postulated previously that in oat seedlings there may exist two different mechanisms of action of phytochrome (Kopcewicz et al. 1986). The first mechanism would be connected with the control of tissue sensitivity to IAA through the P_R form of phytochrome and participates in the regulation of elongation of etiolated seedlings. The second one would be involved in the regulation of the auxin content in seedlings

through the P_{FR} form of phytochrome and participate in the process of deetiolation and growth of the seedlings under natural environmental conditions. This mechanism could also be connected with the activation of other groups of plant hormones which take part in the control of seedling morphogenesis. Thus, it has been decided to investigate in the present work the correlation between the stationary state of phytochrome and the content of endogenous gibberellins in oat seedlings.

MATERIAL AND METHODS

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

Four variants of coleoptiles with different stationary states of phytochrome and four variants of etiolated and irradiated leaf segments were prepared (Table 1, stages 1–4 on Figures). The content of phytochrome was not determined in the leaves.

Table 1

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

Variant of experiments		Content of phytochrome, %		
Number	conditions of experiments	$P_R \pm 8$	$P_{FR} \pm 6$	$P_{Tot} \pm 7$
1	96 hours of darkness	100	0	100
2	96 hours of darkness + 15 min. of red light	20	80	100
3	96 hours of darkness + 15 min. of red light + 2 hours of darkness	18	44	62
4	96 hours of darkness + 15 min. of red light + 7 hours of darkness	12	0	12

1. Coleoptiles with the maximum content of phytochrome in the P_R form ($P_{Tot.} = 100$; P_R — 100; P_{FR} — 0; percent). Nonirradiated etiolated coleoptiles and leaves were used for experiments.
2. Coleoptiles with the maximum content of phytochrome in the P_{FR} form ($P_{Tot.} = 100$; P_R — 20; P_{FR} — 80; percent). Isolated coleoptiles and

leaves were irradiated for 15 minutes with red light and afterwards the contents of phytochrome (coleoptiles) and gibberellins (coleoptiles, leaves) were determined.

3. Coleoptiles with an intermediate content of phytochrome ($P_{Tot.} = 62$; $P_R — 18$; $P_{FR} — 44$; percent). Isolated coleoptiles and leaves were irradiated for 15 minutes with saturating red light and afterwards they were incubated for 2 hours in darkness at +26°C. After this time the contents of phytochrome (coleoptiles) and gibberellines (coleoptiles, leaves) were determined.

4. Coleoptiles with the minimum content of both forms of phytochrome ($P_{Tot.} = 12$; $P_R — 12$; $P_{FR} — 0$; percent). Isolated coleoptiles and leaves were irradiated for 15 minutes with saturating red light, then incubated for 7 hours in darkness at +26°C. Afterwards the contents of phytochrome (coleoptiles) and gibberellins (coleoptiles, leaves) were determined.

The content of phytochrome was determined spectrophotometrically in 100 tips of coleoptiles from the difference in the optical density of the tissue (△ OD) at 660 and 730 nm according to the method described previously (Butler et al. 1959, Kopcewicz and Cymerski 1983). A modified SPECORD UV-VIS double-beam spectrophotometer with a connected convertor of TEC-1 extinction, enabling the numerical reading of the optical density with an accuracy of up to 10^{-3} was used. For the initial irradiation of coleoptiles, the lamp with a 2500 W Xenon burner equipped with a monochromatic filter was used. The density of irradiation was: 170 μW cm^{-2} at 660 and 140 μW cm^{-2} at 730 nm. The irradiation with active monochromatic light during the measurement was done with a Diaprex B-10 projector having a 150 W halogen bulb.

