Prompt and Delayed Chlorophyll Fluorescence of Intact Leaves in the Presence of Photosynthetic Herbicides

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Introduction

It is well-known that photosynthesis, and particularly Photosystem 2, is very sensible to a wide range of stress conditions and could be an early indicator for detecting plant stress. One of the most employed biophysical methods to study the function of Photosystem 2 in vivo and in situ is chlorophyll a fluorescence, or prompt fluorescence (PF). Another signal that is even more sensitive and information-rich than PF is delayed fluorescence (DF), or delayed luminescence (see Box 1). However, DF has not gained the same popularity and attention as PF, partly because the signal is rather complex and hard to interpret.

In order to better understand DF, it is useful to follow the correlation between PF and DF, measured simultaneously from the same sample during the induction period of dark to light adaptation (Goltsev et al., 2003). Further information could be drawn by applying photosynthetic inhibitors to the sample, thus restricting the electron transport and making the system simpler.

In this study, the photosynthetic herbicides diuron and atrazine, which block the electron transport between Q_A and Q_B (see Bowyer et al., 1991), were applied to pea plants and the simultaneously measured PF and DF induction curves were analyzed. Our goal was to elucidate the nature of the different DF peaks appearing in the induction curve.

DELAYED FLUORESCENCE is light emitted by pre-illuminated photosynthetic samples in the dark. The origin of this emission is back transfer of electrons and charge recombination at the reaction centre of Photosystem 2 resulting in excitation of the antenna chlorophylls (see Jursinic, 1986 for review). Since DF is generated by the same molecules as prompt fluorescence, it has the same spectral properties. However, PF decays in a few ns while DF can last for many minutes. The DF decay consists of a number of exponential components in the time scale from s to minutes, related to various steps in the photosynthetic electron-transport chain. The charge recombination is an activation process; therefore it depends on temperature and electromagnetic fields. Thus DF is highly sensitive to the transmembrane electrochemical gradient. On the other hand, factors that affect the yield of fluorescence, such as the various processes of non-photochemical quenching, have a similar impact on DF.

	Prompt fluorescence	Delayed fluorescence
Source	PS2 antenna chlorophyll a	
Transition	first singlet to ground state	
Lifetime	few ns	s to minutes
Excitation mechanism	direct excitation by light absorption or recombination of the primary radical pair	charge recombination after back electron transfer
Sensitivity to electromagnetic fields	weak	strong

Materials and Methods

14-days-old pea plants grown hydroponically under controlled conditions (60 mol photons.m^{-2}.s^{-1}, 23-25°C) were used. The roots were cut under water and the plants were kept on herbicide solution for 12 h in darkness and 8 h under illumination with growth light.

PF and DF induction curves were measured simultaneously from dark-adapted detached leaves using an FL-2006 fluorometer (Test, Russia). The actinic light (1200 mol photons.m^{-2}.s^{-1} at the sample surface) is mechanically modulated by means of a Becquerel-type disc phosphoroscope, providing alternating light and dark periods of about 5 ms duration. PF is registered during the light periods and the DF decay is registered during the dark periods (Zaharieva and Goltsev, 2003).

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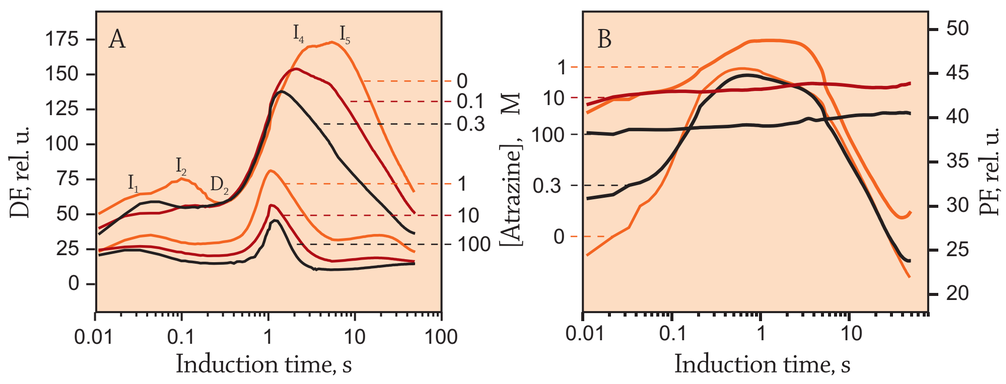

