

Photochemical properties of photosystem 2 in primary leaves of barley seedlings grown under various blue or red irradiances

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Abstract

Photosystem 2 (PS2)-driven electron transfer was studied in primary leaves of barley (*Hordeum vulgare* L.) seedlings grown under various photon fluxes (0.3-170.0 $\mu\text{mol m}^{-2} \text{s}^{-1}$) of blue (BR) or red (RR) radiation using modulated chlorophyll fluorescence. The F_v/F_m ratio was 0.78-0.79 in leaves of all radiation variants, except in seedlings grown under BR or RR of 0.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The extent of the photochemical phase of the polyphasic F_v rise induced by very strong "white light" was similar in leaves of all radiation treatments. Neither radiation quality nor photon flux under plant cultivation influenced the amount of non Q_B -transferring centres of PS2 except in leaves of seedlings grown under BR of 0.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$, in which the amount of such centres increased threefold. Both BR and RR stimulated the development of photochemically competent PS2 at photon fluxes as low as 3 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Three exponential components with highly different half times were distinguished in the kinetics of F_v dark decay. This indicates different pathways of electron transfer from Q_A , the reduced primary acceptor of PS2, to other acceptors. Relative magnitudes of the individual decay components did not depend on the radiation quality or the photon flux during plant cultivation. Significant differences were found, however, between plants grown under BR or RR in the rate of the middle and fast components of F_v dark decay, which showed 1.5-times faster intersystem linear electron transport in BR-grown leaves.

Additional key words: blue radiation; chlorophyll fluorescence; photosystem 2; red radiation.

Introduction

The members of phytochrome and cryptochrome families of photoregulatory pigments are involved in the regulation of synthesis and assembly of the photosynthetic apparatus (Cashmore 1997, Smith 2000). Phytochromes and cryptochromes are excited preferentially by red (RR) and blue (BR) radiation, respectively. The properties of chloroplasts are different in leaves of plants grown under BR or RR (Buschmann *et al.* 1978). In particular, BR promotes the formation of sun-type chloroplasts (Lichtenthaler *et al.* 1980). The regulatory action of RR- and BR-absorbing photoreceptors on the development of chloroplasts is most pronounced at weak irradiances during the growth of plants, since the contribution of photosynthesis is expected to be small under those irradiances (Bukhov *et al.* 1992). Weak irradiances by "white light" during plant growth stimulate the formation of shade-type chloroplasts with increased amount of light-

harvesting complexes and greater thylakoid stacking (Lichtenthaler *et al.* 1982a,b). The development of the photosynthetic apparatus in primary leaves of barley seedlings at photon fluxes from 12 to 0.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$ is highly sensitive to the radiation quality (Bukhov *et al.* 1999). Reduced amounts of chlorophyll (Chl) and of the major light-harvesting complex of photosystem 2 (PS2) were found in chloroplasts of barley leaves developing under weak BR, but not RR. No information is available, however, whether either the radiation quality or the photon flux affects the electron transport in PS2, including the quantum yield of primary charge separation or PS2 heterogeneity (Laverne and Briantais 1996). The relations of PS2 centres to plastoquinone pools differ in the rate of reduction (Schreiber and Krieger 1996).

Chl fluorescence is a powerful tool for the study of PS2 functioning (Govindjee 1995). Upon the onset of

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Abbreviations: BR – blue radiation; Chl – chlorophyll; F_0 and F_m – basal and maximum levels of chlorophyll fluorescence; F_v – variable chlorophyll fluorescence; F_v/F_m – fluorescence ratio characterising quantum efficiency of charge separation in photosystem 2; PS – photosystem; Q_A and Q_B – primary and secondary acceptors of photosystem 2; RR – red radiation.

strong irradiation, the quantum yield of Chl fluorescence several fold increases from F_0 level to the maximum level, F_m .

