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Photocontrol of biological processes in monocotyledonous plants after relaxation of phytochrome system

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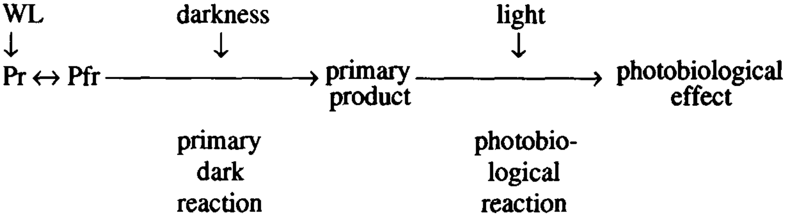
Abstract

Effects of a short-term pre-irradiation of etiolated wheat seedlings by radiation of various wavelengths and various irradiances on the rates of photophospho-rylation, Hill reaction, and chlorophyll accumulation were studied. The curves, constructed for the dependence of inhibitory effect in different processes on pre-irradiance doses, have a saturation level corresponding to photostationary state of the phytochrome system and a pre-threshold site where the inhibition is absent. For an interpretation of the experimental data we suggest a general model that includes both the threshold concept of Mohr and the presence of an additional control mechanism not sensitive to far-red radiation. According to the model, the phytochrome-controlled biological effect is pro-portional to the time during which the level of active phytochrome is higher than the threshold one and may be analysed according to the inhibition degree. The difference in control by red (RR) and green radiation (GR) pretreatments is attributed to the difference of the phytochrome photoconversion rate under RR and GR.

Introduction

Phytochrome system participates in control of various vital functions of plants, in particular the organization of photosynthetic apparatus. Besides a non-de-layed action of phytochrome system, a phytochrome-mediated control is impor-tant, which starts a considerable while after a "white light" (WL) pulse. In the later case the phytochrome control may be represented by the following scheme:



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WL-pretreated phytochrome is converted from the inactive form (Pr) into the active form (Pfr). In the dark, Pfr stimulates a primary dark reaction which proceeds until Pfr exists. This reaction stops after the decomposition of Pfr due to reversion and destruction. In monocotyledonous plants the reversion reaction is absent (Tochver 1975). The primary product is formed as a result of a primary dark reaction and it remains in darkness for a long period of time after the end of phytochrome action (Sharma 1985). The quantity of the primary product determines the value of the photobiological effect in plants under WL.

At present, the physicochemical law-governed nature of conversion of the phytochrome main forms is well-known (*e.g.* Volotovskii 1987), while the intermediate stages which form a signal chain of phytochrome regulation and include a number of reactions from Pfr to the obtained photoeffect are not clear enough (Sharma 1985).

The purpose of the present study was to examine the regularities of the phytochrome-mediated control in accordance with the characteristics of pre-irradiation pulse when the action started after relaxation of the phytochrome system.

Material and methods

Experiments were carried out with 5-d-old etiolated wheat (*Triticum aestivum* L. cv. Scala) seedlings which were thrice pretreated with monochromatic radiant pulses separated by 3-h dark periods. The first batch of plants was pretreated with red radiation (RR) (642 nm), green radiation (GR) (553 nm) or blue radiation (BR) (436 nm), the second one with far red radiation (FRR) (748 nm) after RR, GR or BR. The doses of RR, GR and BR were 4800 μmol m^{-2}, that of FRR was 9600 μmol m^{-2}. After that all the plants were placed into darkness for 18 h. Then they were incubated for 4 h under fluorescent lamps with "white light", WL (30 W m-2). Non-pretreated plants were used as the check ones. The $K_3Fe(CN)_6$-photoreduction (Hill reaction) and non-cyclic photophosphorylation rates were determined by a potentiometric method (Nishimura *et al.* 1962, Zabotin 1970). Chlorophyll content in the leaves was estimated according to Shlyk (1971). Induction transitions of fluorescence (ITF) were examined with a phosphoroscope (Grigor'ev *et al.* 1973).

Results

Our earlier results (Zaitseva *et al.* 1982, Shapiro and Zaitseva 1986) on the effect of short-term pre-irradiation of etiolated plants by radiation of various wave-lengths on the functional activity of photosynthetic apparatus of wheat are summarized in Table 1.

The respective conclusions are: (1) pretreatment with any monochromatic radiation (with the exception of FRR) increases activity of the studied photosynthetic characteristic; (2) pretreatment with FRR after RR or GR lowers the stimulatory effect of RR or GR, (3) the differences between the experimental variants RR + FRR and GR + FRR are small and insignificant, and (4) the activation by BR (λ = 436 nm) is not reversed by FRR.

