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Photoinhibition of Photosystem II In Vivo During Greening of the Wheat Seedlings

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Abstract: The photoinhibition of photosystem II in vivo by analysis of diverse components -initial rate, steady state rate and lag phase-of photosynthetic O_2 evolution curves on greening wheat seedlings after illumination by excess white light (320 W/m^2) was investigated. A sharp reduction in the initial and steady state rates and a simultaneous intense rise in the lag phase of O_2 evolution were observed under the illumination of seedlings by excess light on the lag phase of chlorophyll a biosynthesis (less than 6 h of seedling greening) in comparison with the illumination of seedlings by excess light at the stage of substantial pigment synthesis (> 6 h of seedling greening). It is assumed that photosystem II proteins not completely integrated in thylakoid membranes as chlorophyll-protein complexes of reaction centres at the early stage of wheat seedling greening were more susceptible to excess light.

Key Words: Wheat seedlings, O_2 evolution, photoinhibition, photosystem II

Buğday Fidelerinin Yeşermesi Sırasında In Vivoda Fotosistem II'nin Fotoinhibisyonu

Özet: Kuvvetli ışık (320 W/m^2) uygulanan buğday fidelerinin yeşermesi üzerinde O_2 çıkışı eğrilerinin çeşitli elemanları-başlangıç hızı, anlık hız ve gecikme evresi-analiz edilerek Fotosistem II'nin fotoinhibisyonu araştırılmıştır. Büyük miktarda pigment sentezlenme evresinde uygulanan kuvvetli ışıklandırmaya göre, klorofil biyosentezinin gecikme evresinde kuvvetli ışık uygulamasına bağlı olarak O_2 çıkışının başlangıç ve anlık hızlarında ani bir düşüş, gecikme evresinde ise ani bir yükseliş gözlenmiştir.

Anahtar Sözcükler: Buğday fideleri, oksijenin ayrılması, fotoinhibisyon, fotosistem II

Introduction

The illumination of a photosynthetic system by excess light leads to a stepwise inactivation of photosystem II (PSII). There is consistent in vivo evidence that the major site of photoinhibition is located in PSII (Krause, 1988). Photoinhibition of PSII is shown to be accompanied by a lowering of the yield of maximum variable fluorescence (F_v), as was observed after treatment with photoinhibitory light. Electron transport capacity in PSII was shown to be linearly related to some parameters of variable fluorescence. The activity of PSII thylakoids isolated after photoinhibitory treatments of spinach leaves at 20°C was lowered to the same extent as the F_v/F_m ratio of the leaf discs (Krause et al., 1990). Arising mainly from in vitro studies, 2 mechanisms of photoinhibition of PSII have been proposed: acceptor-side and donor-side

photoinhibition. The acceptor-side photoinhibition is assumed to be caused by strong reduction (overreduction) of the acceptor side, blocking electron flow from Q_A^- to Q_B , followed by double reduction and protonation of Q_A^- (Vass et al., 1992). In donor-side photoinhibition arising from impaired electron donation from the oxygen evolving complex, the cause of PSII inactivation is assumed to be $P680^+$ or Tyr Z^+ (Yegerschold et al., 1990; Eckert et al., 1991). The prevailing mechanism of photoinhibition of PS2 is usually accompanied by light-induced down regulation of electron donation of P680 (Krieger et al., 1992). It was shown that photoinhibition of PSII in vivo is caused by oxidising species on the donor side (Van Wijk and Van Hasselt, 1993) in analogy to the photoinhibition of PSII inactivated by artificial pretreatment, for instance, by Tris-washing.

The situation is much more complicated and unclear in developing cells. The formation of thylakoids is a multistep process. The cores of PSI and PSII are formed first, followed by the formation of light harvesting chlorophyll-protein complexes (Akoyunoglou, 1992; Gasanov et al., 1988). The rate of chlorophyll *a* formation is a determining factor in thylakoid development since most of the polypeptides formed are stabilised by assembled chlorophyll *a* pigment-protein complexes.