Polyphasic kinetics of the variable Chl fluorescence (F_v) rise upon the onset of very strong radiation reflects the filling up with electrons of plastoquinone pools, which differ in the rate of reduction (Strasser *et al.* 1999). Three rise phases were distinguished in that kinetic curve. An initial fast phase reflected the photochemical reduction of Q_A to Q_A^- , while two slower ones were due to removal of electrons from Q_A^- via the so-called "plastoquinone-type" quenching (Kramer *et al.* 1995). The F_v rise under weak actinic radiation allows distin-

guish the population of non- Q_B -transferring PS2 centres from the centres capable of electron transfer from the primary to the secondary quinone acceptor (Cao and Govindjee 1990). Very recently, we have reported that a multiphasic decay of F_v in the dark is a good indicator of different pathways of electron release from Q_A^- , reduced primary electron acceptor of PS2, into active PS2 centres (Bukhov *et al.* 2001a, Egorova *et al.* 2001).

The goal of this study was to examine by means of Chl fluorescence whether photon fluxes of BR or RR applied for plant growth affect the photochemical capacity of PS2, as it was found for total Chl content in leaves (Bukhov *et al.* 1999).

Materials and methods

Plants: Barley (*Hordeum vulgare* L.) seedlings were cultivated in a phytotrone chamber in a mixture of topsoil, sand, and peat (5 : 1 : 1, m:m) at 22/20 °C day/night temperature. The photoperiod was 16 h. BR or RR was provided by colour fluorescence tubes (SS-65 for BR or KS-65 for RR) produced in Russia by the Institute of Light Technique. Irradiance was attenuated with layers of cheesecloth and/or wire-net. Leaf segments were taken at 2-4 cm far from the top of a leaf blade.

Chl content was measured spectrophotometrically after extraction with 80 % acetone according to Arnon (1949).

Chl fluorescence emission from leaves was measured using the PAM-2000 portable fluorometer (Walz, Effeltrich, Germany). Standard «Run 6» and «Run 7» files were used to sample the data. Sampling rate was 1 ms per

point. Actinic RR (655 nm) of different irradiances was obtained from light-emitting diodes. Strong "white light" ($11\,400\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$) was provided by a halogen lamp (150 W, 24 V). It was passed through a Calflex C filter (Balzers) to decrease heat emission. A mechanical shutter with the opening time of 0.5 ms limited irradiation by the lamp. Magnitudes and half-times of different kinetic components of F_v dark decay were obtained from plots of $\lg(F_v)$ as a function of relaxation time. Best linear fits were calculated for each kinetic component (i) providing its half-time as a time at which $F_{v,i}$ reached 50 % of its maximum magnitude which, in turn, was obtained from the intersection of the linear fit with the Y-axis. Leaves were dark-adapted for at least 30 min prior to the measurements of Chl fluorescence. Experiments were done in triplicate. The tables show the means and standard errors for corresponding parameters.

Results

Table 1 demonstrates high photochemical competence of PS2 in leaves of seedlings grown at photon fluxes of 170, 12, or 3 $\mu\text{mol m}^{-2}\ \text{s}^{-1}$ of BR or RR, as manifested by F_v/F_m ratios as high as 0.78-0.79. Slightly lower values of F_v/F_m ratio were found, however, in leaves of plants cultivated

under BR or RR of 0.3 $\mu\text{mol m}^{-2}\ \text{s}^{-1}$. Thus, the radiation quality used for plant growth did not affect maximum quantum yield of charge separation in PS2. In contrast, the Chl *a/b* ratio markedly increased upon the decline in photon flux of BR (Table 1).

Table 1. The effect of radiation quality (BR – blue radiation, RR – red radiation) and photon flux during the cultivation of barley seedlings on maximum quantum yield of charge separation in PS2, F_v/F_m , absolute chlorophyll (*a+b*) content [g kg^{-1} (fr.m.)], chlorophyll *a/b* ratio, the relative magnitudes of photochemical phase of F_v , $(F_0 - I_1)/F_m$, and relative amount of non Q_B -transferring centres of PS2, $(F_0 - F_{pl})/F_v$. Means of three experiments \pm SE.

