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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),

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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electron transport (ET) and the reduction in end electron acceptors at the PSI acceptor side (RE). Additional parameters characterising the PSII 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.

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

Fast kinetics of ChF were measured using a *HandyPEA* fluorometer (Hansatech Instruments Ltd., King's Lynn, Norfolk, Great Britain). The leaves were dark-adapted using leaf clips for 20 min. The dark-adapted leaf samples of 4 mm diameter within each clip were illuminated with 660 nm light of 3500 μmol m^{-2} s^{-1} for 1 s. Descriptions and equations for calculating of JIP-test parameters are explained in Table 1.

Prior to the fluorescence measurements, the leaf-relative chlorophyll content, the flavonol content and the NBI (Nitrogen Balance Index) were measured in the same leaves using *Dualex Scientific+* ™ (Force-A, Orsay, France).

Effects of stress treatments on leaf chlorophyll and flavonol content and ChF parameters were calculated using one-way ANOVA, with the significance of differences being determined by the LSD Fisher *post-hoc* test at the significant level of $p < 0.05$, using STATISTICA v13.1 software (Dell Inc., USA). In addition, a Principal Component Analysis (PCA) was conducted on a correlation matrix to detect variables which could distinguish clusters of experimental cases. For the analyses the variables among investigated parameters (pigment contents and ChF indices) were selected with the highest representativeness (more than 80% in the whole analysis). The analyses were performed for six cases selected by the removal of three extremes. Removing of extremes was based on excluding three outlier cases from analysis at each measuring date.

3. Results

Variable fluorescence curves were constructed to compare differences between stressed and control plants on each measuring day during the experiment (Fig. 1). The first visible changes between variable fluorescence curves were observed on the 21st day of the experiment (Fig. 1C). However, the differences increased on the 28th day, where the K- and J-bands showed significantly higher values for both stressed groups compared to the control. In addition, variable fluorescence transient curves did not show any specific differences between salinity and drought stress (Fig. 1). For ΔVt analysis ($\Delta V_t = ((F_t - F_0)/F_v) - V_{tF}$) fluorescence in control trees on each day of the experiment (was a used as a reference and) equalled 0 (Fig. 2). For salinity-stressed trees on the 7th, 14th and 21st days, changes and peaks observed were not significant when compared to control trees, but on the 21st day the visible positive ΔJ-band appeared (Fig. 2A). On the 28th day of salt treatment the ΔVt curve showed three visible positive peaks – ΔK, ΔJ and ΔI, which could be correlated with damage to OEC and PQ. For drought stress, the significant bands were observed earlier, on the 21st day of experiment (Fig. 2B). On the 28th day the ΔK-band was significantly higher when compared to salinity stressed trees, while the ΔI bands were comparable for both stress factors (Fig. 2) and the differences between $\Delta V_{I\text{-}P}$ in control and both stressed groups were not significant. It could be assumed based on ΔVt that drought affects the PSII earlier than salinity stress.

The phenomenological energy fluxes through cross-sections (CS) of *Tilia cordata* leaves are shown in Fig. 3. On the 14th day of the experiment, a significant decrease in parameters describing energy fluxes per CS was detected in both stressed groups (Fig. 3). The decrease in values was found for absorbed energy (ABS/CS), trapped energy (TR/CS) and electron transport through CS (ET/CS) with simultaneously the same value of dissipated energy (DI/CS). A similar effect was observed on the 21st day, but the highest decrease, particularly the diminished percentage of active RCs (RC/CS), was observed on the 28th day (Fig. 3), on which the most highly dissipated energy and percentage of inactive RCs were also found in drought-stressed trees. According to the overall results obtained for phenomenological energy fluxes, RC/CS, ET/CS and DI/CS were the parameters which significantly changed during the experiment.

On the 7th day, no significant differences between fluorescence indices (F_0, F_m, Area and F_V/F_0), specific energy fluxes (ABS/RC, TR_0/RC, ET_0/RC and DI_0/RC), quantum yields (φ_{Po}, ψ_o, φ_{Eo}, φ_{Do}) and vitality index (PI_{ABS}) were found. The first symptoms of stress were observed as the decrease in F_m for drought and salinity stress. In addition, in drought-stressed trees, a significant decrease in F_V/F_0, ET_0/RC, φ_{Po} and increase in φ_{Do} in comparison to the control was found (Fig. 4B). On the 21st day, the decrease in some parameters (F_m, ET_0/RC, φ_{Po}) was observed in both stressed groups compared to the control, however, the increase in F_0, DI_0/RC and ABS/RC and decrease in ψ_o, φ_{Eo} and PI_{ABS} was noticed, but only in drought-stressed trees (Fig. 4C). Moreover, the decrease in TR_0/RC was found for salinity stressed trees in comparison to the control. The highest changes between stressed trees and the reference group were observed on the 28th day. Each of the quantum yield parameters was equally affected by both stress factors.

