Technical communication

Sensitivity of the relative $F_{pl}$ level of chlorophyll fluorescence induction in leaves to the heat stress

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Abstract

The $(F_{pi} - F_o)/F_v$ value of the fluorescence induction curve is shown to be a more suitable parameter to detect a wider range of heat stress damage to thylakoid membranes as compared to quantities $t_{1/2}$ (time of fluorescence rise from $F_o$ to $(F_o + F_m)/2$ level) and $\tau$ (the fluorescence induction time defined as the area above the induction curve normalized to $F_v = 1$). A method for exact and automatic $F_{pl}$ determination is presented.

A break point in the quality and behaviour of the fluorescence induction curve of barley leaves incubated at 49 °C was reached at the moment (about 240 s) when the transformation of PS II active ($Q_B$-reducing) to PSII inactive ($Q_B$-non-reducing) centres was completed. The meaning of the standard $F_v$ and $F_v/F_m$ parameter was then changed.

The method of $F_{pl}$ determination described here may help to increase the analytical value of the standard chlorophyll fluorometers.

Abbreviations: $F_o$—initial fluorescence; $F_m$—maximal fluorescence; $F_{pi}$—fluorescence at first inflection point ('plateau'); $F_v$—variable fluorescence ($F_v = F_m - F_o$); PSM—plant stress meter; SD—standard deviation

Introduction

The measurement of the chlorophyll fluorescence induction curve is a very useful method for detection of changes in the stress state of the photosynthetic apparatus. Many empirical parameters have been derived from the induction curves—the one most frequently used is the $F_v/F_m$ ratio, interpreted as a measure of the quantum efficiency of PS II photochemistry (Krause and Somersalo 1989, Schreiber et al. 1989, Krause and Weis 1991, Öquist and Hunner 1991). This interpretation of the $F_v/F_m$ value brings no direct information on the heterogeneity of PS II. Some other parameters, however, are in some cases (Naus and Melis 1992) more sensitive indicators of stress and contain information about PS II heterogeneity—especially parameters based on the measurement of the $F_{pl}$ value (Fig. 1).

The advantage of the $F_{pl}$-related parameter $(F_{pl} - F_o)/F_v$ is its clear interpretation as a measure of PS II $Q_B$-non-reducing centres (Guenther and Melis 1990). The existence of the plateau as a characteristic property of the induction curve has been substantiated theoretically (Malkin 1966, Baake and Schlöder 1992).

Sometimes, the $F_i$ value, as the first local
maximum or shoulder, is measured instead of $F_{p2}$ and used in stress-related studies (Neubauer and Schreiber 1989, Leverenz et al. 1990). Cao and Govindjee (1990) have brought evidence for the interpretation of the OID phase of fluorescence induction as representing the $Q_A$ reduction in PSII inactive centres. Similarly, evidence of PSII heterogeneity with respect to the electron transport and fluorescence properties was documented by, e.g. Briantais et al. (1988) and Chylla and Whitmarsh (1989) and surveyed by Govindjee (1990).

Whereas the $F_o$, $F_v$ and $F_m$ values are measured automatically by commercial instruments, the $F_{pl}$ must be evaluated by a different method. One of these methods (but only an approximate one) was described by Shaw et al. (1986).

In this paper, we suggest an unambiguous way for the determination of $F_{pl}$ as the first inflection point in the fluorescence induction curve. The $F_{pl}$ is then defined as a local minimum of the first derivative of the original induction curve (Fig. 1a and b). The method might be of value especially in measurements with whole leaves where the chemical method using FeCN for $F_{pl}$ determination (Melis 1985) cannot be used effectively. An example of $F_{pl}$ determination is presented for the case of heat damage to PSII centres upon incubation of barley leaves at 49 °C.

Materials and methods

Seedlings of spring barley (Hordeum vulgare L., cv. Zenit) were grown in a cultivation chamber under a light intensity of 100 μmol m$^{-2}$ s$^{-1}$ at the regime – light 16 h (22 °C) and dark 8 h (18 °C). After 10 days, in the growth phase of the second leaf (1.2 according to the Feekes macrophenological scale) the central segment (adaxial side) of the primary leaf was used for measurements.

The central segment of the primary leaf blade was excised and immersed in the dark in distilled water of a constant temperature of 49 °C for a time interval from 1 s to 1 h.

The fluorescence induction curve at room temperature was detected using the Plant Stress Meter manufactured by Biomonitor AB S.C.I. (Umeå, Sweden) (Oquist and Wäss 1988). A recording time of 1 s and photon flux density of 400 μmol m$^{-2}$ s$^{-1}$ were used. The parameters $F_o$, $F_m$, $F_v$, $F_v/F_m$ and $t_{1/2}$ are displayed after each measurement. Before measurements, the leaves were dark-incubated for at least 15 min. The fluorescence induction curves were transferred from the memory of the PSM to a personal computer (PC-AT) for further analysis. To load the recorded signal from PSM, a 14 bit A/D converter was used (Super-Lab Card PCL-714 by Advantech Co., Ltd.). The sampling frequency was 7.877 Hz, the number of digital samples was 512.

Determination of the induction parameters $F_{p1}$ and $\bar{t}$

The originally loaded fluorescence induction curve was filtered by a floating average filter (width 15 samples). The first derivative was calculated and filtered again with the same filter. The $F_{pl}$ parameter was evaluated by iterative algorithm from the curve as a fluorescence level at the first inflection point (e.g. at the first local minimum at time $t_{pl}$ on the curve of the first derivative – see Fig. 1b).