Table 1. Effect of monochromatic radiation (RR - red, GR - green, BR - blue, FRR - far-red) pretreatment on the rates of Hill reaction with ferricyanide, photophosphorylation and chlorophyll accumulation in wheat seedlings. Significant differences were 2-4 (*) or 5-7 (**) % between the repeated measurements. (From Zaitseva *et al.* 1982, Shapiro and Zaitseva 1986.)

Pre-irradiation	ITF* [rel. units]	Chlorophyll** [g kg^{-1}(fr.m.)]	Photophosphorylation [mmol(ATP) kg^{-1}(Chl) s^{-1}]	$K_3Fe(CN)_6$-photoreduction in Hill reaction [mmol kg^{-1}(Chl) s^{-1}]
Dark	0.20	0.168	18.4	30.8
FRR	-	-	19.3	28.9
RR	0.42	0.354	55.2	75.3
RR+FRR	0.28	0.278	31.6	50.6
GR	0.38	0.330	51.3	72.7
GR+FRR	0.27	0.271	35.7	53.6
BR	0.26	0.254	37.4	75.2
BR+FRR	0.31	0.258	36.0	72.8

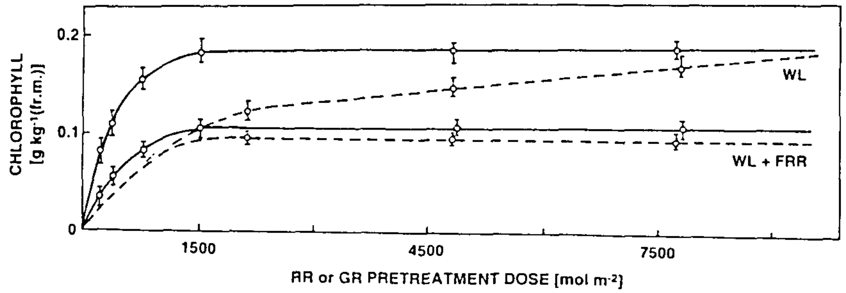

Fig. 1. Influence of monochromatic radiation (*full line* = red, RR, *dashed line* - green, GR) pretreatment dose on chlorophyll accumulation under 'white light' WL or WL + far-red radiation (FRR) (4 h, 30 W m^{-2}) in wheat seedlings.

In the present study we tested the dependence of FRR inhibition on RR and GR pulse doses for the rates of Hill reaction, photophosphorylation and chlorophyll accumulation. The results were qualitatively similar for chlorophyll accumulation (Fig. 1) and both the photochemical processes examined. All the curves had a saturation site but the ways of coming out to it were different for RR and GR. Already with a small dose of RR equal to 600 µmol m^{-2} the curves for WL and WL + FRR were parallel and they reached a plate an at a dose of 1200 µmol m^{-2}. On the contary, under pretreatment GR the curve for WL + FRR reached a plate and at 1200 µmol m^{-2} while the WL curve did not reach it even at 8000 µmol m^{-2}. Curves I coincide with curves II till a certain threshold dose of VL pretreatment is not attained. The threshold doses may be different for different processes and for radiation of different wavelengths. These doses appeared to be 100 μmol m^{-2} in RL and 1500 μmol m^{-2} in GL for Hill reaction and chlorophyll accumulation; for photophosphorylation they are correspond-ingly 150 μmol m^{-2} and 2100 μmol m^{-2}.

Discussion

The qualitative similarity of effects of various pre-irradiations on different photosynthetic characteristics (Table 1) shows that the first phytochrome regulation phase is common for all the processes (see the above scheme: Photoconversion causes formation of a certain amount of Pfr in the system. For interpretation of experimental data we propose a model based on the well-known physicochemical properties of phytochrome (Tochver 1975, Volotovskii 1987):

(1) The controlling effect of phytochrome is determined by the threshold conception (Mohr 1972) according to which the phytochrome-dependent reaction is absent till the Pfr concentration is lower than a certain threshold level characteristic for this reaction. When this threshold level is exceeded the rate of the primary dark reaction does not depend on Pfr concentration. The absence of an inhibitory effect under BR in our studies means that the Pfr threshold concentration in the given conditions is larger that the Pfr photo-stationary concentration at λ = 436 nm, *i.e.* 23 % according to Mohr (1972).

(2) During the period of time when Pfr concentration in the system exceeds the threshold level none of the primary dark reactions approaches the saturation site and they proceed approximately linearly with time. Thus the biological effect of the phytochrome controlling mechanism is proportional to the time during which the Pfr level is higher than the threshold one.

(3) Besides the phytochrome controlling mechanism there is an additional mechanism which is activated by WL and is not sensitive to FRR. An incomplete FRR inhibition of RR and GR action and an activating effect of BR may be explained by the presence of this mechanism in the model.