Figure 1

Induction curves of PF (A) and DF (B) registered from dark-adapted detached leaves of pea plants kept for 20 h on atrazine solution. The concentrations corresponding to each curve are indicated on the Y-axes.

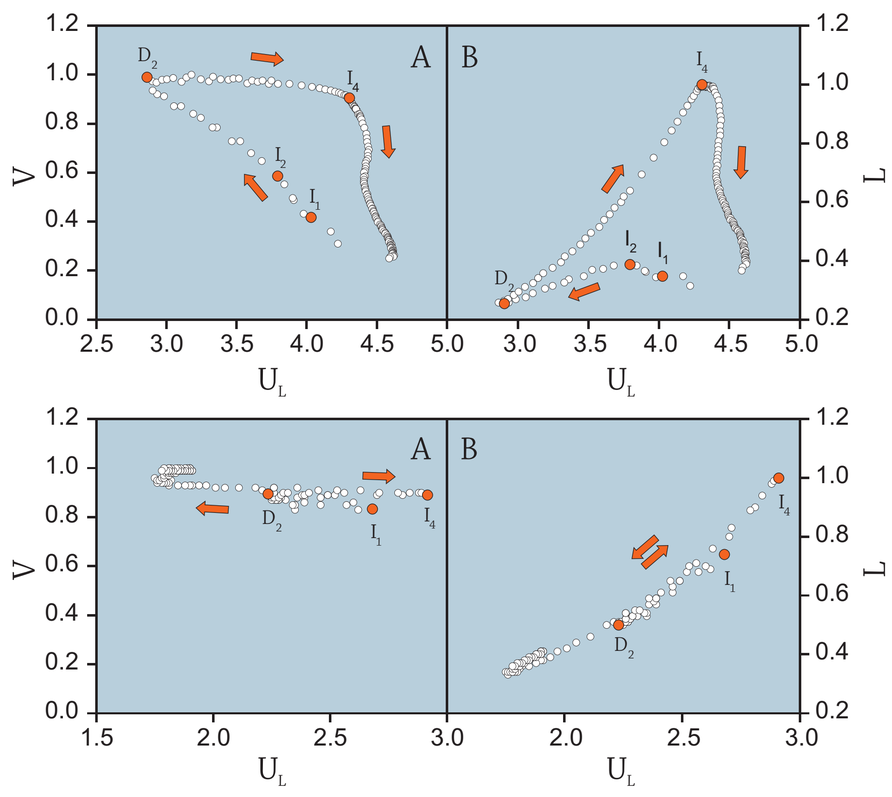

Figure 2

Phase diagrams representing the course of PF (plots A, C) and DF (plots (B, D) as a dependence on the luminescence potential in control pea leaves (A, B) or treated with 10 M atrazine (C, D). The arrows indicate the direction of the phase trajectories in respect with time. The red circles indicate the characteristic points of the DF induction curve.

THE LUMINESCENCE POTENTIAL, U_L, is defined as the sum of the redox potential of the primary quinone acceptor of Photosystem 2, Q_A, and the transmembrane electrochemical potential:

$U_L = E' + H^+$

U_L measures the driving force for the delayed fluorescence, because in the ms scale DF is proportional to the extent of open reaction centres (with Q_A oxidized) and the logarithm of the transmembrane potential.

