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Simultaneous *in vivo* recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*☆

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ABSTRACT

A new instrument (M-PEA), which measures simultaneously kinetics of prompt fluorescence (PF), delayed fluorescence (DF) and modulated light reflection at 820 nm (MR), was used to screen dark-adapted leaves of the resurrection plant *Haberlea rhodopensis* during their progressive drying, down to 1% relative water content (RWC), and after their re-watering. This is the first investigation using M-PEA, which employs alternations of actinic light (627-nm peak, 5000 μmol photons m^{-2} s^{-1}) and dark intervals, where PF-MR and DF kinetics are respectively recorded, with the added advantages: (a) all kinetics are recorded with high time resolution (starting from 0.01 ms), (b) the dark intervals' duration can be as short as 0.1 ms, (c) actinic illumination can be interrupted at different times during the PF transient (recorded up to 300 s), with the earliest interruption at 0.3 ms. Analysis of the simultaneous measurements at different water-content-states of *H. rhodopensis* leaves allowed the comparison and correlation of complementary information on the structure/function of the photosynthetic machinery, which is not destroyed but only inactivated (reversibly) at different degrees; the comparison and correlation helped also to test current interpretations of each signal and advance their understanding. Our results suggest that the desiccation tolerance of the photosynthetic machinery in *H. rhodopensis* is mainly based on mechanism(s) that lead to inactivation of photosystem II reaction centres (transformation to heat sinks), triggered already by a small RWC decrease.

1. Introduction

The chlorophyll (Chl) *a* fluorescence emitted by higher plants, algae, lichens and photosynthetic bacteria upon illumination (prompt fluorescence, PF) carries a lot of information for the structure and function of the photosynthetic apparatus. The discovery of the variable fluorescence by Kautsky [1] showed that it consists of a fluorescence rise until a peak P (F_P) and a subsequent decrease until a steady state S (F_S). In higher plants and algae the fluorescence emitted at room temperature originates, predominantly, from the antenna Chls of photosystem (PS) II. For several decades, the true extremes of the fast rise, i.e., F_0 at the origin O – where all PSII reaction centres (RC) are open, and F_M – the maximal F_P (reached when all RCs are closed), could not be detected due to the poor time resolution and weak illumination, respectively, of the former instruments.

The resolution of the fast fluorescence rise was highly improved with instruments using optoelectronic parts. With modulated light-fluorometers accurate F_0 values could be measured using a weak modulated light beam before illumination with actinic light, and F_M could also be reached under the strong illumination used; though the

Abbreviations: Chl, chlorophyll; DT, drying-time (of leaves); DF, delayed fluorescence; Fd, ferredoxin; FNR, ferredoxin-NADP$^+$ reductase; F_M and F_0, maximum and minimum fluorescence intensity, respectively; J and I, intermediate steps in the Chl *a* fluorescence rise (OJIP) appearing between F_0 and F_M at about 2 and 30 ms, respectively; LES, "light emitting state" (responsible for DF emission by PSII); MR, modulated reflection (here at 820 nm for the determination of P700 and PC oxido-reduction; "modulated" refers to the light beam used); P700, Chl of PSI RC; PC, plastocyanin; PF, prompt fluorescence; OEC, oxygen-evolving complex; Pheo, pheophytine; PQ, plastoquinone; PQH_2, plastoquinol; PS, photosystem; Q_A and Q_B, primary and secondary quinone electron acceptors of PS II; RC, reaction centre; RWC, relative water content (of leaves); Tyr, tyrosine. For abbreviations used in the JIP-test, see Table A1 in the Appendix

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fluorescence rise is measured with a low time resolution, it was shown that the F_0 to F_M rise is multi-phasic, with intermediate steps I_1 and I_2 [2]. The availability of a direct-light-fluorometer (PEA – Plant Efficiency Analyzer, Hansatech Instruments), with the high time resolution of 10 μs and the first reliable point (taken as F_0) at 50 μs (or at 20 μs with later PEA versions, like with the M-PEA used in the present study), allowed to record, on logarithmic time scale, the full fluorescence rise from O to P, with distinct and accurately detected intermediate steps – J (at 2 ms) and I (at about 30 ms); the F_0–F_J–F_I–F_P (F_M) trace is since called as *fast fluorescence rise OJIP* [3,4].

Based on the *theory of energy fluxes in biomembranes* [5], an analysis of the fast OJIP fluorescence rise has been developed, called as *JIP-test* [6] (for reviews, see [7,8]), which links the different steps and phases of the transient with the redox states of PSII and, concomitantly, with the efficiencies of electron transfer in the intersystem chain and to the end electron acceptors at the PSI acceptor side [9]. The model, a "Z-scheme" expressed by energy fluxes, and the mathematical formulation of the JIP-test are presented in Fig. 1 (for the glossary and definition of terms, see Table A1 in the Appendix).

Opto-electronic devices with modulated light beams allow since many years to measure very small absorption changes, as transmission or reflection changes. A modulated light source built in the measuring head of a PEA fluorimeter allowed the measurement of the kinetics of light-induced absorption changes at 820 nm, which are indicators of the redox state changes of the PSI RC (P700) and plastocyanin (PC), simultaneously with the prompt fluorescence PF and with the same time resolution [10]; this means that it became possible to measure simultaneously the redox states of the reaction centres of both photosystems.

