

The use of the chlorophyll fluorescence for estimation of photosynthetic electron transport flow in water stressed pea plants

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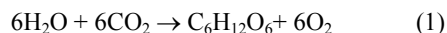
The main act in the photochemical conversion of light energy by plants in the photosynthesis process is the delivery of electron from special chlorophyll molecule from the reaction center of photosystems, after the light absorption at the antennae level. This photochemical reaction is the driving force of the photosynthesis, and is followed by the electron conduction in the molecular transport chain up to the consumer: chemical machinery located in the stroma that performed biochemical reactions of synthesis of organic matter starting from inorganic CO₂ in the chloroplast. In this paper we analyze the photochemical light reactions in photosynthesis from spectroscopic point of view, discussing also the similarities with the photoelectric effect. Starting from the optical and spectroscopic properties of the chlorophyll, main pigment in the photosynthesis, we have used the fluorescence emitted by chlorophyll *in vivo* for the estimation of the quantum yield of photochemistry and the electron flow in the electron transport chain, comparing the values obtained under water stress for pea plants.

(Received November 30, 2005; accepted August 22, 2007)

Keywords: Photosynthesis, Blue green fluorescence, Chlorophyll fluorescence

1. Introduction

Photosynthesis is the process by which plants and bacteria use the energy from sunlight to produce sugar, which cellular respiration converts into ATP. The photosynthetic process uses water and releases the oxygen. The overall reaction of this process may be written according to [1], as:



The conversion of sunlight energy into chemical energy is associated with the actions of the green pigment, the chlorophyll (Chl).

Photosynthesis is a two stage process. In the first one, call Light Dependent Processes (Light Reactions), occurs the photochemical act. Light strikes Chl *a* from reaction centers of photosystems and excites the electrons to a higher energy state. In a series of reactions this energy is converted (along an electron transport process) into energetic carriers ATP and NADPH molecules, used further in the second process call Dark Reactions to reduce the carbon dioxide to carbohydrates [CH₂O]_n, process call carbon fixation.

Photochemical act occurs in the photosystems, complexes of chlorophyll, proteins and other pigments packed into the thylakoids membrane of chloroplast (organelle in the plant cell that conducts photosynthesis) in a specific architecture enabling a very efficient absorption of visible light (cca 80 %). Eukaryotes have two photosystems working in series, Photosystem II and Photosystem I, containing in the reaction center a special molecule of Chl *a* referred as P680 and respectively P700. Both "active" forms of chlorophyll *a* function in

photosynthesis due to their association with proteins in the thylakoid membrane. Light of different colors is efficiently absorbed by leaves at the antennae level by the complex pigment assembly that have different absorption spectra allowing the use of photons of different wavelength shorter than a threshold of 680 nm in PSII or 700 nm in PSI.

In summary, in photosynthesis, light energy causes the removal of an electron from a molecule of Chl and, consequently, there is induced a flow of electrons from water to final acceptor NADP, across a potential difference between lowest (P680) and highest energy states (P700*) of about 1.8V [2,3].

In the last decades, several spectroscopic methods have been considered for characterizing the status of plant and assessing their biomass production. Among these methods, the possible use of fluorescence emitted by plants under laser or daylight excitation has been the object of intense research and appears to be a promising tool [4-6].

Fluorescence from chlorophyll *in vivo* is a very sensitive indicator of the energy status of the photosynthetic system, indicating how the multiple processes of energy absorption and utilization interact.

Chlorophyll *a* solution *in vitro* emits some 30 % of the light absorbed as fluorescence but *in vivo* Chl fluorescence yield is variable and under physiological conditions may go from a maximum fluorescence emission of about 12 % to a minimum of about 2 % from the PSII absorbed photons (Table 1). Most emission from a leaf at normal temperatures is from Chl *a* of PSII; PSI only produce significant fluorescence at low temperatures.

Table 1. Typical utilization of absorbed quanta by PSII when electron transport is optimal or blocked [7].