Cutting and isolating the coleoptiles, as well as filling the measurement cell, were conducted on a thermostatic aluminium plate ensuring a constant temperature of 0°C. All of the handling was done under dim green light. 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 content of gibberellins was determined in about 20 g of both isolated 5 mm-long top segments of coleoptiles and 5 mm-long top segments of first leaves according to the method described previously (Kopcewicz and Poraziński 1973). Frozen material was extracted with 80% methanol for 48 hours at +5°C. Evaporation at +30°C removed the methanol leaving the aqueous residue (water phase). The water phase was adjusted to pH 3.0 with 10% HCl (acid fraction) and partitioned twice with equal volumes of ethyl acetate and once with ethyl ether. The combined ethyl acetate-ethyl eter fraction was evaporated to dryness and the residue (containing free gibberellins) was dissolved in a small

volume of absolute methanol. Thin-layer chromatography was done using chloroform: acetic acid: ethyl acetate (90:5:10 v/v) as the solvent.

The water phase was then readjusted to pH 8.0 with saturated $NaHCO_3$ (neutral fraction) and extracted with ethyl acetate-ethyl ether. The combined ethyl acetate-ethyl ether fraction was evaporated and the residue was kept in 0.4 N HCl for 1 hour at +30°C. After adjusting to pH 3.0, the hydrolyzable gibberellins were extracted with ethyl acetate-ethyl ether. The latter fraction was evaporated and the residue (containing bound gibberellins) was chromatographed similarly as in the case of free gibberellins.

The gibberellin-like substances from both acid and neutral fractions were bioassayed by the lettuce hypocotyl (Frankland and Wareing 1960) and dwarf pea (Mc Comb and Carr 1958) tests. For quantitative determination the lettuce hypocotyl test was used. The content of gibberellins was established 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 was calculated as a mean value of experimental repetitions conducted, and was given as an equivalent of gibberellic acid in 100 g of fresh weight of investigated tissues. It was assumed that each bar on the diagram denots the content of gibberellins in particular chromatogram segments (Rfs), calculated for the appropriate amount of original plant material. The results of the experiments were analyzed statistically and the LSD was calculated with the significance level = 0.01 and 0.05.

RESULTS

During the initial experiments, 4 variants of top segments of coleoptiles differing in the amount of both forms of phytochrome (Table 1) as well as four similar variants of irradiated segments of oat leaves have been prepared.

In the case of coleoptiles, the obtained results show that etiolated coleoptiles containing the maximum amounts of the P_R form of phytochrome (Table 1) were characterized by an increased level of bound gibberellins (Figs. 2 and 3). These coleoptiles contained 3 groups of conjugated and only one group of free gibberellins (Figs. 1 and 2). 15 minute-long irradiation with red light resulting in the photoconversion of phytochrome into P_{FR} form (Table 1) cause of at the same time the appearance of high amounts of free gibberellins (Figs. 1 and 3). In these coleoptiles the level of bound gibberellins, especially at R_f 0.4–0.6 and R_f 0.8–1.0, rapidly dropped (Figs. 2 and 3). Keeping the coleoptiles

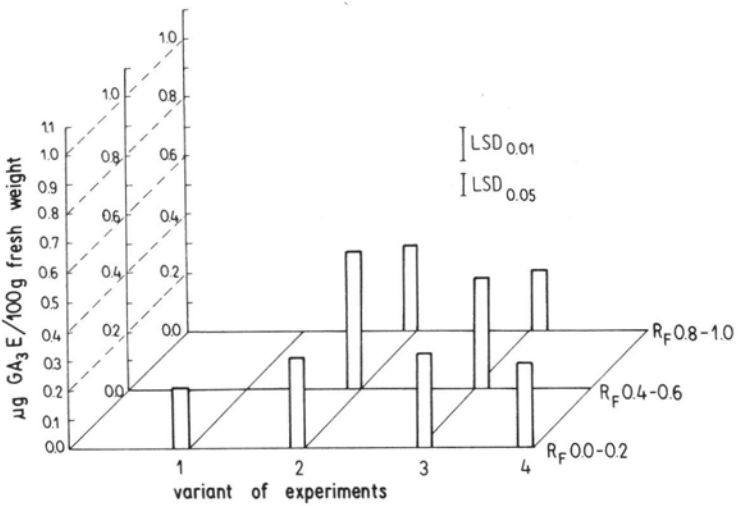

Fig. 1. The content of free gibberellins in oat coleoptiles with different stationary states of phytochrome