By theoretically analyzing the correlation between PF and DF we have found that U_L can be estimated by the ratio between the DF intensity, L, and the variable fluorescence, F_v:

$U_L \sim \ln (L / F_v)$

The simultaneous measurement of PF and DF allows to calculate the U_L values at different times of the induction period. PF and DF can be then plotted against U_L to obtain phase diagrams similar to those shown in Fig. 2. The linear regions on the phase diagrams indicate that only one of the components of U_L (E' or H^+) is responsible for the luminescence changes while the other one is constant. Thus, the luminescence potential provides a tool to distinguish between the changes due to the redox potential of Q_A (the openness of the reaction centres) and the transmembrane potential.

PF & DF induction curves

The herbicides atrazine and diuron had profound effects on the PF and DF induction curves of the treated plants depending on concentration (Fig. 1). The DF changes observed with increasing the herbicide concentration can be summarized as follows:

> DF intensity gradually decreased. The DF decaying in milliseconds is proportional to the extent of open reaction centres, while the closed centres attribute to the longer (seconds) components of the DF decay (see Malkin et al., 1994). The inhibitors provoke closure of the reaction centres and thus diminish DF.

> I_2 and I_5 decreased rapidly and were completely erased at high concentrations. This points out that electron transport and/or open reaction centres are required for these peaks to appear. I_2 has been related to the electron transport between Q_A and Q_B (Goltsev and Yordanov, 1997).

> I_1 and I_4 were less sensitive and were clearly expressed even at saturating herbicide concentrations. It should be assumed that under such conditions the maxima are independent of the electron transport. Therefore, they should be ascribed to transient changes in the transmembrane potential. I_1 may be due to an electrical gradient generated by the photoinduced charge separation (Satoh and Katoh, 1983) and I_4 could be a result of pH formed by cyclic electron transport around Photosystem 1.

> The beginning of the D_2-I_4 rise was not changed at all. This is also an evidence that I_4 reflects the transmembrane proton gradient, which is rapidly built with the activation of Photosystem 1.

Luminescence potential

Figure 2 shows the time courses of the normalized PF (V) and DF (L), as a dependence on the luminescence potential (U_L) which sums the redox potential of Q_A and the transmembrane gradient (Box 2, Goltsev et al., 2003). These phase diagrams can help to understand the contributions of the redox state of Q_A and pH to the changes in PF and DF during the induction period.

In control plants the phase diagrams (plots A, B) exhibited three distinct phases, described below.

1. Beginning to D_2: U_L decreased and PF increased (plot A), supposedly due to the photoinduced reduction of reaction centres. In this phase DF (plot B) was not linearly related to U_L, possibly as a result of changes in the transmembrane electrical gradient induced by charge separation.

2. D_2 to I_4: U_L returned to its initial values, however PF was only slightly changed. Therefore the changes in this phase were not due to the redox state of Q_A, as in the previous one, but due to pH. A strong support for that is that DF rose exponentially with U_L, in accordance with the known exponential dependence of DF on pH (Wraight & Crofts, 1971).

3. I_4 to end: U_L did not change significantly while both PF and DF decreased sharply. Most probably the luminescence changes were not caused by either of the two U_L components, but were a result of non-photochemical quenching that reduces the quantum yield of radiative de-excitation.

In plants treated with 10 M atrazine, which fully blocked the electron transport, the phase diagrams exhibited only one phase. PF was almost constant in the whole induction period (Fig. 2C) even though U_L was changing, and DF (Fig. 2D) was at all times exponentially related to U_L. This clearly shows that the DF changes in inhibited leaves were strictly following the changes of the transmembrane electrochemical gradient.

Conclusions

> DF is a highly sensitive non-destructive probe for the action of photosynthetic herbicides in vivo.

> The DF maxima I_2 and I_5 are related to the electron transport at the acceptor side of Photosystem 2 and require that the reaction centres are open.

> I_1 and I_4 are related to the transmembrane electrochemical potential and thus could be used to measure it in vivo.

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