Mechanisms	Electron transport	
	optimal	blocked
Photosynthesis	84	0
Heat	14	88
Fluorescence	2	12

Once an electron from Chl molecule is promoted into an excited state following photon absorption, the rate of decay of the excited state depends on radiationless transitions, excitation transfer, fluorescence and photochemistry. The associated rate constants for these processes are respectively K_d , K_t , K_f and K_p so that the overall rate constant may be written:

$$K = K_d + K_t + K_f + K_p. \quad (2)$$

Singlet excited states are relatively short lived, that of chlorophyll being about 5×10^{-9} s. The fluorescence quantum yield is expressed according to [3] by the relation:

$$\Phi_f = K_f / (K_d + K_t + K_f + K_p * P) \quad (3)$$

P being the fraction of PSII reaction centres that can accept energy from chlorophyll.

The rate of fluorescent emission, F , is proportional to the light absorbed (I), to the fraction of the energy reaching the PSII (b), with the Chl a concentration and quantum yield (Φ_f);

$$F = I * b * (\text{chl PSII}) * \Phi_f \quad (4)$$

Illuminating a dark-adapted leaf induced a typical Chl a transient fluorescence from the minimal to the maximal fluorescence, the Kautsky curve, that is represented below:

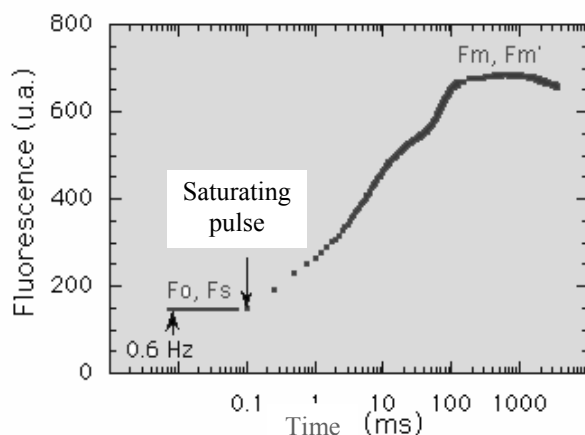


Fig. 1. A typical Kautsky curve - the Chl a fluorescence transients induced by illumination of a darkness leaf with a saturating pulse from FIPAM fluorimeter (from [8]).

At F_0 on the induction curve the PSII reaction centers are fully oxidized, $P = 1$; after saturating light pulse, at maximum fluorescence, F_m , Q_A is fully reduced, $P=0$, so the maximum yield of fluorescence ΦF_m is obtained. The variable fluorescence, $F_v = F_m - F_0$, is the difference between the respective quantum yields and F_v/F_m become proportional to the yield of photochemistry.

Variable fluorescence, is a very important component derived from the fluorescence signals because it reflects the balance of energy between the antenna and the reaction centre and that used by the photochemical and other processes. It is related to the efficiency with which energy is used and therefore to the pathways of chemical processes downstream of the photosystems.

It was shown [9, 10] that from the PSII chlorophyll fluorescence levels it may be estimated the quantum yield of linear photosynthetic electron flow. A linear relationship is obtained in many condition (varying light intensity, CO_2 concentration, during induction of photosynthesis) between the quantum yield of CO_2 assimilation and the photochemical yield of PSII, $\Phi_{\text{PSII}} = (F_m' - F_s)/F_m'$, in the absence of photorespiration [10].

2. Experimental

2.1. Material

Pea plants (*Pisum sativum* L., var. Petit Provençal) were used for the water stress experiment. Plants were grown in a growth chamber under a 16h:8h light-dark photoperiod at $300 \mu\text{mol photons m}^{-2}\text{s}^{-1}$, in pots containing soil regularly watered. Water stress was induced by withholding watering. Variable chlorophyll fluorescence measurements were performed on the 3rd leaf of 3 week old pea plants.

2.2. Methods

We have used for measuring photochemical yield and electron transport flow the saturating pulse method as described by [11, 12] using a modulated chlorophyll fluorimeter PAM 2000 (Walz, Germany) exciting the Chl fluorescence at 650 nm using a light emitting diode (LED).

