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 μmol m⁻² s⁻¹) of blue (BR) or red (RR) radiation using modulated chlorophyll fluorescence. The Fv/Fm ratio was 0.78-0.79 in leaves of all radiation variants, except in seedlings grown under BR or RR of 0.3 μmol m⁻² s⁻¹. The extent of the photochemical phase of the polyphasic Fv 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 Qa-transferring centres of PS2 except in leaves of seedlings grown under BR of 0.3 μmol m⁻² 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 μmol m⁻² s⁻¹. Three exponential components with highly different half times were distinguished in the kinetics of Fv, dark decay. This indicates different pathways of electron transfer from Qa⁻, 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 Fv, 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 μmol m⁻² 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 (Lavergne 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; Fv and Fm – basal and maximum levels of chlorophyll fluorescence; Fv – variable chlorophyll fluorescence; Fv/Fm – fluorescence ratio characterising quantum efficiency of charge separation in photosystem 2; PS – photosystem; Qa and Qb – 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, \( F_{\text{m}} \).

Polyphasic kinetics of the variable Chl fluorescence (\( F_\lambda \)) 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\( \lambda \) to Q\( \lambda^* \), while two slower ones were due to removal of electrons from Q\( \lambda^* \) via the so-called "plastoquinone-type" quenching (Kramer et al. 1995). The \( F_\lambda \) rise under weak actinic radiation allows distinguishing the population of non-Q\( \lambda \)-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 \( F_\lambda \) in the dark is a good indicator of different pathways of electron release from Q\( \lambda^* \), 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 µmol m\(^{-2}\) 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 \( F_\lambda \), dark decay were obtained from plots of \( \lg(F_\lambda) \) 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 \( F_{\lambda,i} \) 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 µmol m\(^{-2}\) s\(^{-1}\) of BR or RR, as manifested by \( F_{\text{r}}/F_{\text{m}} \) ratios as high as 0.78–0.79. Slightly lower values of \( F_{\text{r}}/F_{\text{m}} \) ratio were found, however, in leaves of plants cultivated under BR or RR of 0.3 µmol m\(^{-2}\) 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>
<thead>
<tr>
<th>Growth irradiance [µmol m(^{-2}) s(^{-1})]</th>
<th>( F_{\text{r}}/F_{\text{m}} )</th>
<th>Chl ( (a+b) )</th>
<th>Chl ( a/b )</th>
<th>( (F_0 - I_1)/F_{\text{m}} )</th>
<th>( (F_0 - F_{\text{r}})/F_{\text{r}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR 170</td>
<td>0.78±0.0005</td>
<td>159±137</td>
<td>2.4±0.04</td>
<td>0.62±0.04</td>
<td>0.072±0.023</td>
</tr>
<tr>
<td>12</td>
<td>0.79±0.003</td>
<td>356±49</td>
<td>2.50±0.09</td>
<td>0.58±0.02</td>
<td>0.064±0.014</td>
</tr>
<tr>
<td>3</td>
<td>0.79±0.003</td>
<td>128±17</td>
<td>3.40±0.15</td>
<td>0.60±0.06</td>
<td>0.075±0.012</td>
</tr>
<tr>
<td>0.3</td>
<td>0.73±0.011</td>
<td>17±4</td>
<td>5.30±0.22</td>
<td>0.50±0.05</td>
<td>0.223±0.062</td>
</tr>
<tr>
<td>RR 170</td>
<td>0.79±0.007</td>
<td>1276±114</td>
<td>2.50±0.02</td>
<td>0.63±0.06</td>
<td>0.072±0.016</td>
</tr>
<tr>
<td>12</td>
<td>0.78±0.003</td>
<td>421±76</td>
<td>2.60±0.06</td>
<td>0.61±0.05</td>
<td>0.065±0.009</td>
</tr>
<tr>
<td>3</td>
<td>0.79±0.004</td>
<td>154±12</td>
<td>2.70±0.08</td>
<td>0.58±0.07</td>
<td>0.060±0.011</td>
</tr>
<tr>
<td>0.3</td>
<td>0.74±0.014</td>
<td>113±21</td>
<td>2.80±0.13</td>
<td>0.61±0.07</td>
<td>0.076±0.017</td>
</tr>
</tbody>
</table>
Typical kinetics of $F_r$ 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 $0.3 \mu mol\ m^{-2}\ s^{-1}$, the contribution of photochemical phase into $F_r$ 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 $F_r$ 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 $\mu mol\ m^{-2}\ s^{-1}$, $F_0$, $F_{ph}$, $F_m$, $I_1$, and $I_2$ are the various levels of chlorophyll fluorescence. *Upward arrows* indicate the onset of either strong “white light” of 11,400 $\mu mol\ m^{-2}\ s^{-1}$ (A) or weak RR of 6.2 $\mu mol\ m^{-2}\ s^{-1}$ (B). *Downward arrow* indicates the onset of very weak excitation radiation.