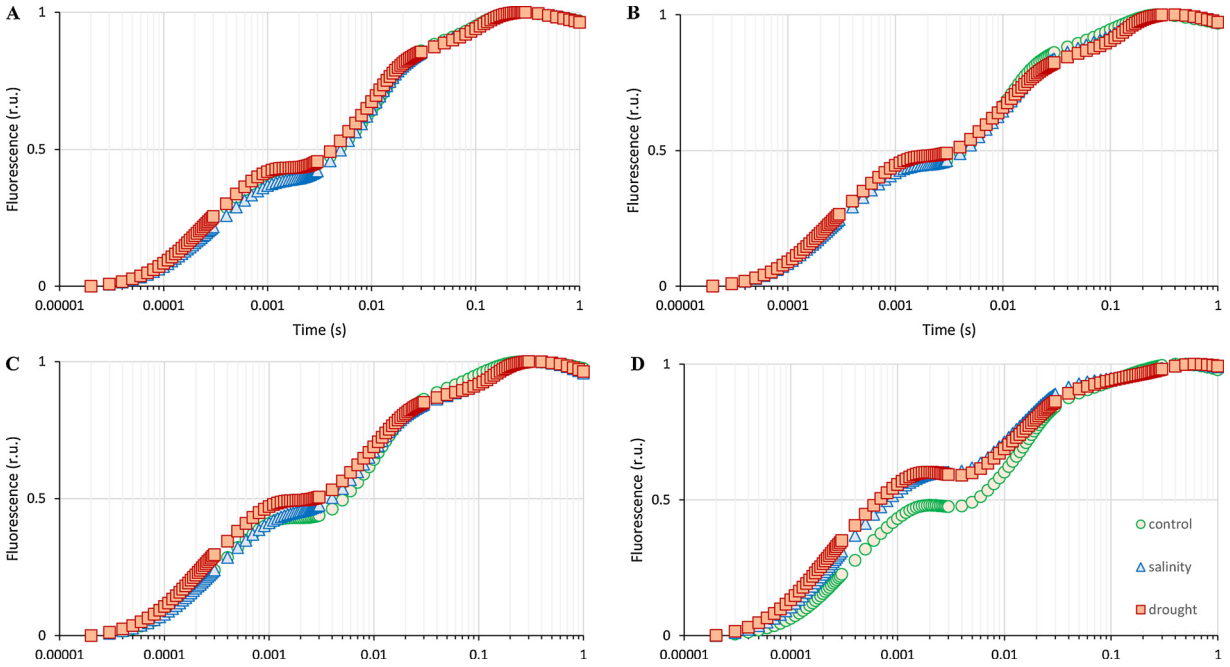

Fig. 1. Variable fluorescence in *Tilia cordata* leaves on 7th (A), 14th (B), 21 st (C) and 27th (D) day of experiment. Values are means (n = 9).

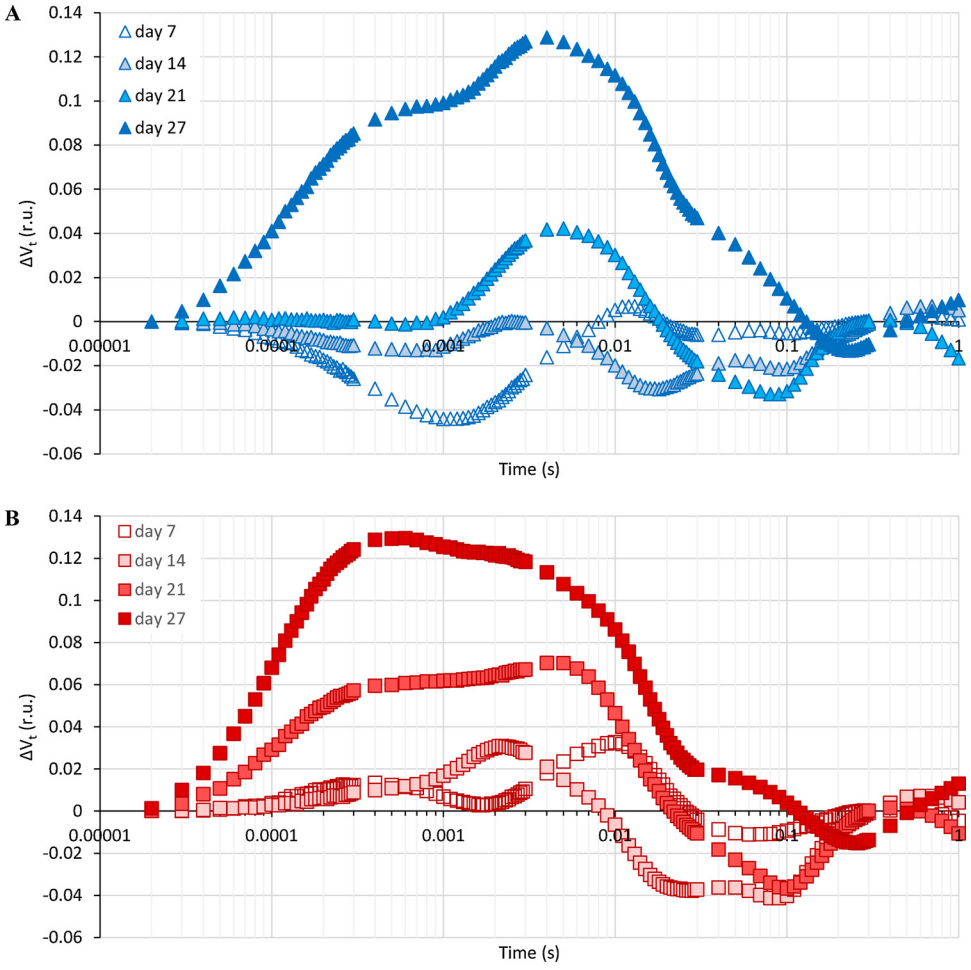

Fig. 2. The effect of salinity (A) and drought (B) on relative variable fluorescence (ΔVt = ((Ft − Fo)/Fv) − VtF) in *Tilia cordata* leaves. For ΔVt analysis fluorescence of control leaves on each day of experiment (was used as a reference and) equalled 0. Values are means (n = 9).