Variable Chl fluorescence measurements were performed under different light irradiance after minimum 30 min adaptation to darkness or 10 min to PAR. Minimal or stationary fluorescence (F_0 , or F_s for dark and light adapted leaves, respectively) was measured previous to the application of 1 s saturating pulse used to induced the maximal fluorescence (F_m or F_m'). Optimum quantum yield of PSII was computed as follows:

$$F_v/F_m = (F_m - F_0)/F_m, \quad (5)$$

The quantum yield of the linear photosynthetic electron flow was computed following [10]:

$$\Delta F/F_m' = (F_m' - F_s)/F_m' \quad (6)$$

The electron transport rate (ETR) was estimated from the relationship:

$$ETR = \Delta F/F_m' \times PAR \times f_a \times 1/2, \quad (7)$$

where $f_a = 0.84$ is the fraction of PAR that is absorbed by the pea leaf. The factor $1/2$ takes into account the light absorption by the two photosystems, PSI and PSII.

Non-photochemical quenching computed from maximal fluorescence levels as:

$$Q_N = (F_m/F_m') - 1 \quad (8)$$

may give also useful information about the dissipation of the excitation energy from PSII. Relaxation kinetics of Q_N allows to discriminate between three main energy dissipation mechanism:

- energetical quenching (qE)-rapidly reversible with halftime $t_{1/2} \approx 15$ seconds
- state transition (qT)- $t_{1/2} \approx 10$ minutes
- inhibition (qI) - $t_{1/2} > 30$ minutes associated to damage of PSII centers

Leaf water deficit (LWD) was estimated on reference plants from the same pot. Leaf discs were cut and weighted immediately and after 24 h of re-hydrating in distilled water at 4 °C. Water deficit was calculated from the difference between the fresh and turgid weights, as percent from the turgid weight [13].

3. Results and discussion

Chlorophyll absorption and fluorescence

Under near-UV irradiation of a leaf, fluorescence emission can mainly be observed in the red and blue domains of the visible spectrum (Fig. 2).

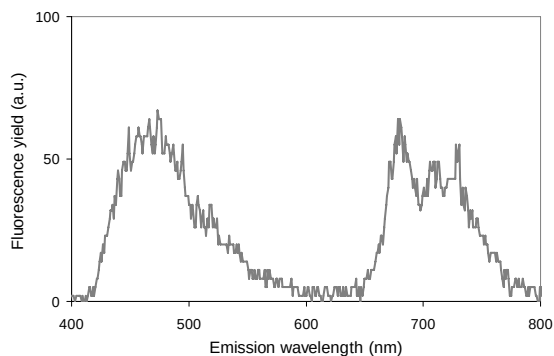


Fig. 2. Fluorescence emission spectrum of a maize leaf under UV (360 nm) excitation.

The red fluorescence, coming from chlorophyll corresponds to one of the de-activation processes of chlorophyll molecules excited by photons. This emission appears in the 650-740 nm wavelengths domain and the signal exhibits two maxims at 690 nm and 735 nm. Beside this red fluorescence, a second emission of similar intensity exists in the blue domain of the spectrum and is characterized by two peaks at 450 nm and 530 nm. The origin of this blue-green (BGF) fluorescence is still under discussion, but increasing evidence is found to associate it with non-photosynthetic parts of the plant tissue including cellular wall components or precursors, skin waxes and vacuolar metabolites [6].

Chlorophyll looks green in white light because it absorbs light in the blue (about 420 nm) and in the red parts (660 nm) of the visible spectrum and transmits and reflects in the green [14]. Each quantum of red light which is absorbed by the chlorophyll molecule raises an electron from the ground state to an excited state and all of its energy is transferred in this process. Absorption of blue light causes even greater excitation (because of the higher energy content of the blue quantum) but the elevated electron then falls back into the "red orbital" too quickly to permit useful chemical work. Thus, whatever the quality of the light absorbed, the electron reaches the same energy level more or less immediately after excitation and all subsequent events derive from this common starting point.