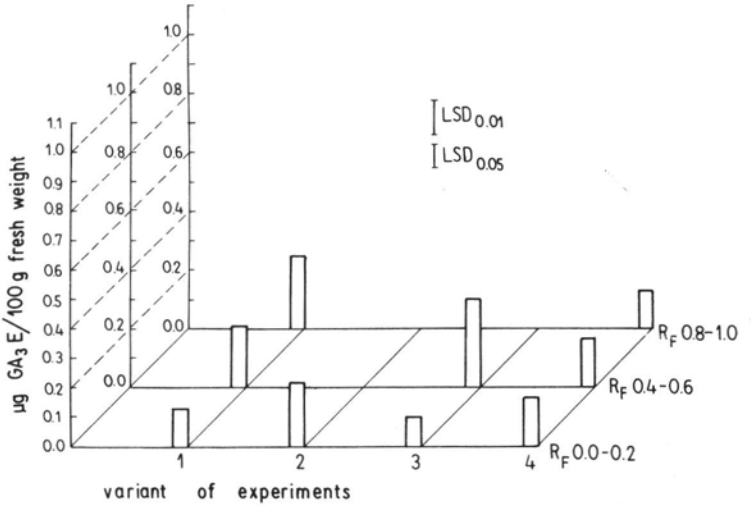

Fig. 2. The content of bound gibberellins in oat coleoptiles with different stationary states of phytochrome

in darkness after irradiation with red light led to the rapid destruction of phytochrome P_{FR}, slower decrease in the amount of phytochrome P_R (Table 1) as well as to the continuous drop in the level of free gibberellins in coleoptiles. At the same time, a slight tendency to increase the amount of bound gibberellins (Figs. 2 and 3) was observed.

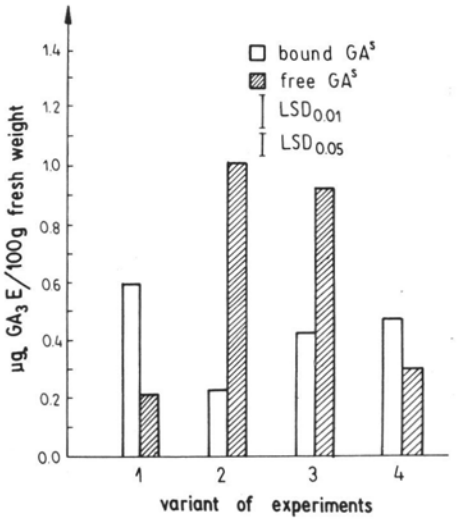

Fig. 3. Total content of free and bound gibberellins in oat coleoptiles with different stationary states of phytochrome

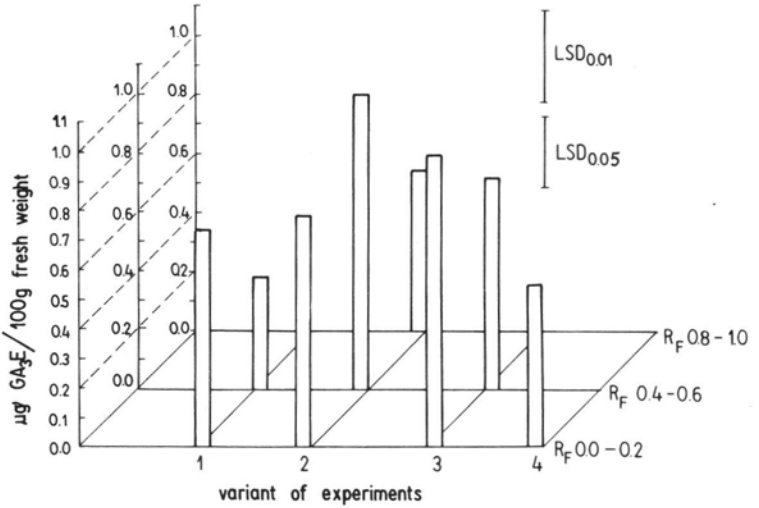

Fig. 4. The content of free gibberellins in leaf segments of etiolated and irradiated seedlings