	Growth irradiance [$\mu\text{mol m}^{-2}\ \text{s}^{-1}$]	F_v/F_m	Chl (<i>a+b</i>)	Chl <i>a/b</i>	$(F_0 - I_1)/F_m$	$(F_0 - F_{pl})/F_v$
BR	170	0.780 \pm 0.005	1593 \pm 137	2.40 \pm 0.04	0.62 \pm 0.04	0.072 \pm 0.023
	12	0.790 \pm 0.003	356 \pm 49	2.50 \pm 0.09	0.58 \pm 0.02	0.064 \pm 0.014
	3	0.790 \pm 0.003	128 \pm 17	3.40 \pm 0.15	0.60 \pm 0.06	0.075 \pm 0.012
	0.3	0.730 \pm 0.011	17 \pm 4	5.30 \pm 0.22	0.50 \pm 0.05	0.223 \pm 0.062
RR	170	0.790 \pm 0.007	1276 \pm 114	2.50 \pm 0.02	0.63 \pm 0.06	0.072 \pm 0.016
	12	0.780 \pm 0.003	421 \pm 76	2.60 \pm 0.06	0.61 \pm 0.05	0.065 \pm 0.009
	3	0.790 \pm 0.004	154 \pm 12	2.70 \pm 0.08	0.58 \pm 0.07	0.060 \pm 0.011
	0.3	0.740 \pm 0.014	113 \pm 21	2.80 \pm 0.13	0.61 \pm 0.07	0.076 \pm 0.017

Typical kinetics of F_v rise upon the onset of very strong "white light" exhibited two distinct phases, the photochemical phase ($F_0 - I_1$) and the thermal one ($I_1 - I_2 - F_m$) (Fig. 1A). In leaves of seedlings grown at high photon fluxes of either BR or RR, both $I_1 - I_2$, and $I_2 - F_m$ phases were clearly distinguished. They were not well separated, however, in leaves of plants grown under low photon fluxes (Fig. 1A). In leaves of all radiation treatments except seedlings grown under $0.3 \mu\text{mol m}^{-2} \text{s}^{-1}$, the contribution of photochemical phase into F_v emission was about 60 % (Table 1). This value is typical for plants with active PS2 (Schreiber and Krieger 1996, Samson *et al.* 1999). The time required by Chl fluorescence to reach F_m level did not vary significantly among leaves of various radiation treatments. This indicated that the light-induced reduction of plastoquinone pool occurred similarly in chloroplasts of leaves, which had been grown under radiation of different qualities and photon fluxes.

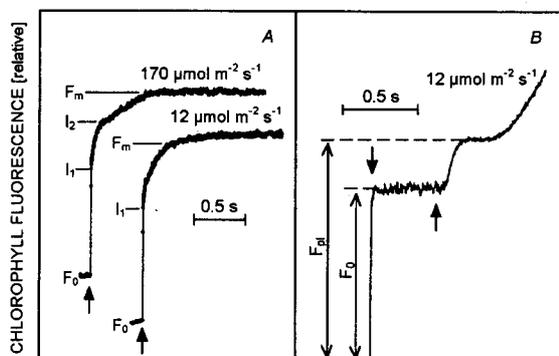


Fig. 1. Original traces of F_v rise initiated by the onset of either strong "white light" (A) or weak red radiation, RR (B) in primary barley leaves grown under blue radiation of 170 or $12 \mu\text{mol m}^{-2} \text{s}^{-1}$. F_0 , F_{pl} , F_m , I_1 , and I_2 are the various levels of chlorophyll fluorescence. Upward arrows indicate the onset of either strong "white light" of $11\,400 \mu\text{mol m}^{-2} \text{s}^{-1}$ (A) or weak RR of $6.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ (B). Downward arrow indicates the onset of very weak excitation radiation.

A plateau (Fig. 1B) rapidly followed the fast initial rise of F_v after the onset of weak actinic irradiation. This part of the rise kinetics is indicative of non- Q_B -transferring reaction centres of PS2 (Cao and Govindjee 1990). Table 1 demonstrates that emission from inactive centres accounted for 6-8 % of the total F_v in nearly all radiation variants. The only exception were leaves of seedlings grown under BR of $0.3 \mu\text{mol m}^{-2} \text{s}^{-1}$, in which the emission from non- Q_B -transferring reaction centres of PS2 exceeded 20 % of the total F_v .