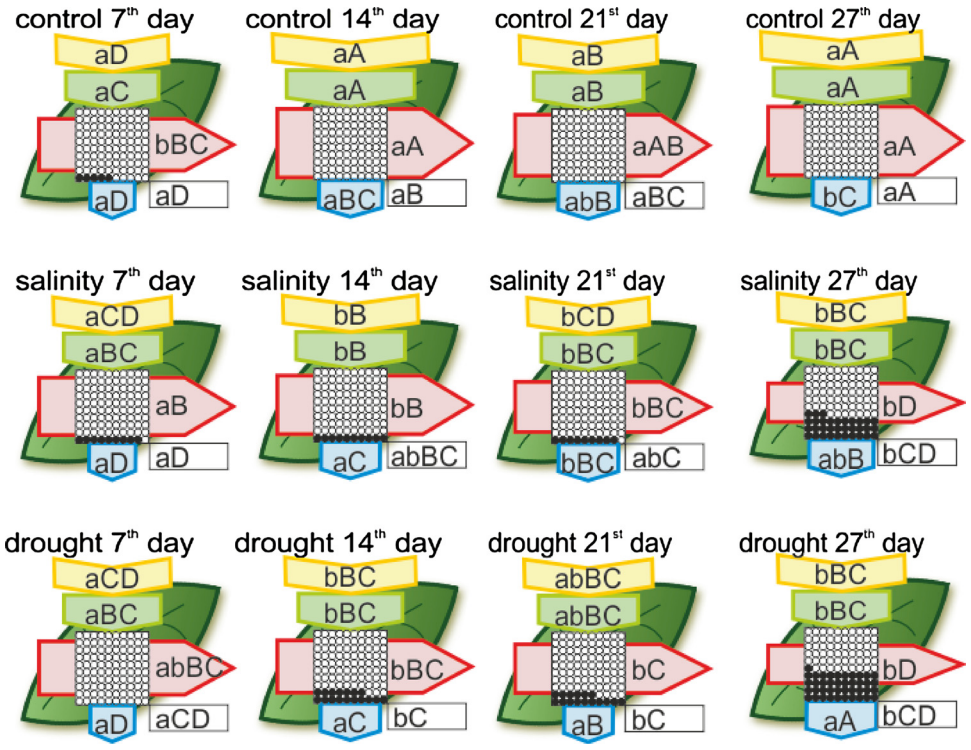

Fig. 3. Leaf model showing phenomenological energy fluxes per excited cross-section (CS) in *Tilia cordata* leaves changing in time under salinity and drought stress. Each relative value of parameters measured is a mean (n = 9) and the width of each arrow corresponds to the intensity of the flux. Yellow arrow – ABS/CS, absorption flux per CS approximated; green arrow – TR/CS, trapped energy flux per CS; red arrow – ET/CS, electron transport flux per CS; blue arrow – DI/CS, dissipated energy flux per CS; circles inscribed in square – RC/CS, % of active/inactive reaction centres. White circles inscribed in squares represent reduced Q_A reaction centres (active), black circles represent non-reducing Q_A reaction centres (inactive), 100% of active reaction centres respond with the highest mean value observed on each day of the experiment. Means followed by the same small (for a column) and capital (for the whole graph) letter for each parameter are not significantly different from each other using the LSD test ($P \leq 0.05$). Letters are inscribed into arrows, except for RC/CS where they are placed in a box in the lower-right corner of the square with circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