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

Fig. 1 shows the rise of Chl fluorescence from $F_0$ level initiated by actinic radiation and related to Q$_{A}$ reduction. $F_r$ 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 $F_r$ initiated in dark-adapted leaves by 1-s pulse of red actinic radiation of 260 $\mu mol\ m^{-2}\ s^{-1}$. Similarly to previous reports (Bukhov et al. 2001a, Egorova et al. 2001), we quantified our results by describing the dark $F_r$ 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 $F_r$ relaxation differed drastically, whereas their magnitudes were similar (Fig. 2).

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

In all radiation treatments studied, $F_r$ 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 irradiation of a given quality even when photon flux was very low. Leaves of barley seedlings grown under BR of 0.3 μmol m⁻² 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, LHCII (Peter and Thornber 1991). Despite a large decrease in the content of LHC2, the Fm/Fv ratio declined slightly in leaves of plants grown under BR or RR of 0.3 μmol m⁻² s⁻¹. These values are consistent with high values of Fm/Fv reported previously for plant mutants lacking LHC2 (Lokstein et al. 1994). High Fm/Fv ratios were observed in the objects deficient in LHC2 probably because of the origin of Fv. We showed recently (Bukhov et al. 2001b) that, unlike F0, Fv is emitted in PS2 of intact leaves as a result of recombination in the radical pair P680⁺ Pheo⁺. The recombination yields excited P680, which then equilibrates with nearest Chl a molecules.

Similar relative magnitudes of individual components of Fv 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 Qh⁻. It provides additional evidence in favour of highly similar assembly of PS2 units in the thylakoids of all radiation treatments studied.

Fast component of Fv dark decay reflects the move-

<table>
<thead>
<tr>
<th>Growth irradiance [μmol m⁻² s⁻¹]</th>
<th>Fslow/Fm</th>
<th>t1/2,slow (ms)</th>
<th>Fmiddle/Fv</th>
<th>t1/2,middle (ms)</th>
<th>Ffast/Fv</th>
<th>t1/2,fast (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR 170</td>
<td>0.41±0.04</td>
<td>935±48</td>
<td>0.29±0.02</td>
<td>47±5</td>
<td>0.32±0.03</td>
<td>10.0±0.7</td>
</tr>
<tr>
<td>12</td>
<td>0.37±0.02</td>
<td>935±67</td>
<td>0.30±0.04</td>
<td>47±7</td>
<td>0.32±0.05</td>
<td>10.0±0.4</td>
</tr>
<tr>
<td>3</td>
<td>0.31±0.04</td>
<td>778±106</td>
<td>0.34±0.05</td>
<td>87±11</td>
<td>0.39±0.03</td>
<td>9.0±1.3</td>
</tr>
<tr>
<td>0.3</td>
<td>0.42±0.03</td>
<td>978±32</td>
<td>0.28±0.04</td>
<td>91±9</td>
<td>0.29±0.05</td>
<td>9.0±0.6</td>
</tr>
<tr>
<td>RR 170</td>
<td>0.39±0.03</td>
<td>862±54</td>
<td>0.31±0.04</td>
<td>136±10</td>
<td>0.30±0.04</td>
<td>15.0±1.4</td>
</tr>
<tr>
<td>12</td>
<td>0.32±0.01</td>
<td>1087±79</td>
<td>0.32±0.03</td>
<td>130±16</td>
<td>0.36±0.02</td>
<td>17.0±2.1</td>
</tr>
<tr>
<td>3</td>
<td>0.31±0.05</td>
<td>990±34</td>
<td>0.33±0.05</td>
<td>138±9</td>
<td>0.35±0.04</td>
<td>16.0±0.9</td>
</tr>
<tr>
<td>0.3</td>
<td>0.32±0.03</td>
<td>982±61</td>
<td>0.36±0.02</td>
<td>119±14</td>
<td>0.30±0.05</td>
<td>13.0±0.5</td>
</tr>
</tbody>
</table>

ment of oxidised plastoquinone molecules from the plastoquinone pool towards PS2 reaction centres, which had no bound Qb 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 μmol m⁻² 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 Qh⁻-binding site of PS2 complex.
References