Fig. 1 shows the rise of Chl fluorescence from F_0 level initiated by actinic radiation and related to Q_A

reduction. F_v relaxed to F_0 level few seconds after actinic radiation had been turned off, thus manifesting Q_A^- re-oxidation. Fig. 2 demonstrates the kinetic analysis of the dark decay of F_v initiated in dark-adapted leaves by 1-s pulse of red actinic radiation of $260 \mu\text{mol m}^{-2} \text{s}^{-1}$. Similarly to previous reports (Bukhov *et al.* 2001a, Egorova *et al.* 2001), we quantified our results by describing the dark F_v relaxation in terms of three exponential decay components representing different pathways of electron transfer from Q_A^- . The half times of the fast, the middle, and the slow components of F_v relaxation differed drastically, whereas their magnitudes were similar (Fig. 2).

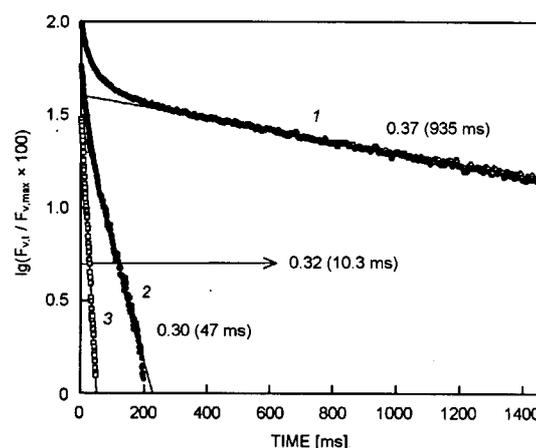


Fig. 2. Semi-logarithmic plots of dark decay of F_v after 1-s application of red actinic radiation pulse of $260 \mu\text{mol m}^{-2} \text{s}^{-1}$ (1) and de-convoluted middle (2) and fast (3) components of that decay. F_v was measured in primary leaves of barley seedlings grown under blue radiation (BR) of $12 \mu\text{mol m}^{-2} \text{s}^{-1}$. Numbers adjacent to the curves indicate the relative magnitudes and half times (in parentheses) of individual components of F_v dark decay. Solid lines represent linear fits calculated for each individual component of F_v decay. The initial curve is the average of 8 measurements done with different leaves.

In all radiation treatments studied, F_v dark decay kinetics was well fitted by a sum of three exponentially decaying components. Table 2 summarises the relative magnitudes and half times of those components obtained with leaves of barley seedlings grown under BR or RR of various photon fluxes. No statistically significant differences were found between the relative magnitudes of individual decay components in leaves grown under different irradiations. Half times of the slow component were also similar in leaves of all radiation treatments. The influence of radiation quality on their half times was, however, obvious for two decay components of the three, namely, the middle and the fast components.

Discussion

The absolute Chl content and, consequently, the amount of PS2 units dramatically declined in leaves with the decrease in photon flux of RR and, particularly, BR, which is in agreement with our previous report (Bukhov *et al.* 1999). However, the data on Chl fluorescence summarised in Table 1 clearly show that the PS2 units possessed high photochemical capacity. The stimulus provided by either BR- or RR-absorbing photoreceptor acting independently appears, therefore, to be sufficient for the assembly of functionally active PS2 in leaves of plants exposed for a long time to radiation of a given quality even when photon flux was very low. Leaves of barley seedlings grown under BR of $0.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ were the exception, as the amount of PS2 centres lacking the capability to reduce secondary electron acceptor increased dramatically in those leaves.

We reported earlier (Bukhov *et al.* 1999) that Chl *a/b* ratio significantly increased in BR-grown leaves following the decline in irradiance during plant cultivation (see Table 1). This indicates loss of the major Chl *a/b*-bound light-harvesting complex of PS2, LHC2 (Peter and

Thornber 1991). Despite a large decrease in the content of LHC2, the F_v/F_m ratio declined slightly in leaves of plants grown under BR or RR of $0.3 \mu\text{mol m}^{-2} \text{s}^{-1}$. These values are consistent with high values of F_v/F_m reported previously for plant mutants lacking LHC2 (Lokstein *et al.* 1994). High F_v/F_m ratios were observed in the objects deficient in LHC2 probably because of the origin of F_v . We showed recently (Bukhov *et al.* 2001b) that, unlike F_0 , F_v is emitted in PS2 of intact leaves as a result of recombination in the radical pair $\text{P680}^+ \text{Pheo}^-$. The recombination yields excited P680, which then equilibrates with nearest Chl *a* molecules.