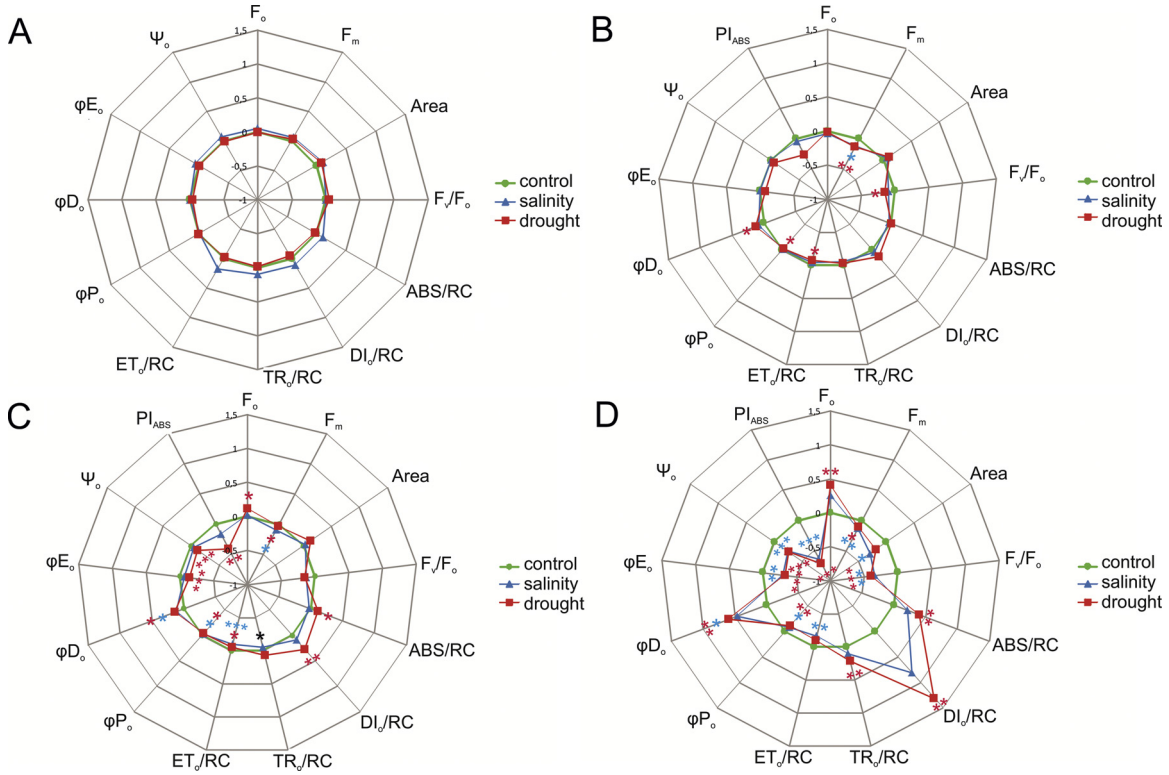

Fig. 4. Changes in the shape of the spider plot JIP-test parameters induced by salinity (Δ) and drought (□) stress applied to *Tilia cordata* measured on 7th (A), 14th (B), 21 st (C) and 28th (D) day of experiment. Values are means (n = 9). Asterisks (*, **, ***) denote significant differences between controls and stressed plants according to Fisher LSD test at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively. Significant differences between control and salinity (blue) or drought (red) stress treatment were denote by a different colour of asterisks. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The drought stress caused the increase in TR_0/RC, ABS/RC and F_0 (Fig. 4D). In addition, salinity and drought stress caused the decrease in F_m, PI_{ABS} and F_V/F_0 parameters as well as the increase in DI_0/RC. The Area parameter was significantly diminished only in salinity-stressed trees (Fig. 4D). DI_0/RC, φ_{Do} and φ_{Po} seem to be the most sensitive and responsive parameters and may act as effective stress markers. Although the relative variable fluorescence curves based on means of each stress group measurements showed minor negative ΔI-bands (Fig. 2), the fluorescence parameter concerning I-step (δ_{Ro}) did not reveal significant differences.

Trees under drought stress revealed significantly lower chlorophyll content when compared to the control and the salinity-stressed groups on the 7th day of the experiment (Fig. 5A). This tendency was similar on the 14th and 21st days. At the end of the experiment, the highest content of chlorophyll was found in reference trees, while significantly lower and the lowest values were observed in salinity- and drought-stressed trees, respectively (Fig. 5A). In each experimental group an increase in chlorophyll content was observed on the 14th and 21st days, for drought and salinity the decrease was found on the 28th day, and simultaneously, a continuously increasing tendency in the reference trees was observed. There were no significant differences in flavonol content and the Nitrogen Balance Index between experimental groups and consecutive days along with stress treatment (Fig. 5B, C).

The Principal Component Analysis (PCA) was performed on a correlation matrix for each day of the experiment to detect early indicators of drought and/or salinity stress (Fig. 6). For this reason, the parameters which grouped cases into clusters consisting of trees under different treatment were highly desirable. Based on the analysis performed for the 7th day of the experiment no pattern was observed, which could suggest the differentiation of cases into clusters (Fig. 6B). However, the φ_{Po} and φ_{Do} parameters showed negative correlation and mostly conditioned PC 2 (Fig. 6A). On the 14th day, based on variable correlation analysis, the increase in φ_{Do} and DI_0/RC as well as the decrease in φ_{Po} seemed to be the first indicators of drought (Fig. 6C, D). The major factor differentiating salinity-stressed plants on the 21st day was the decrease in F_m while other parameters (particularly φ_{Do} and DI_0/RC) stayed at the control level at the same time (Fig. 6E, F). In drought-stressed plants on the 21st day, the most important factors differentiating the cases were still the increase in φ_{Do} and DI_0/RC and the decrease in φ_{Po} (Fig. 6E, F). All parameters obtained from ChF analyses conditioned PC 1, while parameters describing proper photosynthetic performance were grouped opposite to those indicating damage to PSII (Fig. 6G). Those variables grouped cases into stressed and control groups by the PC 1 axis. In addition, the only variables differentiating stressed plants along the PC 2 axis were the flavonol content and NBI.