Very similar results to those presented above have also been obtained in the case of oat leaf segments. Etiolated leaves contained high amounts of bound gibberellins (Figs. 5 and 6) whereas irradiation with red light caused the appearance of great amounts of free gibberellins (Figs. 4 and 6). Similarly as in coleoptiles in the leaves, 3 groups of bound gibberellins

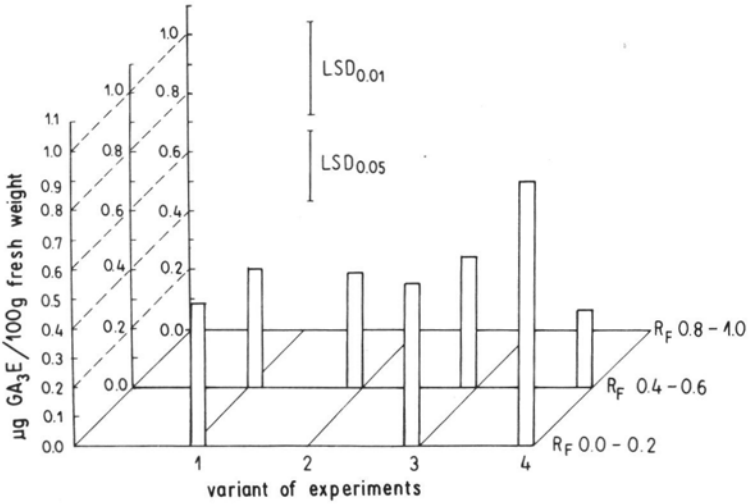

Fig. 5. The content of bound gibberellins in leaf segments of etiolated and irradiated oat seedlings

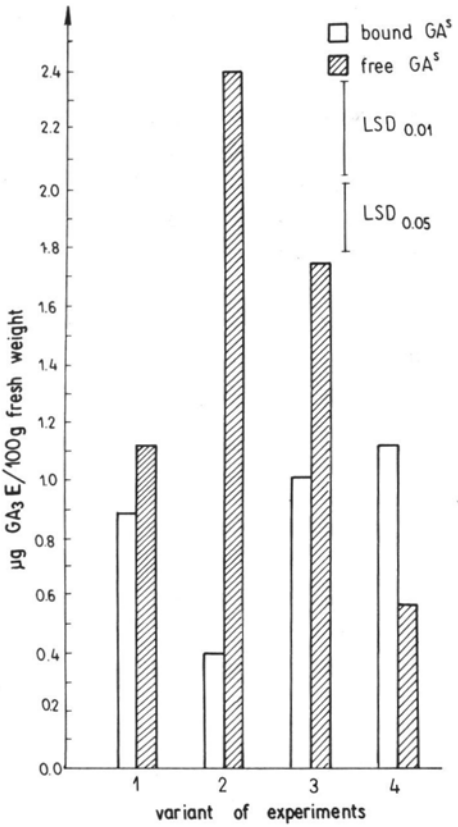

Fig. 6. Total content of free and bound gibberellins in leaf segments of etiolated and irradiated oat seedlings

and 3 groups of free gibberellins have been found. The leaves, however, contained on average, twice the amounts of gibberellins as in the case of coleoptiles.