Some of the excitation energy of chlorophyll is lost by radiationless de-excitation and some is used to drive the "chemical" reactions of photosynthesis (ATP synthesis, NADP reduction, etc.). Some energy is also dissipated as fluorescence. Fluorescence (which takes about 10^{-9} s to discharge) derive from the lower more persistent "excited state one" rather than "excited state two" which decays in about 10^{-13} s. For this reason chlorophyll fluorescence is red, regardless of the quality of the exciting light. It is shifted to longer wavelength than the red absorption peak (Stokes shift) because electrons fall to the ground state from the lowest levels of excitation (the rapid cascade of heat dissipation) and must therefore give rise to photons of lower energy content.

Variable chlorophyll fluorescence measurements in water stressed plants

Variable chlorophyll fluorescence measurements were performed at leaf level using a PAM 2000 fluorimeter as described in Experimental conducting to the assignment of F_0 , F_m (for dark adapted leaves) and F_s and F_m' (for light adapted leaves) levels. Results obtained for pea plants under good physiological conditions (control) and submitted to progressive water stress to induce a mild (30 % water deficit) or a strong water stress (50 % water deficit) are presented in Fig. 3.

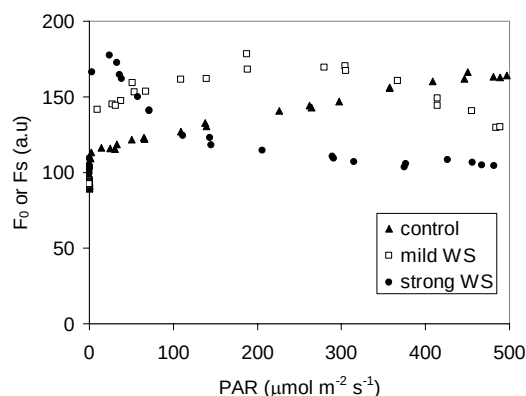


Fig. 3. Stationary chlorophyll fluorescence measured under different light irradiation for a control pea plant and a mild and strong water stressed plant.

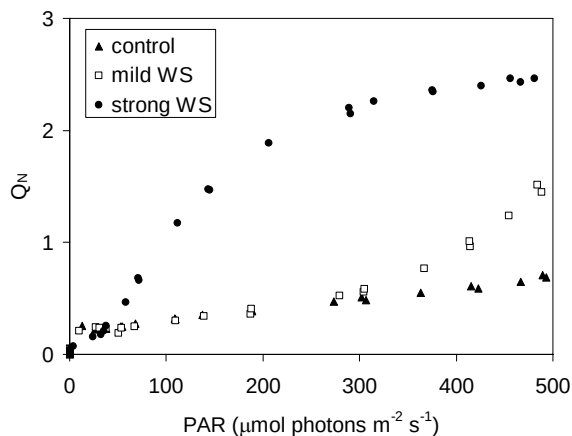


Fig. 4. Non-photochemical quenching fluorescence computed following relation (7) for control and water stressed plant.

Analyzing the results presented in Fig. 3 it might be seen that in water stressed plants there is an increasing of stationary fluorescence at increasing leaf irradiance comparing to the control plants up to a certain level, followed then by a decrease of F_s level. This decrease of F_s is correlated to the development of higher non-photochemical quenching as seen from Fig. 4. We conclude that in water stressed plants there is an important reduction of electron transport chain, as indicated by the increase of F_s and photo-electrons produced by Light Reactions cannot be used in the Dark Reactions. Consequently the mechanisms of dissipation of excess excitation became active as seen by the increase of non-photochemical quenching (Fig. 4).