4. Discussion

The effects of drought and salinity on the photosynthesis process range from the changes in pigment composition, via the restriction on CO_2 diffusion into the chloroplast, limitations on stomatal opening mediated by shoot-root-generated hormones and on the mesophyll transport of CO_2, to alterations in leaf photochemistry and carbon metabolism (Chaves et al., 2009; Fusaro et al., 2014). Our study revealed that mechanisms of plant reaction to both salinity and drought stresses were similar at the early stage of plant exposure to those stress factors. Besides dehydration, salt stress could induce hyper-osmotic and hyper-ionic stress, an ion toxicity (particularly caused by Cl^-) when plants are exposed to high concentrations of salt for long periods (Kalaji and Pietkiewicz, 1993; Munns, 2002; Chaves et al., 2009). At high salinity, salts build up in leaf tissues to excessive levels. However, how the salts exert their toxicity remains unknown. Salts may build up in the apoplast and dehydrate the cell, they may build up in the cytoplasm and inhibit enzymes involved in carbohydrate metabolism, or they may build up in the chloroplast and exert a direct toxic effect on photosynthetic processes (Munns and Tester, 2008). Although drought

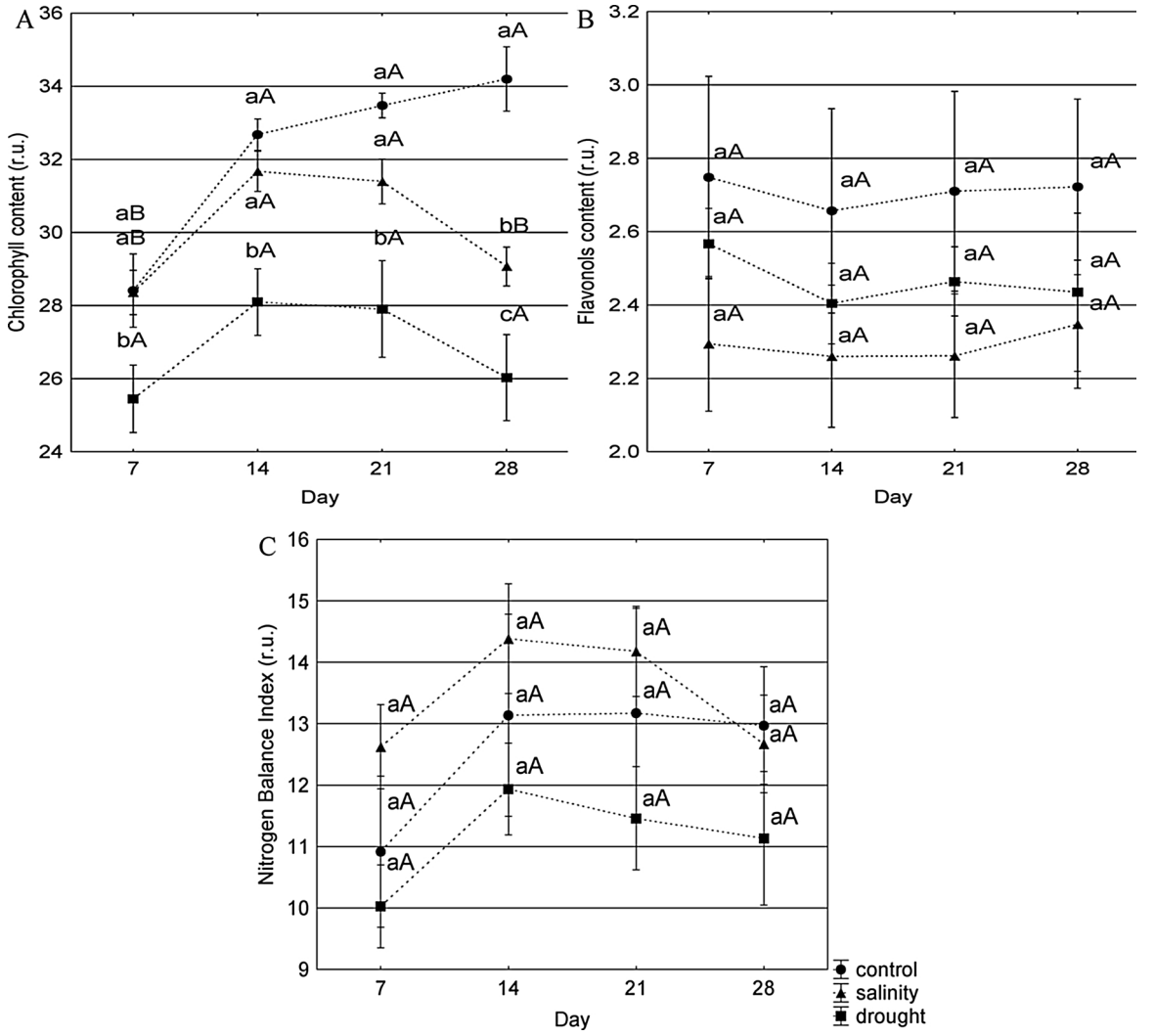

Fig. 5. The effect of salinity and drought on the relative chlorophyll content (A), flavonol content (B) and Nitrogen Balance Index (C) in *Tilia cordata* leaves. Values are means ± SE (n = 9). Lower case letters denote significant differences between variants on each day separately, whereas capital letters denote significant differences between days of treatment for each variant separately according to one-way ANOVA, post-hoc LSD test (P ≤ 0.05).

stressed trees showed earlier reduction in their vitality and photosynthesis efficiency as compared to those growing under salinity stress, changes observed in both cases revealed the same pattern.

