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Can chlorophyll-*a* fluorescence parameters be used as bio-indicators to distinguish between drought and salinity stress in *Tilia cordata* Mill?

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ABSTRACT

Chlorophyll-*a* fluorescence measurements have been used (for many years) to study the function and performance of photosynthetic machinery of various plants. However, only few recent works have shown that this tool can be useful to identify stress factor that affects or/and limits plant growth. The aim of our research was to identify chlorophyll fluorescence parameters which can be used as indicators to drought or/and salinity stress in *Tilia cordata* Mill. Young potted trees (1–1.5 m high with a trunk of 2–3 cm in diameter) were exposed to drought stress or salinity stress for 4 weeks under semi-controlled conditions in a greenhouse. Chlorophyll-*a* fluorescence measurements were conducted every week four times during the stress treatment. The fluorescence parameters based on OJIP transient curves were calculated in order to ascertain which of them was affected by drought and/or salinity. We found that salinity and drought had similar mechanisms of action on the light-depend photosynthesis phase, however the response of photosystem II to applied stress occurred earlier in drought-stressed plants. Changes appeared as damage to the oxygen-evolving complex and reaction centres with a simultaneous increase in dissipated energy. Since both stress factors induced similar photo-inhibitory influence on the light-depending photosynthesis phase, the chlorophyll-*a* fluorescence tool cannot be recommended as a bio-indicator to distinguish between drought and salinity stress effects in *Tilia cordata* Mill.

1. Introduction

Chlorophyll-*a* fluorescence (ChF) analysis has recently become a popular method to detect environmental stress in plants, including drought and salt stress (Percival, 2005; Fini et al., 2009; Šajbidorová et al., 2014; Salvatori et al., 2014; Guo et al., 2016). The 'JIP-test' developed by Strasser et al. (2004) gives the possibility to gain a deeper insight into photosynthetic apparatus conditions under particular types of stress as it enables scientists to distinguish the effect of stress on particular phenomena involved in light absorption and its conversion to biochemical energy. High-time resolution measurements of so-called prompt (or 'fast') fluorescence (Strasser et al., 2010) when plotted on a logarithmic time scale allow a curve of ChF changes to be plotted, as well as a dataset for calculation of several JIP-test parameters to be provided. These parameters describe energy fluxes occurring inside and around the RC of PSII (Strasser et al., 2004, 2010), so-called 'specific energy fluxes' when expressed per active RC, and 'phenomenological energy fluxes' when expressed per an excited CS. In this way, it is possible to evaluate the consecutive energy fluxes of average photon absorption (ABS), exciton trapping (TR), energy dissipation (DI), electron transport (ET) and the reduction in end electron acceptors at the PSI acceptor side (RE). Additional parameters characterising the PSII

Abbreviations: ChF, chlorophyll-*a* fluorescence; CS, cross section of tested sample; ET, electron transport; ETC, electron transport chain; F_0, minimal fluorescence yield of the dark-adapted state; F_m, maximal fluorescence yield of the dark-adapted state; F_v/F_m (φ_{Po}), maximum quantum yield of PSII photochemistry; F_V/F_0 ($(=F_m\text{-}F_0)/F_0$), value proportional to oxygen-evolving complex activity; OEC, oxygen-evolving complex; PSII, photosystem II; PQ, plastoquinone pool; Q_A, primary quinone acceptor of PSII; RC, reaction centre of PSII; RWC, relative water content

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behaviour define quantum yields and efficiencies (or probabilities): the maximum quantum yield of primary photochemistry (φ_{Po}), quantum yield of electron transport (φ_{Eo}), probability that a trapped exciton moves an electron into the ETC beyond Q_A (ψ_o), probability that an electron is transferred to reduce end electron acceptors at the PSI acceptor side (δ_{Ro}) (Strasser et al., 2004, 2010). The density of active RCs (Q_A reducing RCs) per cross-section at point 0 can be defined by the calculated specific absorption flux, ABS/RC, and the experimentally accessible phenomenological absorption flux, $ABS/CS_0 = F_0$ (Strasser et al., 2004).

The above-mentioned parameters are susceptible to different stressors and therefore have recently been used for stress detection (Christen et al., 2007; Kalaji et al., 2011; Guha et al., 2013; Salvatori et al., 2015). Previous research has shown that, in case of drought or thermal stress, a new step arises in the fast fluorescence curve at the time of 300 μs, called the K-step, or K-band, when only a marked shift in the fluorescence at 300 μs is noted (Strasser et al., 2004; Oukarroum et al., 2007; Brestic and Zivcak, 2013). The appearance of the K-band is ascribed to restricted electron flow from OEC to the RC (Strasser et al., 2004; Brestic and Zivcak, 2013). Finally, an integrative parameter, the so-called performance index, PI_{ABS}, introduced by Strasser et al. (2004), is used for the assessment of the influence of stress on plants (Hermans et al., 2003; Živčák et al., 2008; Swoczyna et al., 2010). PI_{ABS} expresses the photosynthetic machinery potential for energy conservation from photons absorbed by PSII in relation to the reduction of intersystem electron acceptors, and, based on calculations of the density of active RCs, the probability of photon trapping by RCs and the efficiency of electron movement beyond the Q_A (Strasser et al., 2004, 2010).

Portable fluorometers calculating fast fluorescence parameters enable abundant information about the photosynthetic machinery performance of numerous samples to be obtained in a relatively short time (Kalaji et al., 2014a,b). This is very important for practitioners who are obliged to make quick decisions concerning agro-technics or tree maintenance. The possibility of distinguishing the background of stress reaction of trees using the ChF method would be very advantageous.