DISCUSSION

The photoconversion of phytochrome P_R into P_{FR} form is accompanied by the destruction of the initial large fraction of phytochrome found in the coleoptiles of etiolated oat seedlings (Kopcewicz and Cymerski 1983). The irradiation of etiolated seedlings stops their intensive elongation and begins the onward process of deetiolation. Phytochrome might control these processes, through, among others, the regulation of endogenous hormone levels and/or the change of tissue sensitivity to hormones. It has been shown previously (Kopcewicz et al. 1986) that in oat seedlings there may exist two different mechanisms of action of phytochrome. The results obtained in this work confirm such an assumption. It has become evident that similarly as in the case of auxins (Kopcewicz et al. 1986) gibberellins are also regulated by phytochrome. The changes in the content of gibberellins both in coleoptiles and in the leaves of oat seedlings are correlated with the stationary state of phytochrome. Similarly as in case of auxins (Kopcewicz et al. 1986), it has been shown that etiolated tissues containing the maximum amounts of P_R form of phytochrome are characterized by an increased level of bound gibberellins. Saturated irradiation with active light resulting in the conversion of phytochrome into P_{FR} causes, similarly as in case of auxins (Kopcewicz et al. 1986), the appearance of a high amount of free forms of hormones.

Thus, it seems, that releasing hormones from their bound forms has an iniversal character. The above process is closely correlated with the photoconversion of phytochrome into P_{FR} form. So, it may be an important aspect of the mechanism of action of phytochrome in the process of seedling deetiolation. It is characteristic, at the same time, that with the proceeding disappearance of phytochrome P_{FR} in darkness after irradiation, there also follows a successive lowering in the amount of both free auxins (Kopcewicz et al. 1986) as well as free gibberellins (Figs. 3 and 6). So, the occurrence of increased amounts of free forms of plant hormones in young seedlings is closely correlated with the increased level of phytochrome P_{FR}. This may be of essential importance to seedling morphogenesis, however, it requires further detailed investigations. A similar influence of red light on the transformations of gibberellins in wheat leaves was observed by Beevers et al. (1970).

The results obtained in this work show that although the hormonal

situation after irradiation in different organs of seedlings is generally similar (Figs. 3 and 6) one may, however, observe some differences. Comparing coleoptiles and leaves it is clearly seen that in coleoptiles, the release of non-polar gibberellins (R_F 0.4–0.6 and R_F 0.8–1.0) absolutely predominates (Figs. 1 and 2) whereas in leaves (Figs. 4 and 5) the situation is more complicated. It may be suspected that in leaves, the increase in the total amount of gibberellins after irradiation (Fig. 6) may be the result of both releasing them from bound forms as well as from synthesis of these substances. It is characteristic that generally leaves contain twice as much gibberellins as coleoptiles (Figs. 3 and 6).

The interesting question still remains whether the observed changes in the content of hormones correlated with the transformation of phytochrome are the result of separate interaction of the phytochrome system with every hormone apart individually or only on one of them, the one which is a specific intermediary. This problem should be solved in the course of further investigations. In order to better understand the mechanisms of photomorphogenesis of monocotyledonous seedlings, fuller information concerning not only the influence of light through phytochrome on the content of endogenous hormonal substances but also on the influence of exogenously applied hormones on the morphogenesis of these plants are necessary.

The results of present work point, however, to the existance of a correlation between phytochrome and various plant hormones in the control of growth and development of young oat seedlings.

Acknowledgments

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Fitochrom i endogenne substancje giberelinopodobne w etiolowanych i napromieniowanych siewkach owsa

Streszczenie

Badano zawartość substancji giberelinopodobnych w koleoptylach owsa różniących się między sobą stanem stacjonarnym fitochromu, jak również w etiolowanych i napromieniowanych wycinkach liści. Etiolowane koleoptyle i liście zawierające maksymalne ilości fitochromu w formie P_R charakteryzują się jednocześnie podwyższoną zawartością związanych giberelin. Napromieniowanie koleoptyli i liści światłem czerwonym, powodując fotokonwersję fitochromu do formy P_{FR}, prowadzi jednocześnie do pojawienia się dużych ilości wolnych giberelin. Uwalnianie hormonów z form związanych skorelowane z powstawaniem w wyniku fotokonwersji fitochromu P_{FR} stanowić może ważny przejaw mechanizmu działania fitochromu w procesach deetiolacji siewek. W pracy dyskutuje się współzależności między fitochromem i hormonami roślinnymi w kontroli fotomorfogenezy młodych siewek roślin jednoliściennych.