In our experiment, both drought and salt stresses significantly affected PSII performance. These effects were visible in the variable fluorescence curve (Fig. 1) presented for each day as well as in relative variable fluorescence curves presented for each stress factor separately (Fig. 2). Although significant differences between both stress and control variable fluorescence curves were scarcely visible on the 28th day of the experiment, an increase in fluorescence at K- and J- bands was observed. The changes in OJIP fluorescence rise kinetics could be revealed by calculating differences between variable fluorescence curves (ΔVt) based on subtracting the normalised fluorescence values (between O and P) recorded in stressed plants from those recorded in control plants (Kalaji et al., 2014a,b). This analysis revealed that in case of drought there was a significant increase in ΔK- and ΔJ-band on the 21st and 28th days, while salinity evoked the same effect only on the 28th day. Interestingly, ΔK-band was higher for drought stressed plants on the 28th day while the ΔJ-band was at similar level for both stresses. The appearance of ΔK-band could occur due to the reduced efficiency of OEC resulting in an imbalance between the electron flow from the OEC to the RCs and towards the PSII acceptor side (Strasser, 1997; Guha et al., 2013). This is consistent with some studies in which salt or drought stress provoked serious changes in the fluorescence curve shape, and additional steps emerged (Oukarroum et al., 2007; Kalaji et al., 2011; Wang et al., 2012; Brestic and Zivcak, 2013).

The leaf pipeline model is widely used to describe environmental and/or anthropogenic pressure on plants (Kalaji et al., 2011; Mehta et al., 2010; Zushi et al., 2012). Phenomenological energy fluxes model (Fig. 3) revealed a significant increase in energy dissipation (DI/CS), decrease in active RCs (RC/CS), trapped (TR/CS) and absorbed energy (ABS/CS) as well as energy transfer (ET/CS) per cross-section in stressed trees on the 28th day of the experiment. The most visible changes over the whole experiment were found for energy dissipation (DI/CS), active RCs (RC/CS) and energy transfer (ET/CS). These results with additional increasing tendency of specific energy fluxes (ABS/RC, DI_0/RC, TR_0/RC) with the exception of ET_0/RC (Fig. 4) are in agreement with/concur with findings presented by Christen et al. (2007) and Strasser et al. (2010) for drought-stressed plants. Strasser et al. (2010) referred the increase of specific energy fluxes to a decrease in active RCs. The increase in F_0 and decrease in F_m could suggest the possibility of dissociation of the light-harvesting complex (LHC) from the PSII and the disruption of energetic connectivity (Havaux, 1993; Strasser and Stirbet, 1998; Strauss et al., 2006; Oukarroum et al., 2007, 2009). This facilitates an avoidance of the over-reduction of the PQ and protection of the PSII from damage (Zivcak et al., 2013). These findings could be additionally supported by a decrease in the Area parameter on the 28th day as Area refers to the pool of free plastoquinone (Kalaji et al., 2011).

Numerous experiments are consistent with our findings showing the increase in F_0 in case of drought stress. (Percival and Sheriffs, 2002; Christen et al., 2007; Fini et al., 2009; Swoczyna et al., 2015). Additionally, from the 21st day onward, the visible symptoms of simultaneous φ_{Po}, φ_{Eo} and ψ_o decrease were observed as well as the increase in φ_{Do} in both stressed groups. The decrease in φ_{Eo} and ψ_o corresponds to the inhibition of ET beyond Q_A (Misra et al., 2001; Mehta et al., 2010; Kalaji et al., 2011; Jafarinia and Shariati, 2011). The reduction of active RCs could be caused by their transformation into excitation traps not forwarding the excitons to the plastoquinone, but dissipating all the trapped energy as heat (φ_{Do}) (Misra et al., 2001; Kalaji et al., 2011; Jafarinia and Shariati, 2011). These protective mechanisms seem to be switched on as a consequence of an overloaded PQ. In addition, the supposition of RCs transformation into traps could be additionally supported by the decrease in ET_0/RC which was the only one parameter exhibiting the decrease among all parameters describing specific energy fluxes. The decrease in quantum efficiencies of consecutive processes on the acceptor side of PSII (φ_{Po} also known as F_v/F_m, $ET_0/TR_0 = \psi_o$) has also been reported in previous studies on tree species (Percival et al., 2006; Oukarroum et al., 2007; Bussotti et al., 2010; Wang et al., 2012).

Chlorophyll degradation was found in both stressed groups, however drought-stressed trees exhibited more advanced degradation of chlorophyll when compared to salt-stressed specimens. Low chlorophyll content in woody plants is referred to low nitrogen (N) availability rather than to drought stress (Rubio-Covarrubias et al., 2009). Nevertheless, poor water availability in the soil may enable sufficient N uptake (Rubio-Covarrubias et al., 2009). Moreover, severe drought may lead to chlorophyll degradation due to early leaf senescence in seriously stressed plants (Grassi and Magnani, 2005; Gallé and Feller, 2007). High salinity stress may also result in diminished chlorophyll content when osmotic constraints limit water availability for plants (Beinsan et al., 2003; Fusaro et al., 2014).