Fig. 5 shows the optimum and effective photochemical quantum yield as computed from measured fluorescence levels using the relations 5 and 6. It might be seen from Fig. 5 that in control plant the optimum quantum yield has values of around 0.84, typical for pea plants under good physiological conditions. This optimum

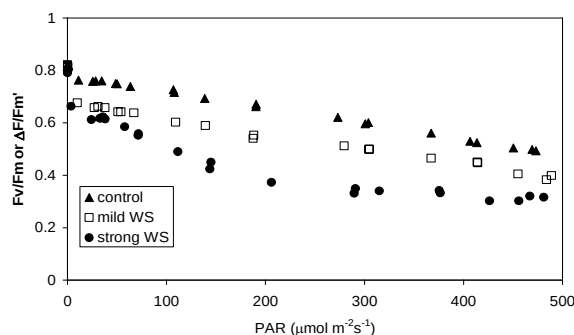


Fig. 5. PSII photochemistry quantum yield estimation at different leaf irradiation for control and water stressed plants.

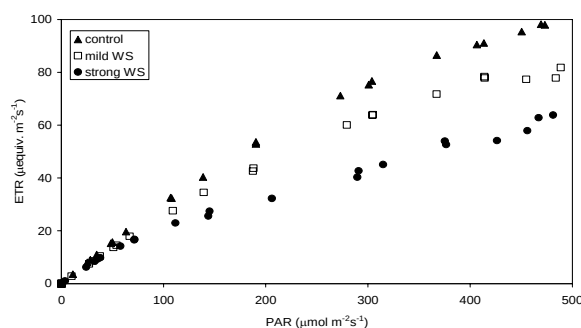


Fig. 6. Electron transport flow estimation function of leaf irradiance for control and water stressed pea plants.

PSII quantum yield decrease to 0.79 in the strong water stressed plant and not significantly in mild stressed plant. Significant decreases of PSII effective quantum yield may be observed for both mild and strong water stressed plants in the presence of light, starting from very low irradiance (PAR around $10 \mu\text{mol m}^{-2} \text{s}^{-1}$).

Fig. 6 shows the values obtained for computing the electron flow using quantum yield presented in Fig. 5 and equation 7. It might be seen that in a control plant there is a quite linear relationship between electron flow and photon flux density (PAR) up to a certain level and start to deviate at high PAR (around $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ here, but it depends on plants and condition of growth).

In water stressed plants the electron flow obtained at same PAR is lower than that for control plant and saturation is attained at lower irradiance. Excess excitation is dissipated by different mechanism. However, this capacity of dissipation is limited and if exceeded when the light intensity is too high, or use of photoelectron is limited, photoinhibition can occur and photosynthetic machinery can be damaged.

In summary, in photosynthesis, photoelectrons are generated in photosystems, following the absorption of light and have several similitudes with the photoelectric effect taking place in inorganic matter, as described by Einstein, 1905:

- 1) A photoelectric current appears in the molecular circuitry if the consumer (final acceptor) exists for

closing molecular circuitry; electron flow may be measured.

- 2) It requires the absorption of photons of a certain energy (maximal wavelength threshold around 680-700 nm). Energy of photons of shorter wavelength is lost as heat.
- 3) The number of generated photo-electrons (electron flow) depends on the light intensity (number of photons) but not on its wavelength (still a dependence of wavelength exists in the leaf absorption properties).

When discussing the dependence of photoelectron current on light irradiation it must be pointed out that the current is highly regulated by complex mechanisms, depending on the chemical need of electrons in the biochemical Calvin cycle.

4. Conclusions

Photosynthesis is a complex process taking place in plants, functioning due to the interconnected molecular machinery, well optimized in terms of efficiency, protected against harmful products of his work (reactive oxygen species) and self regulated by very complex reaction networks. Chlorophyll fluorescence may be successfully used to determine *in vivo* and *in situ* the quantum yield of photochemistry and electron flow, allowing nondestructive, rapid and sensitive insight into the functioning of complex photosynthesis process and vegetation monitoring.

Acknowledgements

The authors acknowledge the financial support of CNCIS for this study, through an AT grant (no. 149/2004). We thank G. Dima and S. Dinu for the reading of the manuscript.

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