T. cordata is a typical broadleaf forest species. It grows naturally in stable forest habitats; however, it may also occur in the open landscape. Due to its aesthetic values, it is often planted in cities. However, urban environments provide unfavourable conditions for tree growth and performance (Sieghardt et al., 2005). Urban trees are usually affected by drought stress evoked by both extended transpiration, due to a higher atmospheric vapour pressure deficit, and restricted soil volumes which unable a sufficient amount of water to accumulate (Bühler et al., 2006). Additionally, in Central and Northern Europe, NaCl is commonly used in the winter for road and pavement de-icing (Sieghardt et al., 2005; Swoczyna et al., 2010) and its accumulation in soils triggers osmotic constraints in plant water uptake despite snow melting and spring precipitation. These stress factors seriously endanger the condition of urban trees.

The aim of our experiment was to identify which fluorescence parameters could be used as an evident signal of water stress in contrast to parameters affected by salt stress. In this study, we focused our attention on changes in the state and yield of photosynthetic apparatus in time of exposure to drought or salinity stress to find the possible marker parameter/s of either stress.

2. Materials and methods

Young *T. cordata* trees obtained from a commercial supplier were potted into 5-l two-cover pots and placed in a computer-controlled greenhouse. The soil was a typical horticulture mixture containing 49–59% organic matter; pH of 6.5; $N\text{-}NO_3$, $N\text{-}NH_4$, P, K, Ca, Mg, Cl were > 1000, 66, 219, 1229, 1056, 700 and 41 mg dm^{-3}, respectively. The maximum light intensity was 1200 μmol m^{-2} s^{-1}, the temperature of the day ranged between 25 and 30 °C, during the night between 14 and 18 °C. Nine specimens, 1–1.5 m high with a trunk of 2–3 cm in diameter, were chosen for the experiment. The experiments were carried out in June (2nd–30th). Stress conditions were set after two weeks of acclimatisation. The plant material was divided into 3 groups, 'control', 'salinity stress' and 'drought stress', with 3 specimens in each group. For the drought group, watering was stopped from June 2nd (1st day of experiment). For the salinity group, watering with saline solution in a concentration of 120 mmol NaCl was carried out per 0.5 dm^3 of solution every 2 days starting from June 2nd. For the control group, a regular water regime was applied, 0.5 dm^3 of water every 2 days. The amount of applied water was calculated as the amount needed to obtain 70% of field capacity. Three fully developed leaves were marked on each tree from 3 different sides. Consecutive measurements were carried out at 11:00–11:30 a.m. approximately once a week, *i.e.*, on 9th June, the 7th day of experiment, 16thJune, the 14th day of experiment, 23rd June on the 21st day of experiment and the last measurements were performed at 28th day of experiment.

Fast kinetics of ChF were measured using a *HandyPEA* fluorometer (Hansatech Instruments Ltd., King's Lynn, Norfolk, Great Britain). The

Table 1
Selected JIP-test parameters calculated on the basis of fast fluorescence kinetics.

Fluorescence parameters	Description
$F_I = F_{30ms}$	fluorescence at 30 ms after illumination of a dark-adapted sample
$F_J = F_{2ms}$	fluorescence at 2 ms after illumination of a dark-adapted sample
$F_K = F_{300}$	fluorescence at 300 μs after illumination of a dark-adapted sample
$F_v/F_m = \varphi_{Po} = TR_0/ABS = (F_m - F_0)/F_m$	maximum quantum yield of PSII photochemistry
$F_v/F_0 = (F_m - F_0)/F_0$	maximum ratio of quantum yields of photochemical and concurrent non-photochemical processes in PSII
$V_I = (F_{30ms} - F_0)/(F_m - F_0)$	relative variable fluorescence at 30 ms after illumination of a dark-adapted sample
$V_J = (F_{2ms} - F_0)/(F_m - F_0)$	relative variable fluorescence at 2 ms after illumination of a dark-adapted sample
$V_K = (F_{300} - F_0)/(F_m - F_0)$	relative variable fluorescence at 300 μs after illumination of a dark-adapted sample
$M_0 = 4\ (F_{300} - F_0)/(F_m - F_0)$	approximated initial slope of the fluorescence transient, expressing the rate of RCs' closure
$ABS/CS_0 = F_0$	obtained from measurements as initial fluorescence
$ABS/RC = (TR_0/RC)/(TR_0/ABS)$	specific absorption flux per RC
$TR_0/RC = M_0/V_J = 4\ (F_{300} - F_0)/(F_{2ms} - F_0)$	trapped energy flux per RC
$DI_0/RC = ABS/RC - TR_0/RC$	energy dissipation flux per RC
$ET_0/RC = (TR_0/RC)\ (1 - V_J)$	electron flux per RC
$ET_0/TR_0 = \psi_o = (F_m - F_{2ms})/(F_m - F_0)$	probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A,
$RE_0/ET_0 = \delta_{Ro} = (F_m - F_{30ms})/(F_m - F_{2ms})$	probability that an electron from the electron transport chain is transferred to reduce end electron acceptors at the PSI acceptor side
$RC/CS_0 = \varphi_{Po}\ (V_J/M_0)\ (ABS/CS_0)$	density of active RCs (Q_A reducing RCs) per cross section at point 0
$PI_{ABS} = RC/ABS \times \varphi_{Po}/(1 - \varphi_{Po}) \times \psi_{Eo}/(1 - \psi_{Eo})$	performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors
Area	density area over the ChF transient delimited by a horizontal line at F_m

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