Based on these results it could be assumed that both drought and salinity stresses induce disturbances in chloroplast photochemistry. When the rate of absorption of light energy by photosynthetic pigments exceeds the rate of its consumption, the absorbed light energy accelerates the process of photoinhibition. Takahashi and Murata (2008) reported an excess of light energy absorbed by photosynthetic pigments to accelerate photoinhibition through suppression of the repair of photodamaged PSII and not by acceleration of photodamage to PSII. It was reported that the primary photodamage to PSII occurs at the OEC. Following photodamage to OEC, the potential for damage to the PSII RCs upon light absorbed by photosynthetic pigments will increase owing to a lack of electron donation from the OEC to oxidised PSII RCs (Takahashi and Badger, 2011). To cope with excessive light, plants have developed a mechanism which dissipates absorbed energy as heat. DI_0/RC and φ_{Do} are parameters which refer to the rate of energy dissipation by PSII. DI_0/RC and φ_{Do} values obtained in our experiment additionally support the statement that both salinity and drought stresses induce photoinhibition. Results from PCA support this theory (Fig. 6). The combined effect of excessive light in June (data not shown) and stress factors resulted in damage to OEC followed by damage in RCs. In addition, protective mechanism against excess light were utilised resulting in the increase in energy dissipation in stressed trees. This phenomenon was visible on the 14th day of the experiment (Fig. 6C) and corresponds mostly to drought-stressed trees by the increase in DI_0/RC, φ_{Do}, TR_0/RC, ABS/RC. Moreover, on the 21st day F_0 was shifted. This suggests the effect of prolonged stress triggers the dissociation of the LHC from the PSII and the disruption of energetic connectivity. From the 7th to

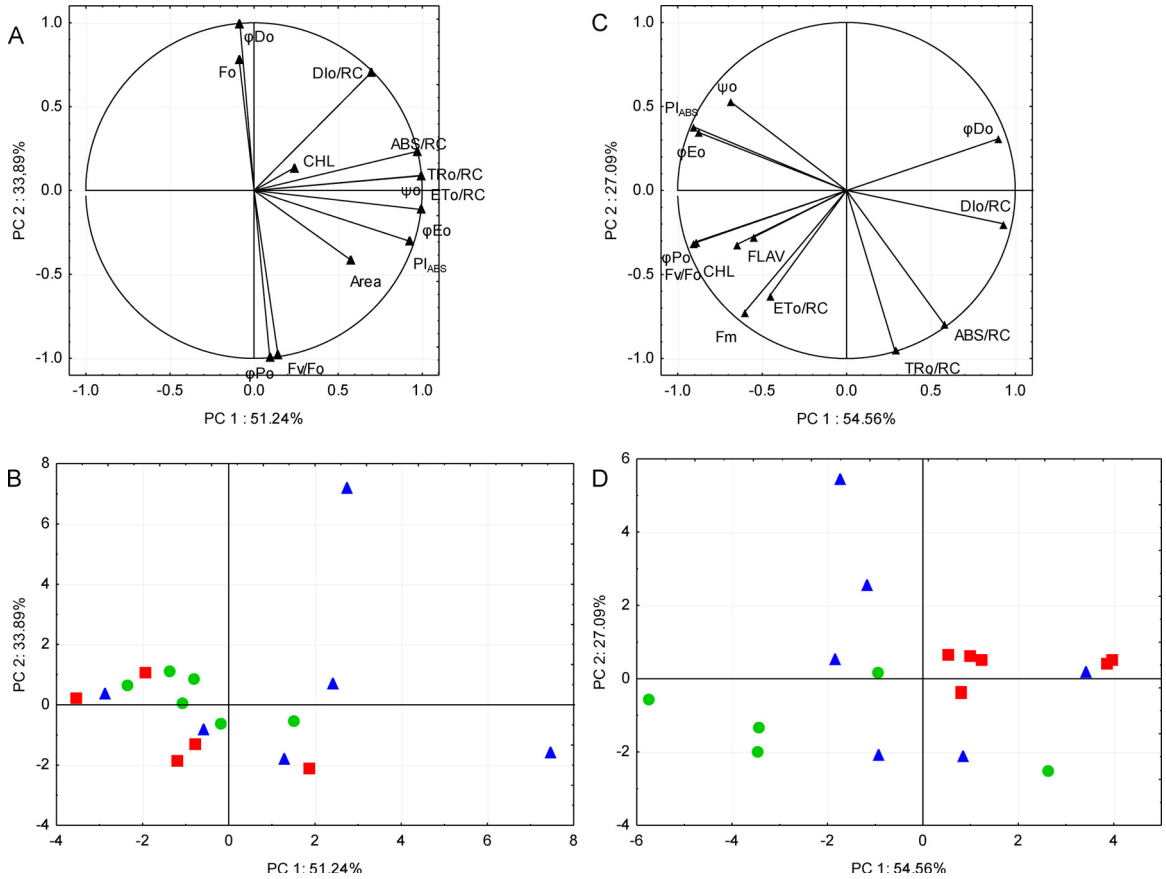

Fig. 6. Principal Component Analysis on parameters obtained during the experiment for the control (○), salinity-stressed (Δ) and drought-stressed (□) *Tilia cordata* plants on the 7th (A, B), 14th (C,D), 21 st (E, F) and 28th (G, H) days of the experiment. Correlation between variables along two PCA axes (A, C, E, G) and ordination of case along two PCA axes (B, D, F, H) were presented for 6 representative cases. Variables with low representativeness were removed to obtain an overall representation of over 80%.