Similar relative magnitudes of individual components of F_v dark decay found in plants grown under BR or RR of any given photon flux indicate that the radiation climate during plant growth did not influence the relative contribution of different routes of electron release from Q_A^- . It provides additional evidence in favour of highly similar assembly of PS2 units in the thylakoids of all radiation treatments studied.

Fast component of F_v dark decay reflects the move-

Table 2. The effect of radiation quality and photon flux during the cultivation of barley seedlings on both relative magnitudes of the slow (F_{slow}/F_v), middle (F_{middle}/F_v), and fast (F_{fast}/F_v) components of dark F_v decay and their half-times. Means of three experiments \pm SE.

	Growth irradiance [$\mu\text{mol m}^{-2} \text{s}^{-1}$]	F_{slow}/F_m	$t_{1/2,\text{slow}}$ [ms]	F_{middle}/F_v	$t_{1/2,\text{middle}}$ [ms]	F_{fast}/F_v	$t_{1/2,\text{fast}}$ [ms]
BR	170	0.41 \pm 0.04	935 \pm 48	0.29 \pm 0.02	47 \pm 5	0.32 \pm 0.03	10.0 \pm 0.7
	12	0.37 \pm 0.02	935 \pm 67	0.30 \pm 0.04	47 \pm 7	0.32 \pm 0.05	10.0 \pm 0.4
	3	0.31 \pm 0.04	778 \pm 106	0.34 \pm 0.05	87 \pm 11	0.39 \pm 0.03	9.0 \pm 1.3
	0.3	0.42 \pm 0.03	978 \pm 32	0.28 \pm 0.04	91 \pm 9	0.29 \pm 0.05	9.0 \pm 0.6
RR	170	0.39 \pm 0.03	862 \pm 54	0.31 \pm 0.04	136 \pm 10	0.30 \pm 0.04	15.0 \pm 1.4
	12	0.32 \pm 0.01	1087 \pm 79	0.32 \pm 0.03	130 \pm 16	0.36 \pm 0.02	17.0 \pm 2.1
	3	0.31 \pm 0.05	990 \pm 34	0.33 \pm 0.05	138 \pm 9	0.35 \pm 0.04	16.0 \pm 0.9
	0.3	0.32 \pm 0.03	982 \pm 61	0.36 \pm 0.02	119 \pm 14	0.30 \pm 0.05	13.0 \pm 0.5

ment of oxidised plastoquinone molecules from the plastoquinone pool towards PS2 reaction centres, which had no bound Q_B at the moment of termination of irradiation (Eaton-Rye and Govindjee 1988, Egorova *et al.* 2001). The middle component reflects the same movement proceeding severely reduced plastoquinone pools with low availability of oxidised plastoquinones (Egorova *et al.* 2001). Clear difference was found between BR- and RR-grown plants in the rates of those components (Table 2). This indicates more rapid diffusion of oxidised plastoquinone to PS2 complexes in thylakoids of BR-grown plants compared to the RR-grown ones. The relative acceleration of plastoquinone diffusion is likely related to the higher fluidity of lipid matrix in thylakoids of BR-

grown plants. It has previously been reported that in BR-grown plants the thylakoid membranes contain much more unsaturated fatty acids than in plants grown under RR (Manuil'skaya *et al.* 1985).

In summary, our results clearly demonstrate the ability of either BR or RR at photon flux densities as low as $3 \mu\text{mol m}^{-2} \text{s}^{-1}$ to control the development of photochemically active PS2 in leaves irradiated for several days. RR exhibits its specific action through the accumulation of Chls likely incorporated into light-harvesting complexes, which is markedly enhanced compared to BR at very low photon fluxes. Most pronounced specific action of BR is the acceleration of plastoquinone diffusion to Q_B -binding site of PS2 complex.

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