All redox reactions of the photosynthetic electron transport between PSII and PSI and all electron transfer reactions in the RC of PSII (donor and acceptor side) are reversible. The accumulation of electrons in the electron transport chain between PSI and PSII leads to back electron transfer and charge recombination in PSII RC, resulting in the re-excitation of the RC and the repopulation, by fast energy transfer, of the excited chlorophyll state of PSII antenna. The light emission from the repopulated excited Chls is delayed (hence, denoted as delayed fluorescence, DF) compared to the prompt fluorescence PF that is emitted before the utilization of the excitation energy in the primary photochemical reaction (for a recent review see [11]).

With a new instrument (M-PEA, Multifunctional Plant Efficiency Analyser) that measures, simultaneously with PF, the modulated reflected beam (MR) at 820 nm, the light phase of a PF transient can be interrupted by short dark intervals, ranging from μs to ms, during which the DF kinetics are recorded with the same data acquisition as PF and MR. The recombination reactions that provoke the DF signal depend on the redox state of the PSII primary electron quinone acceptor (Q_A), which is reflected in the relative variable prompt fluorescence $V_t = (F_t - F_0)/(F_M - F_0)$. The redox state of Q_A depends on the redox states of the electron transport chain carriers, which, in turn, depend on the redox state of PSI RC (P700) that is reflected in the MR kinetics. Therefore, the simultaneous measurements of PF, DF and MR allow collecting and correlating complementary information for all three domains of the photosynthetic electron transport – PSII electron donor side, electron transport between PSII and PSI, and PSI electron acceptor side.

The understanding of drought stress adaptation of plants is a main goal of international research projects in Agronomy. Though the choices of agronomical techniques are limited and determined by the location, the choice of the crop is wide and can be further increased by breeding, molecular modifications and seeds selection; this requires detection and quantification of stress and stress tolerance. *In vivo* multi-parametric bio-spectroscopy allows screenings of thousands of samples in the field, or over big agricultural areas by remote sensing, and then be complemented with biochemical methods in the laboratory for calibration reasons, as well as for a further investigation in crucial cases.

Observing and learning from existing cases in nature that exhibit exceptional survival strategies against drought can become essential for engineering of transgenic plants, a strategy often adopted today. Several cases of wild types, extraordinary in respect to drought stress tolerance, are known. Most lichens, many mosses and some ferns tolerate complete desiccation with high vitality when they are

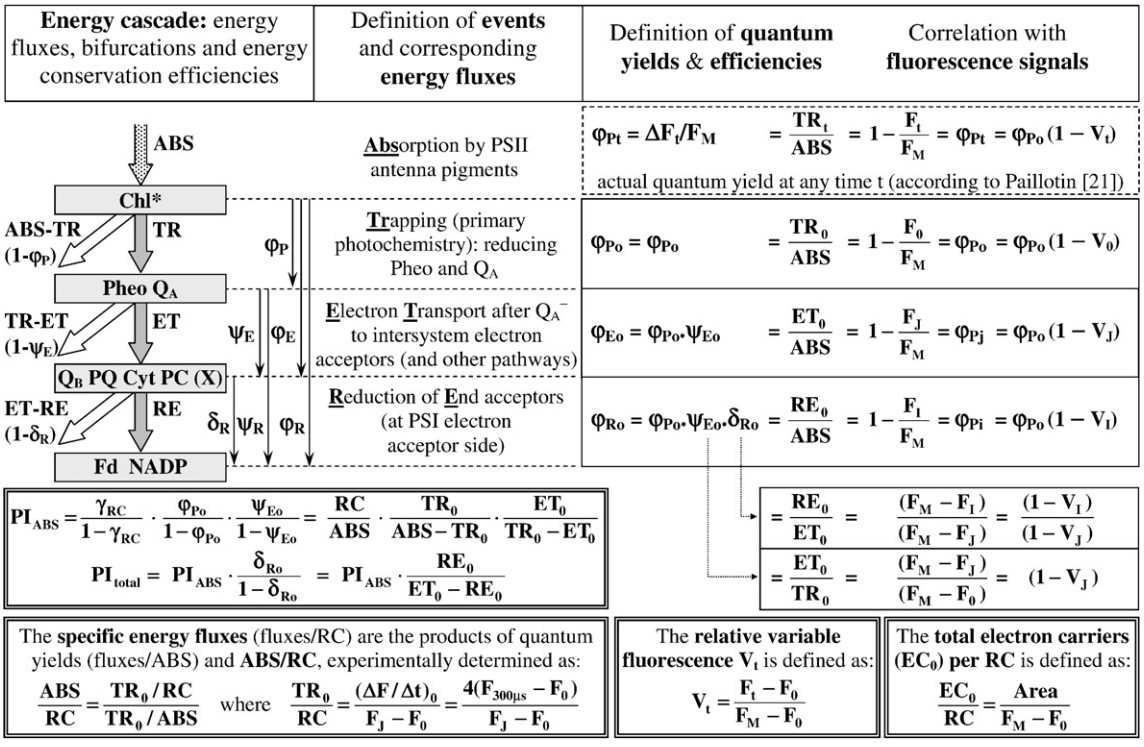

Fig. 1. A schematic presentation of the JIP-test (modified after [9]). For details see Material and methods section.