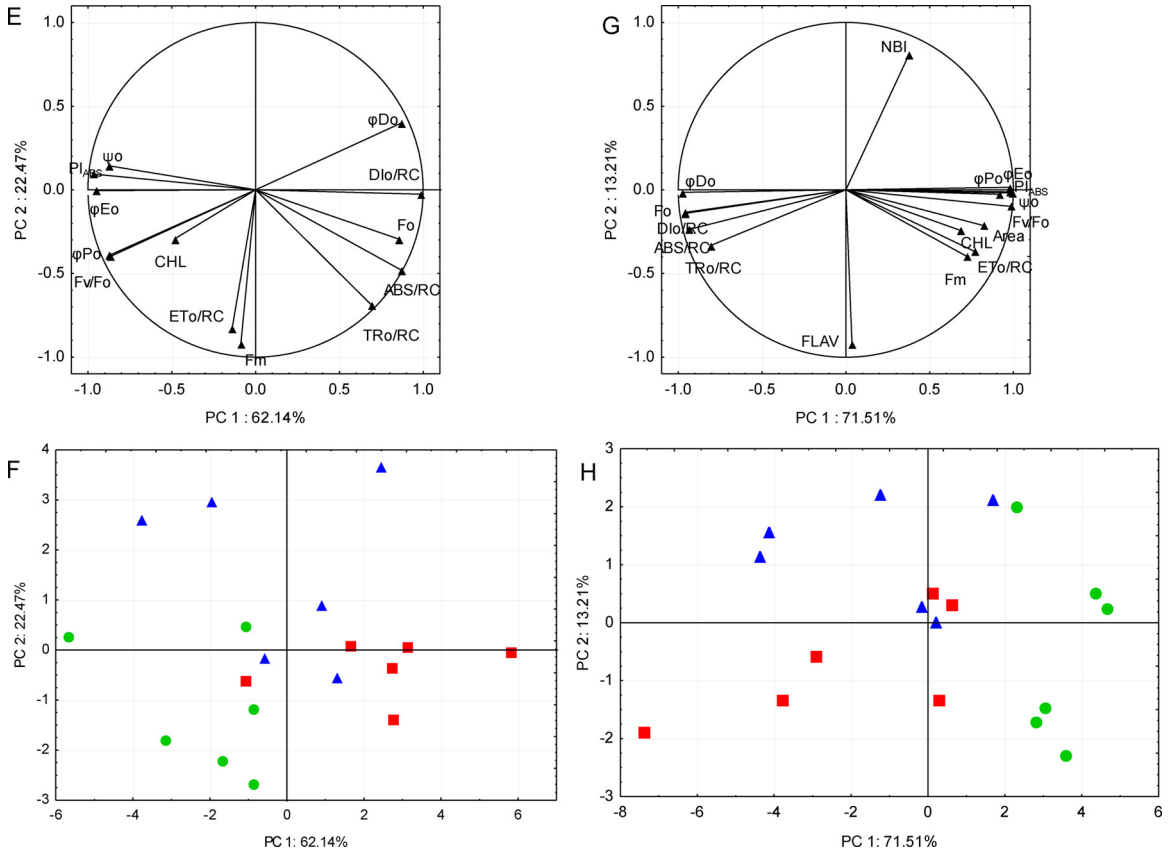

Fig. 6. (*continued*)

the 28th days the increasing contribution of PC 1 in distinguishing clusters appeared. At the end of the experiment, the established ordination of variables corresponding to worse efficiency of photosynthesis apparatus was on the opposite site to those corresponding to better efficiency. On the 28th day there were two variables (NBI and FLAV) which distinguished salt-stressed trees from those stressed by drought. However, this phenomenon could be associated with a more advanced degradation of chlorophyll. Despite the fact that there were no visible mechanistic differences in response of photosynthetic apparatus to salt and drought stress, the specific response could appear at a different plant organisation level.

5. Conclusions

In conclusion, there were no specific indicators among the chlorophyll fluorescence parameters clearly predicting which stress factor affects *Tilia cordata*'s physiological performance. The response of the *Tilia cordata* photosynthetic apparatus to salt and drought stress showed the same pattern, however symptoms of drought stress were detectable earlier. Both stress factors did not affect the IP phase of chlorophyll fluorescence transient, which is related to the reduction of first acceptors of PSI. It could be assumed that the impact of drought and salinity was mainly due the photo-inhibitory effect. We conclude that chlorophyll-*a* fluorescence parameters cannot be used as bio-indicators which allow the effects of drought and salinity stress in *Tilia cordata* to be distinguished.

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