According to this working hypothesis, in this study we investigated the photoinhibition of PSII in vivo by illumination with excess light at different stages of the biosynthesis of chlorophyll *a* and the development of thylakoids in greening etiolated wheat seedlings.

Materials and Methods

Growth conditions and photoinhibition treatment.

Etiolated wheat (*Triticum durum*) seedlings were grown from seeds soaked for 24 h at 25 °C in complete darkness for 7 days. After 7 days' growth, the seedlings were transferred to white light (60 W/m²) for greening with different time intervals (4, 6, 12, 18 and 24 h). For control, 7-8 day old seedlings grown under light were used. The photoinhibition of seedling leaves at different stages of development was performed in a specially designed chamber with constant temperature, air and humidity. For photoinhibition treatments, seedlings were exposed in the chamber to illumination with white light (320 W/m²) for 1 to 20 min.

Measurement of oxygen evolution rate. Leaf discs (4 x 5 mm) from the parts of seedlings illuminated with excess light were cut immediately after illumination and transferred for photosynthetic O₂ evolution measurement. Photosynthetic oxygen evolution relative rates were measured on a polarised uncovered Haxo-Blinks type platinum electrode. Under the illumination by a 500 W projection lamp with a lens system and heat filter the electrical signal obtained from the electrode was amplified and recorded. Oxygen evolution relative rates were obtained from values after 5 min of illumination when a fairly constant rate had been reached (see Figure 1a).

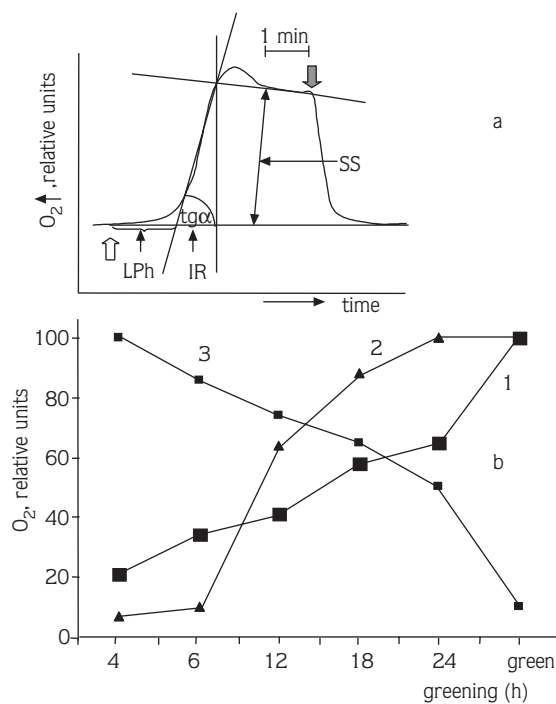


Figure 1. Kinetic curve of 12 h greening etiolated seedlings (a) and time courses (b) of different components of oxygen evolution in greening wheat seedlings

a : ↑ - light on ↓ - light off

Designation of components:

SS – steady state level of O₂ evolution;

IR – initial rate of O₂ evolution

LPh – lag-phase, distance between “light on” and initial point of O₂ evolution

b: 1 – SS; 2 – IR; 3 – LPh

Results and Discussion

Figure 1a illustrates the kinetic curve of photosynthetic O₂ evolution of 12 h greening wheat seedlings leaves in response to illumination and the time course of different components of O₂ evolution at different stages of greening (Figure 1b). At the initial stage of the kinetic curve immediately after the illumination of wheat seedling leaves we observed a lag-phase (LPh) in the rate of O₂ evolution, which takes place at a different time (1 min for 12 h greening seedlings), as indicated in Figure 1a. Then there was a rapid increase in oxygen evolution, represented as an initial rate (IR), followed by a fairly constant rate, represented as the steady-state (SS) stage of the O₂ evolution kinetic curve (see Figure 1a).