According to the suggested model both the phytochrome and non-phytochrome mechanisms participate in the control under an WL pulse irradiation. Under WL + FRR the contribution of non-phytochrome mechanism remains unchanged because it is not sensitive to FRR. On the contrary, the phytochrome contribution is considerably reduced because in this case a superthreshold level of Pfr is maintained for a considerable shorter period of time. Hence, the degree of inhibition is determined by the phytochrome mechanism effect only and it is its good indicator. The degree of inhibition for the investigated processes are shown in Table 2.

Inhibition degree is proportional to the time during which the Pfr concentration is higher than the threshold one. The destruction rate in monocotyledonous plants is constant (Tochver 1975) and thus this time is proportional to the difference between Pfr concentration attained at the end of the WL pulse (Pfr*) and the threshold concentration (T) of Pfr. Hence, the ratio of the inhibition value for GR and RR (GR*/RR*) may be determined by the following expression:

$$\frac{GR^*}{RR^*} = \frac{Pfr^*(GR) - T}{Pfr^*(RR) - T} \qquad (1)$$

If in Eq. (1) the T value could be neglected, the GR*/RR* ratio would depend only on the Pfr accumulation kinetics under the WL pulse but not on the type of the investigated process. However, our experimental results demonstrated different GR*/RR* values for different processes (Table 2). Eq. (1) shows that the larger GR*/RR* value corresponds to the lesser threshold value of the investigated process. Moreover, the difference between the thresholds is considerable, since even a small change of the ratio GR*/RR* requires a considerable alteration of the threshold value. Thus in wheat (Table 2) the highest Pfr threshold level is necessary for photophosphorylation control, the lowest level for chlorophyll accumulation and photosynthetic electron transport.

Table 2. Inhibitory effect of far-red radiatioon (FRR) on the action of red (RR) or green (GR) radiation on some photosynthetic characteristics. It is difference between variants WL and WL + FRR: RR* = RR - (RR + FRR). GR* = GR - (GR + FRR).

Variants	ITF* [rel. units]	Chlorophyll** [g kg^{-1}(fr.m.)]	Photophosphorylation [mmol(ATP) kg^{-1}(Chl) s^{-1}]	$K_3Fe(CN)_6$-photoreduction in Hill reaction [mmol kg^{-1}(Chl) s^{-1}]
RR*	0.14	0.076	23.2	24.7
GR*	0.11	0.059	15.6	19.1
GR*-RR*	0.79	0.79	0.67	0.77

The dependence of the effects of a short-term irradiance on the pulse dose may also be explained by our model. Curves constructed for a single-pulse WL pretreatment (Fig. 1) demonstrated the overall effect of phytochrome and non-phytochrome mechanisms, and curves for the WL + FRR variant only the non-phytochrome mechanism. Thus the difference between both curves corre-sponded to the sole phytochrome mechanism. The threshold of Pfr was not attained at low pretreatment doses the phytochrome mechanism was not started, and therefore both the curves coincided. When the threshold dose was exceeded, the threshold level of Pfr was attained and both the curves diverged. The threshold dose for GR was much larger than that for RR because the phytochrome photoconversion rate in RR was much larger than that for GR (Mohr 1972). In our model the ratio of the pretreatment threshold doses is approximately estimated by the ratio of absorption coefficients of inactive phy-tochrome. For the wavelegths used in these experiments, *i.e.* 642 and 553 nm, it is 13.5 (Smith and Daniels 1981). This is in accordance with the ratios of threshold doses of GR and RR - in our experiments 15 and 14, respectively. The Pfr threshold concentration necessary for photophosphorylation control was larger than that for Hill reaction and chlorophyll accumulation, while the thresholds of the two latter reactions coincided. Similar results were achieved by an indirect way; they agree with the results of Oelze-Karow and Mohr (1978).

For certain pretreatment doses a photostationary state of the phytochrome system is attained, and a further increase of dose does not change the action of the phytochrome mechanism. At these doses the difference between the curves for WL and WL + FRR is constant and theis plateans are parallel. A much larger dose of BR than RR is necessary to attain the photostationary state (Mohr 1972).

So a satisfactory interpretation of the experimental data by the suggested phytochrome control model necessitates the existence of an additional mechanism. Probably protochlorophyllide is a receptor of this additional mechanism. Its absolute inertness in FRR could be accounted for an absence of protochlorophyllide absorption in this spectral region. With GR pulse doses sufficient for attaining the photostationary state of phytochrome system the GR controlling effect is close to the RR controlling effect. At the same time, when the pre-irradiation dose is reduced, the phytochrome control effectiveness in GR is diminished considerably faster than in RR. This fact accounts for the conflicting opinions about the existence of phytochrome control in GR. The suggestion that this control in GR (Galston *et al.* 1983) is absent should be attributed to the low irradiances only.

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