The change in the O_2 evolution initial rate as a function of the illumination time by excess white light is shown in Figure 2. As expected, there was an inhibition of the initial rate of O_2 outburst by preillumination with excess light. This photoinhibition of the initial rate of O_2 evolution induced by excess light is observed clearly in wheat seedlings preilluminated for 1 and 3 min at the early stage of greening for 4 and 6 h, respectively (Figure 2). This photoinhibition effect is markedly decreased in seedlings greening for a long time. The photoinhibition of the initial rate of O_2 evolution is observed after 7 and 13 min of preillumination with strong light in the seedlings greening for 12 and 24 h, respectively (Figure 2).

Figure 3 shows the response of the steady-state rate of O_2 evolution to preillumination with excess light. Photoinhibition of the steady-state stage of O_2 evolution was much clearer at early stages of seedling greening at the time when the lag-phase in chlorophyll *a* biosynthesis was usually observed (Figure 3). The dependence, similar to the initial rate of O_2 evolution under preillumination with strong light, was observed for the steady-state stage of O_2 evolving ability. An increase in wheat seedling

greening time results in an increase in seedlings' stability to preillumination with strong light. The apparent reduction in the preillumination of the steady-state rate of O_2 evolution was observed in the seedlings with a maximal rate of chlorophyll *a* biosynthesis (more than 12h greening), as seen in Figure 3.

In contrast, the effect of excess light on the behavior of the lag-phase stage of O_2 evolution differed strikingly between seedlings greening for short and long times prior to preillumination with photoinhibitory light (Figure 4). In this case a strongly increasing period of lag-phase of O_2 evolution induced with excess light was observed in seedlings greening within the lag-phase of chlorophyll *a* biosynthesis, compared with at a time that exceeds the time of lag-phase chlorophyll *a* accumulation (Figure 4). As usually the photoinhibition of the lag-phase of O_2 evolution of seedlings greening for 4 and 6 h is increased by 20%-30% only after 1-3 min of illumination with strong photoinhibitory light whereas the rate of photoinhibition of the same parameters of 12 h greening seedlings varied between 10% and 15% after 7 min of illumination with excess light intensity.

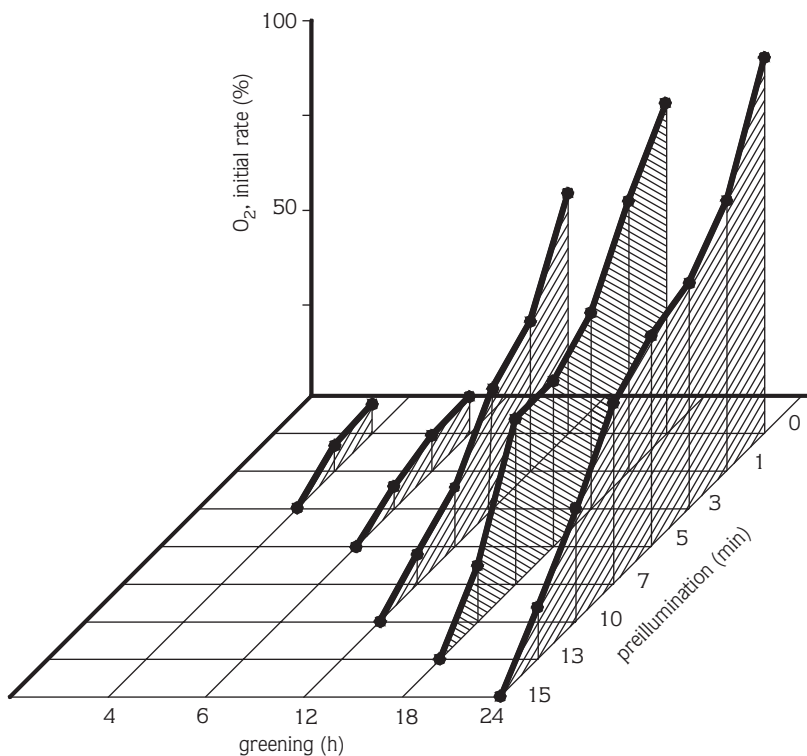


Figure 2. Effect of strong light on the initial rate of O_2 evolution during greening of the wheat seedlings.

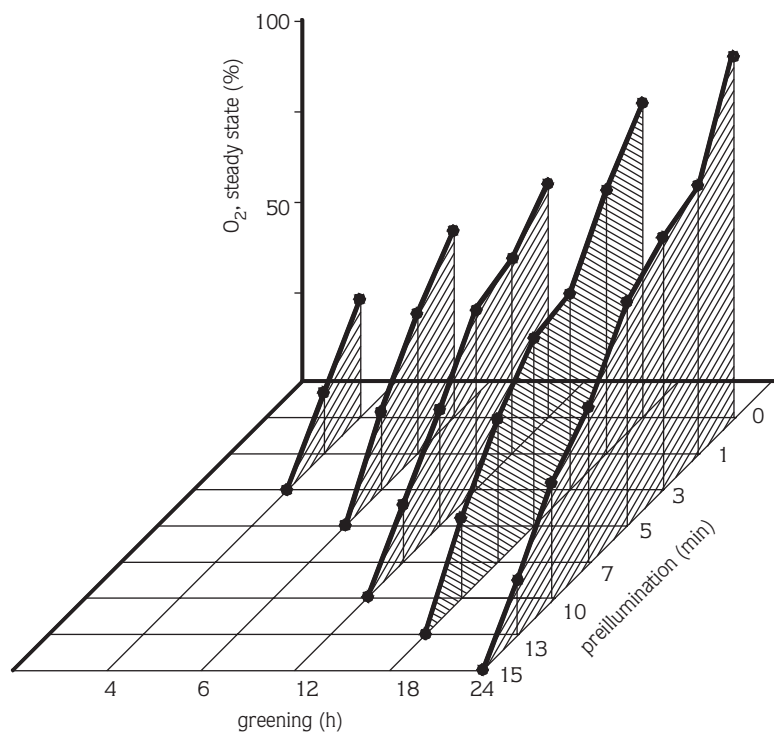


Figure 3. Effect of strong light on the O₂ evolution steady-state components during greening of the wheat seedlings.

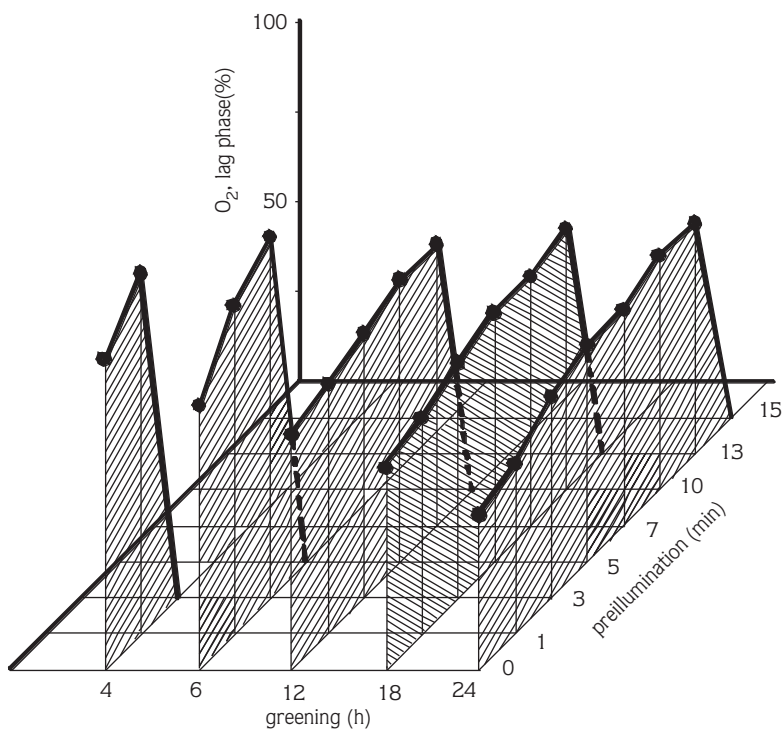


Figure 4. Effect of strong light on the lag-phase components of the O₂ evolution kinetic curve during greening of the wheat seedlings.

It is evident now that photosynthetic membrane biosynthesis takes place during a complicated multistage process (Arntzen and Briantais, 1975; Argyroudi-Akoyunoglou and Akoyunoglou, 1979; Gasanov et al., 1988). Primary thylakoids are known to be the starting membrane structure underlying chloroplast lamellas' membrane. This initial step shows the PSI and PSII reaction centre, cytb₆/f complex, CF₁-CF₀ complex and oxygen evolving complex component formation (see Figure 1b, initial time of greening). Nevertheless, most pigment-protein complexes, and other components, remained unconnected to the reaction centres (Gasanov et al., 1988; Schovefs et al., 1998). As Figure 1a shows, this step of photosynthetic machinery formation is connected with a long lag-phase of O₂ evolution, a slow initial rate of O₂ outburst and a low intensity of steady-state level of O₂ evolution.

Photosynthetic electron transport system formation follows the path of special integration of PSI and PSII-type loci and noncyclic electron flow components with further formation of a conjugated noncyclic electron transport chain. This step requires longer illumination and is not conjugated with the structural rearrangements (>30 min and <6 h greening time, Figure 1b).

There are sharp increases in all characteristics of O₂ evolution kinetics after 6 h of greening of etiolated leaves (Figure 1b).

The illumination (greening) simultaneously triggered the transformation of protochlorophyllide *a* to chlorophyllide *a*. Chlorophyll *a* accumulation presented a lag-phase whose length was twice as long in young leaves as that in old ones. After the lag-phase of chlorophyll *a* accumulation chlorophyll *b* is accumulated (Gassman, 1973; Schovefs et al., 1998). Characteristics of the lag-phase during the development of chloroplasts in greening etiolated leaves give information about the status of plastids.

Next, completing stages assures the highest organisational level and light-harvesting complexes assemblage and incorporation into the membrane with PSI and PSII peripheral antenna formation (Gasanov et al., 1988; Schovefs et al., 1998). At this step of greening (more than 6 h) the lag-phase of O₂ evolution and initial

rate and steady-state level of O₂ evolving capacity are approaching that of green seedlings (Figure 1b).

Strong illumination, the intensity of which is higher than required for saturation of photosynthesis, inhibits photosynthetic reactions. In the green plants PSII is the first to react to such an effect. One can see that the appropriate state of the system may give rise to the development of the so-called acceptor or donor mechanism of photoinhibition (Kyle et al., 1984; Styring et al., 1990; Prasil et al., 1992; Mamedov and Gasanov 1993, 1994). The results obtained demonstrate that a short period of strong illumination had a dramatic effect on the investigated characteristics of the O₂ evolution of photosynthesis (Figure 2-4). The 3 characteristics influenced differently by the time of strong illumination were investigated. At the first level of greening (<6 h) the photoinhibition can be explained by a incomplete integration of Q_A and Q_B binding proteins D₂ and D₁ into the PSII reaction centre. The dramatic photoinhibition of initial rate and steady-state level of O₂ evolution and particularly the increasing time of the lag-phase of O₂ evolving capacity confirm this explanation. Another explanation for the results observed is that the lack of light-harvesting chlorophyll-protein complexes might prevent normal light absorption and light energy migration in thylakoids.

On the other hand, the photoinhibition of the initial rate and steady-state level of O₂ and a sharp increase in the time of the lag-phase of O₂ evolution during the first phase of illumination with excess light on the etiolated seedlings greening less than 6 h indicate that the reaction centre of PSII or some site near it might be damaged. Any damage on this level reflects an effect on the water splitting system. It is possible to suppose that at the initial stage of greening of seedlings the stability of the Mn-cluster or its ability to function normally considerably increases the extent of photoinhibition. This assumption makes it possible to localise the primary damage in the course of photoinhibition induced by the donor side on the Mn-cluster itself or in its environment.

It seems reasonable to conclude that the inhibition of O₂ evolution in greening seedlings in vivo with strong light is caused by a structural disruption on the donor and/or acceptor sides of the PSII.

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